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FOREWORD

The four independent reviewers estimated each paper and recommended 78 articles for publishing at the proceedings consisted of 2 volumes, which started life as presentations at the Annual 18th International Scientific Conference "Research for Rural Development 2012" held at the Latvia University of Agriculture, in Jelgava, on 16 to 18 May 2012.

In the retrospect of four months later, we can count the Conference as a great success. The theme – Research for Rural Development - attracted participation more than 200 researchers with very different backgrounds. There were 114 presentations from different universities of Lithuania, Estonia, Ukraine, South Africa and Latvia.

Thank you for your participation! I'm sure that you have learned from the presentations and discussions during the conference and you can use the outcomes in the future.

The cross disciplinary proceedings of the Annual 18th International Scientific Conference "Research for Rural Development 2012" is intended for academics, students and professionals researching in the area of crop production, animal breeding, agricultural engineering, agrarian and regional economics, food sciences, veterinary medicine, forestry, wood processing, water management, environmental engineering, landscape architecture, information and communication technologies. The proceedings will be useful for researchers in educational sciences, too. The papers are grouped according to the sessions in which they have been presented.

Finally, I wish to thank Organizing and Scientific Committee and the sponsors for their great support to the conference and proceedings.

On behalf of the Organizing Committee
of Annual 18th International Scientific Conference
"Research for Rural Development 2012"

Ausma Markevica
Latvia University of Agriculture

CONTENTS

INFLUENCE OF SOWING TYPE, TIME AND RATE ON THE BUCKWHEAT YIELD FORMING ELEMENTS

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Abstract
There is a lack of an actual information concerning cultivation of buckwheat (*Fagopyrum esculentum*), on the best methods of sowing and seeding rates. The aim of the study was to investigate changes in the buckwheat yield forming elements depending on sowing type, time and rate. Trials were established in farm "Araji", Kraslava area, during 2010. Previous crop was spring barley. Buckwheat cultivar 'Aiva' was used in the field trial. Sowing was done on six different dates – May 15, 20, 25 and 30, and June 4 and 9. Two different types of sowing were used – drill sowing, with 4 sowing rates 200, 300, 400, 500 fertile nutlets per 1 m^2, and the column sowing with the three sowing rates 150, 250, 300 fertile nutlets per m^2. Data analyses were run using MS Excel Two factor analyses of variance. Time of sowing and seed rate had a significant influence on the buckwheat yield forming elements. Evaluating the performance of plant density at column sowing, it was found that plants survival rate was higher than that in drill sowing. The highest individual productivity of the plant was observed in plots sown in columns. The yield of buckwheat was on average 30-50% higher in the plots that were sown from June 4th to 9th if compared to those sown from May 15th to 20th, i.e. earlier sowing times were not the most suitable for buckwheat sowing in 2010.
Key words: buckwheat, sowing time, sowing rate, yield forming elements.

Introduction

Buckwheat (*Fagopyrum eskulentum*) is an important groats plant in temperate climate zone. Buckwheat is widely used in the food industry. Buckwheat products have good dietetic properties because of its easily digestible nutrients. In recent years, the amount of buckwheat growers and cultivated areas has increased in Latvia. Comparing the rates of 1997 and 2011, the area has been increased by 100%, reaching 10.2 thousand ha (Saimniecības ..., 1997; Latvijas statistika..., 2011). Most common problem for the buckwheat growers is relatively low yields of this crop. There is a lack of the actual information about buckwheat growing and factors influencing the yield. There is an insufficient information of the effect of buckwheat sowing time and rate on the yield forming elements.

The optimal time of the buckwheat sowing is one of the main preconditions for harvesting high yields. Assuming that the influence of all agricultural methods of crop growing form 100%, sowing term covers 43%, fertilizing 23%, soil cultivation – 10%, rate and way of sowing – 12%, crop maintenance 4% (Slobodyan, 2004).

There are two main opinions about the sowing date in literature. Some authors recognize 2 or 3 different sowing dates (Анохин, 1960), while others conclude that buckwheat must be sown as early as possible at single, best sowing time.

Choice of optimal sowing date should be based on the longterm field observations and objective sources of information. There is considered that the sowing time is positively dependent on the meteorological conditions in April and May. The sowing rate influences crop density and the individual plant productivity.

Increase in the sowing rate leads to diminished individual plant productivity. Contrary, the reduced sowing rate positively influences the crop – the individual plant productivity increases. It could be explained by plant competition inside the crop. Therefore, correctly established sowing rate provides optimal plant density, and, thereby, increases the plant productivity (Zakarackas, 1999).

Buckwheat sowing rate vary in a relatively wide range. in conventional system in areas with optimal moisture conditions, the preferable sowing rate is about 400 seeds per m^2 applying drill sowing method, and 300-350 seeds per m^2 by column sowing method. In areas with unstable water provision and field areas free of weeds, sowing rates usually become lower, respectively 200-250 and 150-200 seeds per m^2 (Kalinova et al., 2003).

Buckwheat plant branches very well (except of some cultivars) and at favorable conditions an inadequate by low seeding rate has been compensated by increasing productivity of individual plants (Tadina et al., 2007). If there are unfavorable conditions for optimal seed germination, buckwheat sowing rate must be increased by 50-100 seeds per m^{-1} (Bryan et al., 2006).

Some sources of literature propose the column sowing method as the most optimal technique for the buckwheat (Bryan et al., 2006; Kwiatkowski et al., 2004). However, this technique provides the yield increasing only at a low-input agricultural level. This method should not be considered as a key to obtaining high yields. For example, field trials in 1953-1971

showed that the average yield, using the column sowing method was 1.32 t ha^{-1}, independently of the previous crop, while obtained average yield, using drill sowing was 1.86 t ha^{-1} (Ефименко, Барабаш, 1990)

Some authors haveconcluded that all parameters of plant productivity were significantly higher when the column sowing method was used if compared with other sowing methods (Ефименко, 1990; Чашкова, 2007). While different results have been published by V. Upmanis (1957). Observations during two years have showen that highest yields were obtained by column sowing method, as recommended by Д. Ефименко and И. Барашкин. The yield was respectively 1.97 t ha^{-1} when column sowing method was used, and 1.27 t ha^{-1} when drill sowing was used (Upmanis, 1957).

The yield forming elements of buckwheat are: number of branches, number of inflorescences and nutlets per plant. Also plant height is an important morphological parameter. In the agro-climatic conditions of Latvia, data on the influence of sowing type, time and rate on the yield forming elements are not well documented.

The aim of the current study was to investigate the influence of sowing date and rate in different sowing types on buckwheat yield forming elements. Part of the research about the buckwheat yield has been already described in 2011 (Vilcans et al., 2011).

Materials and Methods

Two-factor field trials (A factor – sowing date, B factor – sowing rate) were arranged in farm "Araji", Kraslava region, (latitude: N 55º 86´; longitude: E 27º 04´) in 2010. Buckwheat cultivar 'Aiva' was used and sown using two different sowing types, drill sowing and column sowing, on six different sowing dates.

Factor – A sowing dates:

1st – called 15th May,
2nd – called 20th May,
3rd – called 25th May,
4th – called 30th May,
5th – called 4th June,
6th – called 9th June.

Factor – B seeding rates:

drill sowing – 200, 300, 400 and 500 fertile nutlets per 1 m^2,
Column sowing – 150, 250 and 300 fertile nutlets per 1 m^2.

The trial was randomly spaced; in total, 42 plots werearranged in 4 replications. The plot size – 3 × 15 m. Soil parameters - silt loam l (organic matter content 22.5 g kg^{-1}, soil reaction pH KCl – 5.8, P – 74.23 mg kg^{-1}, K – 149.36 mg kg^{-1}. Previous crop was spring barley.

Traditional soil tillage with 25 - cm - deep autumn plowing was done immediately after the harvesting of spring barley. Soil was cultivated three times before sowing in spring. One week before the second cultivation, weed treatment was done with the herbicide Roundup Eco s.c. (glyphosate, 360 g L^{-1}). All nitrogen fertilizer was incorporated before sowing together with the soil cultivation. For compound fertilizer NPK 16-16-16 with 200 kg ha^{-1} rate was used N 32 kg ha^{-1}; P – 14 kg ha^{-1} and K – 26 kg ha^{-1} rate was used. Buckwheat was sown with mechanical seed-rows-ploughshare 4-m-wide drill – Nordstein Liftomatik, with normal 12 – cm - width line-spacing. Variants sown in columns was composed of two rows with a distance between rows of 12 cm, but that between the columns – 38 cm; depth of buckwheat sowing was 4 cm. The yield was harvested with a grain harvester Massey Ferguson 525, header width - 3.5 m. The yield was accounted as 100% pure nutlets' yield at the moisture content of 140 g kg^{-1}.

Buckwheat plant density was established by counting the plants in one constant 0.5 m^2 area of each plot after germination. Ten plant samples were taken randomly from each plot for biometrical analysis at the end of vegetation. Number of branches per plant (No), plant weight (g), stem length (cm), number of inflorescences (No), and grain weight per plant (g) were measured in laboratory.

Phenological observations during the growing season:

- the phenological phase starts when 10-15% of the plants have reached the development stage, the full phase means the time of 75% of the plants are in the phase.
- the beginning of the phase and full phase was recorded: first seedling phase, flowering phase, technical maturity (ripe nutlets from 70 to 75%), harvest maturity (ripe nutlets 90 - 95%).

Two-factor analysis of variance, and correlation and regression analysis methods were used for data processing. Meteorological data was obtained from Daugavpils Hydro-meteorological Station (HMS) which is the nearest HMS to the farm. Meteorological conditions of the 2010 growing season can be described as warm and wet with periodic substantial rainfall. When plants reached technical maturity phase (70 - 75% of brown nutlets on the plant), the weather conditions of high temperature and extraordinary high amount of precipitations contributed to the restoration of plant growth, which extended the growing season, and made it difficult to choose an appropriate time for buckwheat harvesting.

Results and Discussion

Phenological observations. There was determined dependence of the length of developmental phases and

terms of buckwheat ripening on sowing dates. Plants of late sowing had faster development, phenological phases were shorter, and ripening accelerated. Sprouts of the main early sowing on 20th and 25th May emerged in 15-17 days, whereas late, 30th May and 04th June, sowing sprouts emerged in 9-12 days as sown in moist and optimum warm soil. The sprouts of early sowing of 25th May and late sowing of 30rt May 2010 emerged on the same day. The passing of their further development phases was simultaneous (Fig. 1).

During plant development (Fig. 1) there were two certain critical periods in relation with precipitation. The first critical period of insufficient water supply was at the beginning of germination, the second critical period lasted from the beginning of flowering up to the beginning of seed-kernel filling.

The height of plants mostly depended on sowing rate. Plants sown using high rates were higher depending on sowing dates by 17 cm on average if compared with plants sown in low rates in all variants. According to sowing date, the plants of the main late sowing date (4th June) turned out to be higher, i.e. 118 cm in column sowing method and 119 cm in drill sowing, while late spring date (9th June) plants were 91 cm in height by column sowing and 102 cm in height by drill sowing (Fig. 2).

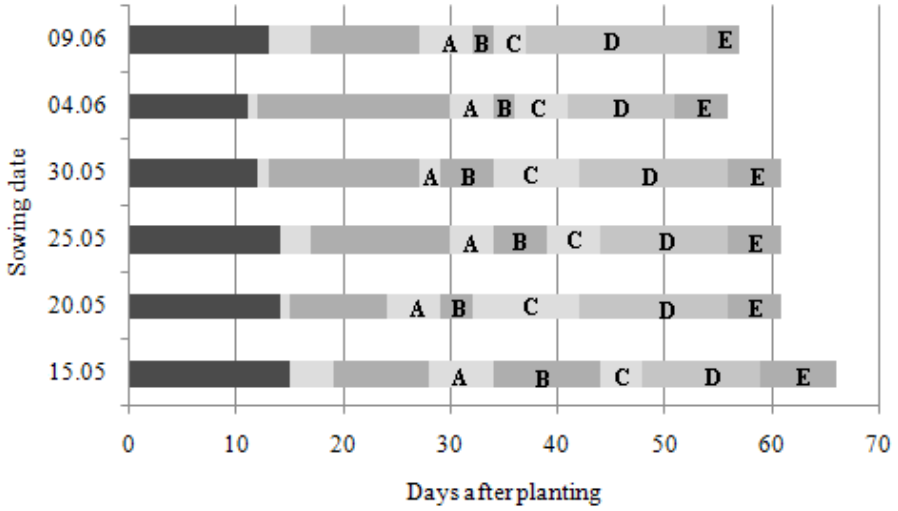

Figure 1. Length of buckwheat phenological phases and vegetation period depending on sowing date.

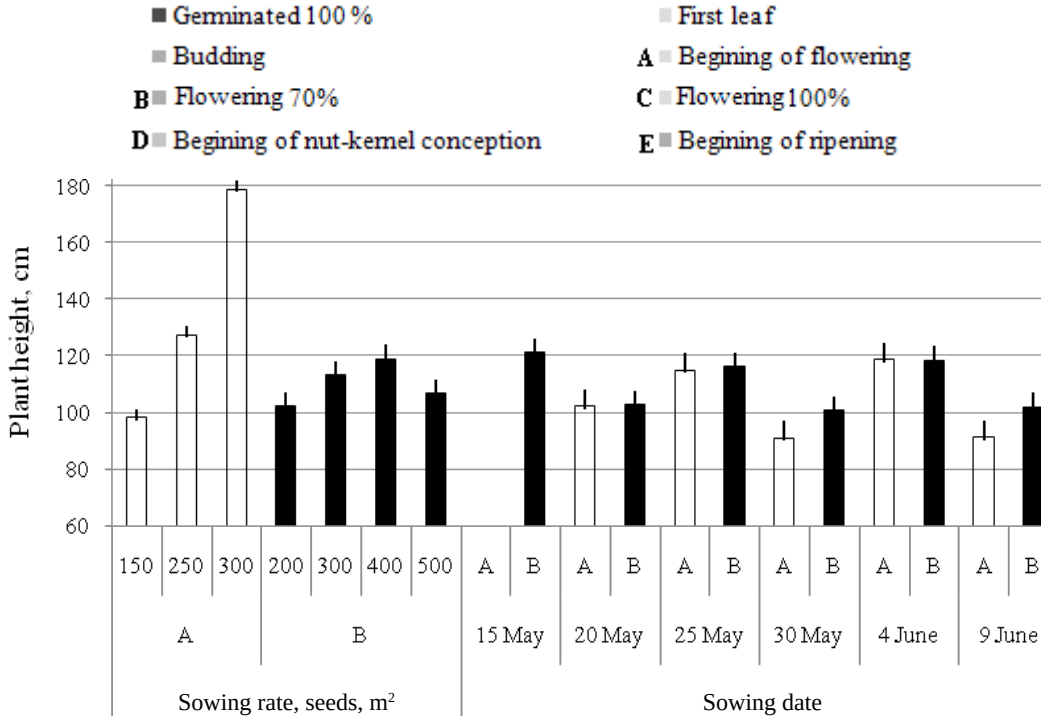

Figure 2. Influence of sowing rate, time and type on buckwheat plant height:
A – column sowing, B – drill sowing.

It was observed that the height of the plants had an impact on the number of branches. The correlation coefficient between the plant height and the number of branches was r = |-0.42|>$r_{0.05}$= 0.25. Plant height increased, but the number of branches decreased with increased sowing rates.

The number of branches. Buckwheat in plots with a smaller number of plants per unit area have all the preconditions for the establishment of additional lateral branches, which in turn will promote greater photosynthetic surface development and a greater number of inflorescences. The correlation between the number of branches and number of the inflorescence confirmed this assumption (Fig. 3)

Sowing date and sowing method had an impact on the number of branches. Plants sown by column sowing had by 1.77 branches more than those sown by drill sowing method. Differences were observed also between plants sown using different sowing rates. The low sowing rate increased the number of branches and contributed to increase in plant productivity. This could be explained by more branches with more productive inflorescences which increased plant productivity under favorable conditions.

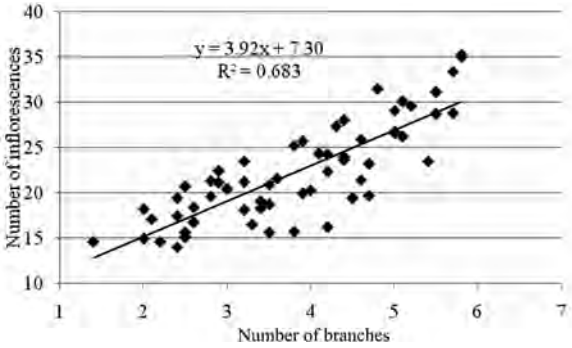

Figure 3. Correlation between the number of branches and inflorescence the number of per buckwheat plant (p<0.0001).

Plant inflorescences. Sowing date also affected the flowering time. The flowering stage of plants sown in the middle of May started 21-23 days after seedling emergence. On the plots sown at the end of May and beginning of June flowers appeared 28 days after seedling emergence, but in later sowing (4th of June) the flowers appeared 30-32 days after germination.

There was observed a close correlation between the number of branches and the numbers of inflorescences: r = |0.82|> $r_{0.05}$ = 0.25; in 68% of cases the changes in inflorescence number might be explained by the changes in the number of branches. Analysis of correlation between the number of branches and inflorescence number showed that the sowing time, rates and sowing type in various interactions affects the closeness of the correlation. It was observed that in the direction from lower sowing rates (200 seeds per m^2) to larger on (400 seeds per m^2) correlation density increase.

Nutlets weight per plant. Correlation analysis between the number of inflorescences and the nutlets weight showed the tendency to increase the nutlets weight per plant with the increased number of inflorescences (Fig. 4)

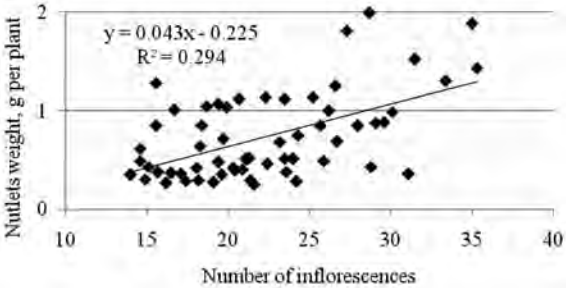

Figure 4. Correlation between the number of inflorescences and the nutlets weight per plant (p<0.0001).

A correlation was found between such important yield forming structural elements (Fig. 4) as the number of inflorescences per plant and the nutlets weight per plant (r = 0.54 > $r_{0.05}$ = 0.25). Optimum growth conditions were provided by column sowing method if compared with drill sowing method. Individual plant productivity was higher when compared with the plants sown by column sowing method with seeding rates above 200 nuts. m^2.

Increasing of plants productivity affected the nutlets' quality indicators - huskiness increased, but 1000 nutlet weight decreased (Fig. 5).

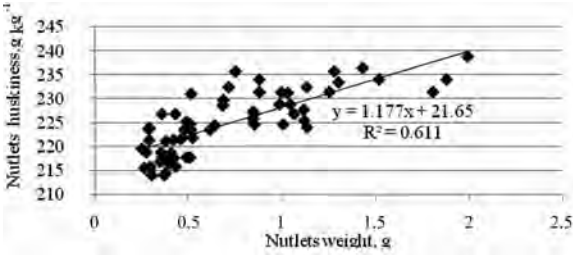

Figure 5. The correlation between the nutlet weight per one plant and nutlets huskiness (p <0.0001).

It was observed that nutlets huskiness growed with increase in individual plant's productivity. It is a negative trend from the agronomical point of view, because it may cause problems during yield harvesting, which further can reduce product quality.

Survival of plants till harvesting was higher in trials sown by column sowing method with low Evaluating the plant density we found that the plant survival was higher in trials seeding rates (150 seeds m^{-2}) if compared with high seeding rates, the same

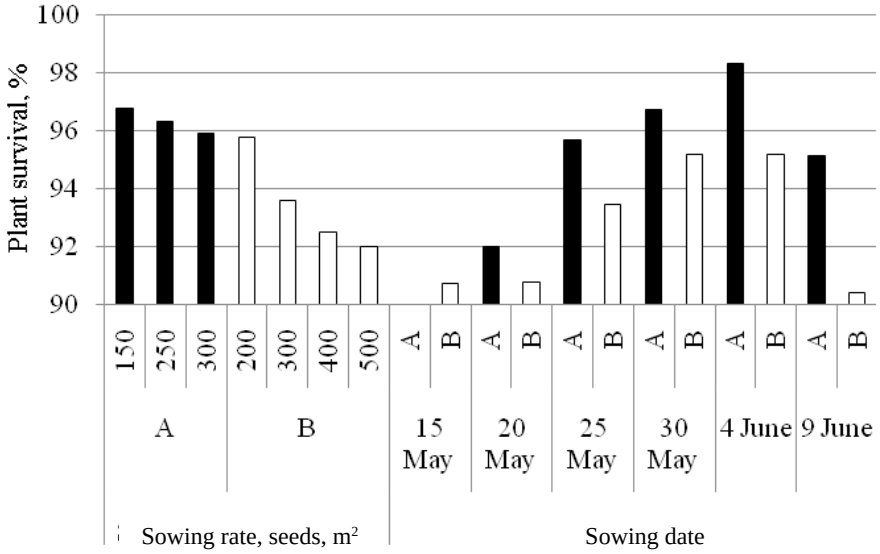

Figure 6. Plant survival in plots depending on different sowing times, rates and methods.
A■ – column sowing; B□ – drill sowing.

effect was observed in the trials sown by drill sowing method. Plant losses were lesser when lower sowing rates (200 seeds m^{-2}) were used. Conditions were not suitable for the establishment of an optimum plant density for sowings of 15th and 20th of May. The greatest difference was obtained in the trial with a higher seeding rate (Fig. 6).

Evaluating the plant density we found that the plant survival was higher in trials sown by column sowing method if compared with the drill sowing method. This trend is attributed to the fact that column sowing method is more suited to the optimal growth of buckwheat (Upmanis, 1957). There is an evidence of the plant competition which leads to losses of the part of the emerged plants if compared with the plots sown with different sowing rates for each sowing method. As a result of the higher density it is more likelihood to plant losses.

Productivity of plants. Sowing rate is the primary cause of plant density, but plant density is closely related to individual plant productivity. It follows that increased sowing rate results in a reduced individual plant productivity, and vice versa, a reduced sowing rate causes increase in the individual plant productivity. The choice of a correct sowing rate is an important precondition to obtain on optimal plant density, as well as the corresponding plant productivity. It should be noted that buckwheat yield not always depends on plant density in a sowing. Buckwheat yield formation elements in 2010 developed in close relationship with meteorological conditions. Plants sown in earlier sowing times suffered from moisture insufficiency and higher air temperature from beginning of flowering to beginning of the nutlets filling, if compared to plants sown at later times (30th of May to 9th of June). It was also found that meteorological conditions during the 2010 growing season were not suitable for a high buckwheat yield formation. Investigations will be continued.

Conclusions

1. Plants of late sowing dates pass development phases faster and accelerate the term of buckwheat ripening.
2. It was observed that the plant survival was higher in trials sown by column sowing method if compared with the drill sowing method.
3. Use of lower sowing rates (150 seeds. m^{-2} for column sowing and 200 seeds. m^{-2} for drill sowing) contributed to increased individual plant productivity - the number of branches and inflorescences per plant. A close relationship ($p<0.0001$) between the number of branches and inflorescences per plant was found.
4. Higher plant density after emergence caused higher plant losses till harvesting. Plant losses in plots sown in columns were less if compared with drill sown plots.
5. Sowing date and method had a significant influence on the number of branches. Plants sown by column sowing method had by 1.77 branches more than the plants sown by drill sowing method.

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EFFECT OF ORGANIC AND CONVENTIONAL PRODUCTION SYSTEM ON THE QUALITY OF SPRING WHEAT

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Abstract
Field trials with the spring wheat (*Triticum aestivum* L.) were carried out on the experimental fields of Estonian University of Life Sciences in 2011. The yield quality (volume weight, 1000 kernel weight, falling number, gluten content, gluten index, gluten content in dry matter) of spring wheat was studied in cultivar 'Vinjett'. The wheat was part of the five-year crop rotation experiment where red clover (*Trifolium pratense* L.), spring wheat, peas (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) undersown with red clover were following each other. There were three treatments which followed the crop rotation. In one treatment mineral fertilizers and pesticides were used, but other two treatments were organic without any synthetic agrochemicals. In mineral fertilizing treatments, the background of P25 and K95 kg ha^{-1} the N amount varied from 0 to 150 kg ha^{-1}. Herbicides Sekator and MCPA 750 were used in conventional part. The aim of this research was to study red clover's after-effect and different N fertilizer amounts' influence on the spring wheat yield quality. In treatments where no mineral fertilizers were used and which only followed the red clovers after effect had higher gluten index and gluten content in dry matter. Higher mineral nitrogen amounts and organic treatments decreased spring wheat 1000 kernel weights and volume weights. The wet gluten content and falling number increased with increase of the amount of mineral N.
Key words: 1000 kernel weight, falling number, gluten, mineral fertilizing effect, volume weight, *Triticum aestivum* L.

Introduction

The main focal point used in advertising organic food is its beneficial impact on human health (Heaton, 2001), and thus consumers believe that crops grown under organic conditions are healthier and with better quality than conventionally grown products (Hughner et al., 2007), but L. Gennaro and G. Quaglia (2003) mention that an improved nutritional profile of organic vs. conventional crops is not ascertained.

In organic farming animal manures, green manures, compost and varied crop rotations are applied, instead of readily soluble mineral fertilizers, in order to optimize the soil biological activity (Matt et al., 2011). Nitrogen (N) fertilization is still one of the most effective environmental factors which determines the grain quality; therefore, a proper management of N fertilizer is essential to ensure high quality grain production (Abedi et al., 2011).

A proper crop rotation is important in all cropping systems in order to maintain the biological diversity, yields, crop quality and soil parameters. Prior to spring wheat was red clover (*Trifolium pratense* L.) which enriches the soil with organics and nitrogen. In a sowing year, red clover leaves up to 160 kg of N ha^{-1} (Viil and Võsa, 2005) to the soil and has a positive effect on the formation of productivity elements of crops not only in the first year but also in the second year, which determines the productivity of the crop rotation links (Skuodienė and Nekrošienė, 2007). Red clover is one of the most widely grown leguminous green manure in Estonia.

The aim of this research was to study red clover's after-effect and different N fertilizer amounts' influence on the spring wheat (*Triticum aestivum* L.) yield quality. Cereal grain is the main food in the world; therefore, it is essential that we fully understand the nutritional implications of cereal grain consumption upon human health and well being (Cordain, 1999).

Materials and Methods

Field trials with the spring wheat cultivar 'Vinjett' (bred in Sweden) were carried out on the experimental fields of the Department of Field Crops and Grassland Husbandry located at Eerika (58°22'N, 26°40'E), Estonian University of Life Sciences in 2011. There were six treatments – organic (Organic) and organic with manure after-effect (Organic M), $N_0P_0K_0$, $N_{50}P_{25}K_{95}$, $N_{100}P_{25}K_{95}$ and $N_{150}P_{25}K_{95}$. The wheat was part of the five-year crop rotation experiment where red clover, spring wheat, peas (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) were following each other. Wheat was sown according to the norm 200 kg ha^{-1}, 600 germinate able seeds per $1m^2$. Fields were fertilized with different fertilizers: Kemira Grow How Power N:P:K – 5:14:28, and AN (ammonium nitrate) 34.4 N:P:K – 34:0:0. Rotation link potato received in one organic part 40 tonnes of composted cattle manure per hectare. The treatments that had received $N_0P_0K_0$ and mineral fertilizers, were sprayed with herbicides Sekator (preparation norm – 40 g ha^{-1}, active ingredients 50 g kg^{-1} amidosulfuron and 12.5 g kg^{-1} iodosulfuron) and MCPA 750 (preparation norm – 0.125 L ha^{-1}, active ingredient 750 g L^{-1} dimethylamine salt) The experiments were laid out in four replications. The size of each test plot was 60 m^2. The soil of the

experimental field was *Stagnic Luvisol* by World Reference Base (2002) classification (Deckers et al., 2002), the texture of which is sandy loam with a humus layer of 20-30 cm.

The soil analyses were carried out at the laboratories of the Department of Soil Science and Agrochemistry, Estonia University of Life Sciences. The trial soil was slightly acidic – pH KCl 6.0; C_{ORG} – 13.3 g kg^{-1}; mobile phosphorus – 0.124 g kg^{-1} (ammonium lactate); mobile potassium – 0.182 g kg^{-1} (ammonium lactate); calcium – 1.15 g kg^{-1} (ammonium lactate); magnesium – 0.146 g kg^{-1} (ammonium lactate) and nitrogen – 1.40 g kg^{-1} of soil.

Compared to the average temperatures of many years, the average temperatures in 2011 were higher. June and July were extremely warm months. Monthly precipitation amounts were much lower than on average. It was a growing season that can be characterized by high temperatures and drought (Table 1).

1000 kernel weight, volume weight, falling number, wet gluten content, gluten index and gluten content in dry matter were calculated as average of 4 replications. 1000 kernels were counted manually and then weighed. Volume weight was measured with the 1-litre measuring cylinder. Wet gluten content, gluten index and gluten content in dry matter were determined by ICC standard methods 137/1 and 155. Falling number was determined by ICC standard method 107/1. Gluten index is found by centrifuging wet gluten. Wet gluten is placed on the sieves which are put into a centrifuge. Liquid gluten is then separated from the solid gluten by centrifuging. Solid gluten is used to calculate the gluten index percent from the wet gluten.

Experimental data were processed by Statistica 7.0 software (Anova, Fisher LSD test) (Statsoft, 2005).

Results and Discussion

L. Sarunaite et al. (2009) points out that the wheat is a valuable crop in organic farming and therefore much effort is put into optimizing its quality. Wheat is usually grown after the best pre-crops in the crop rotation.

Spring wheat 'Vinjett' 1000 kernel weight, volume weight and falling numbers are shown in Table 2. 1000 kernel weight, measure of the size of a grain, depends on the variety of genetic characteristics, growth conditions, and fertilization (Koppel and Ess, 2007). The volume weight is one of the oldest and frequently used wheat quality indicator which is influenced by many factors (Gaines et al., 1997). Volume weight shows the grain weight per unit volume, usually per litre (g L^{-1}) or per hectolitre (kg hL^{-1}) (Tamm et al., 2008). In Estonia the volume weight must be at least 750 g L^{-1} (Ingver, 2007) that the producers could sell

Table 1

Average monthly temperatures (°C) and precipitation (mm) in Estonia during the vegetation period

Month	Temperatures, °C		Precipitation, mm	
	Average of 2011*	Average of 1966-1998**	Sum of 2011*	Average Sum of 1966-1998**
May	11.0	11.6	58.4	55.0
June	17.2	15.1	35.2	66.0
July	19.9	16.7	48.2	72.0
August	15.8	15.6	54.6	79.0
May-August	16.0	14.8	196.4	272.0

* – according to the Eerika weather station.
** – Jaagus, 1999.

Table 2

Spring wheat 'Vinjett' average 1000 kernel weight, g, volume weight, g L^{-1}, and falling number, sec, in 2011

Treatment	1000 kernel weight, g	Volume weight, g L^{-1}	Falling number, sec
Organic	32.5bc	724.9c	263a
Organic M	31.5ab	718.6ab	258a
$N_0P_0K_0$	33.7c	734.8d	284b
$N_{50}P_{25}K_{95}$	33.6c	733.9d	299c
$N_{100}P_{25}K_{95}$	31.1ab	721.9bc	317d
$N_{150}P_{25}K_{95}$	30.3a	714.6a	300c

Means followed by a different letter in the same column are significantly different ($p<0.05$).

Table 3

Spring wheat 'Vinjett' average wet gluten content, g kg^{-1}, gluten index, %, and gluten content in dry matter, g kg^{-1} in 2011

Treatment	Wet gluten content, g kg^{-1}	Gluten index, %	Gluten content in dry matter, g kg^{-1}
Organic	145a	89.2b	329c
Organic M	156b	85.2ab	322c
$N_0P_0K_0$	158b	86.1ab	326c
$N_{50}P_{25}K_{95}$	214c	83.0a	310b
$N_{100}P_{25}K_{95}$	258d	86.3ab	285a
$N_{150}P_{25}K_{95}$	291e	82.9a	280a

Means followed by a different letter in the same column are significantly different ($p<0.05$)

their grain yields for food processing companies. Usually the 1000 kernel weight and volume weight are higher in organic farming than in conventional one (Ingver, 2007), but in Table 2 can be seen that 1000 kernel and volume weight remained statistically lower in organic treatments and in treatments where higher amounts of mineral N were used. So it can be said judging by the results that in years when growing season can be characterized by high temperatures and drought, the lower amounts of mineral fertilizers and agrochemicals can ensure the higher 1000 kernel and volume weight. The average 1000 kernel weight in 2011 was between 30.3 grams ($N_{150}P_{25}K_{95}$) up to 33.7 grams ($N_0P_0K_0$). In all grown treatments the kernels in 2011 remained smaller compared to the average years of 1966-1998. The volume weight was also lowest in treatment $N_{150}P_{25}K_{95}$ (714.6 g L^{-1}) and highest in treatment $N_0P_0K_0$ (734.8 g L^{-1}). All volume weights in 2011 remained smaller than required.

The Hagberg Falling Number measurement is widely used for assessing the baking quality of wheat flour. Falling number value of 250-350 seconds or longer indicates low enzyme activity and very high quality wheat (Sorenson, 2006). The falling number increased statistically with the increase of mineral N (Table 2). The previous experiments have also shown that fertilization increases the Hagberg Falling Number (Lloveras et al., 2001; Kindred et al., 2005; Tein et al., 2010). The average falling numbers were all over 258 seconds which indicate good baking quality flour.

The gluten is the grain proteins that determine the viscoelastic properties of dough (Schofield, 1994). The spring wheat 'Vinjett' different gluten results are shown in Table 3. The wet gluten content, as falling number, increased significantly with the increase of mineral N. The wet gluten content was between 145 g kg^{-1} (Organic) up to 291 g kg^{-1} ($N_{150}P_{25}K_{95}$). The wet gluten content in grains must be at least 260 g kg^{-1} in order to get high quality fluffy bread (Ingver, 2007).

The gluten index and gluten content in dry matter decreased statistically with the increase of mineral N. The optimum gluten index is 60-90% (Talgre et al., 2009). The gluten index was higher than 82% in all treatments ranging between 82.9 ($N_{150}P_{25}K_{95}$) up to 89.2% (Organic). The crop rotation treatments had more than 15 g kg^{-1} higher dry matter contents than in N fertilized treatments. It is a well known fact that organic crops usually have higher dry matter contents than conventional crops because organic crops tend to have more dry nutrients inside the grain (Lairon, 2010)

Conclusions

1. 1000 kernel weights and volume weights remained statistically lower in organic treatments and in treatments where higher amounts of mineral N were used. The average 1000 kernel weights in 2011 were between 30.3 grams ($N_{150}P_{25}K_{95}$) up to 33.7 grams ($N_0P_0K_0$). The volume weight was also lowest in treatment $N_{150}P_{25}K_{95}$ (714.6 g L^{-1}) and highest in treatment $N_0P_0K_0$ (734.8 g L^{-1}).
2. The falling number increased statistically with the increase of mineral N. The average falling numbers were all over 258 seconds.
3. The wet gluten content increased significantly with the increase of mineral N ranging between 145 g kg^{-1} (Organic) up to 291 g kg^{-1} ($N_{150}P_{25}K_{95}$).
4. The gluten index and gluten content in dry matter decreased statistically with the increase of mineral N. All the gluten indexes were higher than 82% and the crop rotation treatments had more than 15 g kg^{-1} higher dry matter contents.
5. In treatments where no mineral fertilizers were used and which only followed the red clovers after effect had higher gluten index and gluten content in dry matter. Higher mineral nitrogen amounts and organic treatments decreased spring wheat 1000 kernel weights and volume weights. The wet gluten content and falling number increased with increase of the amount of mineral N.

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IMPACT OF HARVEST TIMING AND CULTIVAR ON BIOGAS OUTCOME FROM WINTER WHEAT SILAGE

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Abstract
Biogas can be produced from industrial by-products, household waste and raw materials of agricultural origin. Agricultural resources can be agricultural by-products, for example, manure as well as biomass of energy crops. The objective of the trial was to evaluate the methane outcome from the winter wheat (*Triticum aestivum* L.) silage depending on the variety and the growth stage during the harvest. The trial was carried out in State Stende Cereals Breeding Institute in the autumn of 2009. The biomass of three varieties of winter wheat, harvested at three stages of maturity - at the beginning of flowering (GS 60-62), early milk ripeness (GS 70-72), and early yellow ripeness (GS 80-82) - was ensiled in laboratory conditions. The silage was analysed 180 days after it had been ensiled. The biogas and methane outcome in laboratory conditions (in Germany) was determined for samples of silage made from winter wheat variety 'Skalmeje' at all harvesting times according to VDI 4630 method. The theoretically obtainable methane outcome was calculated for silage samples of all varieties by using the results of chemical composition analysis (crude protein, crude fibre, crude fat, N-free-extracts). The highest methane outcome from one ton of winter wheat silage was acquired by harvesting and ensiling the biomass during the flowering stage. However, evaluating the methane yield from one hectare, the best results were obtained by harvesting and ensiling the biomass at the early milk stage of ripeness and at the stage of early yellow ripeness.
Key words: winter wheat silage, growth stages, cultivars, chemical composition, methane outcome.

Introduction

Energy security or the ability to ensure the necessary energy resources is one of the priorities in the European Union. Latvia can currently ensure slightly less than one third of the necessary energy by using the local energy resources. The trials have to be carried out in the local conditions of Latvia in order to establish effective methods for using biomass in creation of new alternative types of fuel that could fully or partially substitute fossil fuels. In recent years Latvia has experienced a rapid increase in the construction of biogas factories. However, there is a problem of raw material availability. Biogas can be produced from different raw materials, including from materials of agricultural origin. The raw materials from agricultural sector can be agricultural by-products as well as energy crops (crops with high biomass yield). In Latvia similarly to the trend in Europe there is a willingness to use mostly maize (*Zea mays* L.) for the production of biogas, but it is important to cultivate different energy crops in order to ensure the crop diversity in the farms and to reduce the risk for the biogas producers in periods when the maize harvests are poor. Austrian scientists confirm that the sustainable biogas production from energy crops is not ensured by maximal yields of one species, but from a sustainable and environmentally friendly crop rotation system (Bauer et al., 2007). Winter wheat (*Triticum aestivum* L.) can also be included in the crop rotation as the winter wheat silage could be used for biogas production. Winter wheat is a suitable cereal crop for the conditions of Latvia because the cultivation technology is well-developed and the producers have accumulated a great amount of experience.

The task of the trial was to evaluate the methane outcome from the winter wheat silage depending on the variety and the stage of maturity during the harvest.

Materials and Methods

The trial was carried out in State Stende Cereals Breeding Institute in the autumn of 2009 in the sod-podzolic loam soil that had following characteristics: pH KCl 5.6-6.0, content of organic matter - 24 g kg^{-1}, content of available for plants P - 100 mg kg^{-1}, and that of K – 150 mg kg^{-1}. Three winter wheat varieties – 'Mulan', line '99-115', and 'Skalmeje' – were examined during the trial. The biomass of cereals was harvested during three growth stages: at the beginning of flowering (GS 60-62), early milk ripeness (GS 70-72), and early yellow ripeness (GS 80-82). Winter wheat biomass was harvested by manual mower, each time determining the weight of the green mass on the field by using the scales ACCULAB SV–30 with the accuracy of 0.005 kg. An average sample was taken from all four replicates in order to make a silage and analysis. The silage was analysed 180 days after ensiling. The content of dry matter (DM) (LVS ISO 712 – 2003) and ash (XY) (ISO 5984:1978), g kg^{-1}, was determined and these results were used to calculate the content of organic dry matter (ODM), g kg^{-1}. During the analysis of silage samples, the content of (1) crude protein (XP), g kg^{-1} (LVS EN ISO 5983-2:2009), (2) crude fibre (XF), g kg^{-1} (ISO 5498:1981), and (3) crude fat (XT), g kg^{-1}

(ISO 6492:1999), was determined. The analysis of chemical composition of the silage was performed at the Scientific Laboratory of Agronomic analyses of Latvia University of Agriculture.

Theoretical methane outcome of cereals was calculated by using the following equation mentioned in scientific literature (Amon et al., 2007) (Formula (1)):

$$MEV = 5.904 \times XP + 3.79 \times XF + 1.352 \times BEV, \quad (1)$$

where

MEV – methane outcome Nm^3 $(t\ ODM)^{-1}$,

BEV – N-free extracts, g kg^{-1}, calculated with Formula (2):

$$BEV = 1000 - XP - XF - XT - XY. \quad (2)$$

The biogas outcome from the silage samples made from biomass of winter wheat variety 'Skalmeje' for all harvests was determined in BiNoLab laboratory (in Germany) according to VDI 4630 method. The biogas and methane outcome is expressed in normal cubic metres – Nm^3 $(t\ ODM)^{-1}$ (Nm^3 – cubic meter of a gas at 0 °C temperature and 1013 mbar pressure). The methane yield from the winter wheat silage acquired from one hectare was calculated using Formula (3):

$$MR = MEV \times OV, \quad (3)$$

where

MR – methane yield, Nm^3 $(ha\ ODM)^{-1}$,

OV – yield of organic dry matter of winter wheat silage, t ha^{-1}.

In the calculation of the harvest of winter wheat silage from 1 ha, the loss of wheat biomass in the ensiling process was taken into account.

The mathematical evaluation of data was carried out, using the two factor analysis of variance.

Results and Discussion

Biomass dry matter yield from energy crops that are cultivated specifically for biogas production is one of the most important indicators. Evaluating the dry matter yield of winter wheat biomass depending on the harvesting time, the highest results were obtained from the winter wheat that was harvested at the early milk ripeness stage (GS 70-72) and at the early yellow ripeness stage (GS 80-82). The lowest yield of the dry matter of biomass was acquired by harvesting the wheat at the flowering stage (GS 60-62). Evaluating the cultivated winter wheat varieties from the view point of their suitability for biogas production, it was determined that a significantly higher dry matter yield was obtained from line '99 – 115' and variety 'Skalmeje', if the wheat biomass was harvested at GS 60-62 and GS 80-82. If the wheat was harvested at GS 70-72, there were no significant differences of the biomass dry matter yield as regards 95% significance (Table 1).

Harvested plant biomass was ensiled in laboratory conditions. Depending on the sample, the losses during the ensiling process were 2.07-5.53%. These losses were taken into account when calculating the silage yield from 1 ha. The regularity of the silage yield changes depending on harvesting time and variety of wheat was the same as regularity described previously regarding the changes in dry matter yield of biomass. The highest yield of organic dry matter (ODM) from the silage was acquired when the silage was made from the wheat harvested at the early milk ripeness (GS 70-72) and at the early yellow ripeness stage (GS 80-82) (Fig. 1). It was also observed that the variety 'Skalmeje' and the new breeding line '99 – 115' ensured the highest dry matter yield of silage from 1 ha, when the silage was made at GS 60-62 and GS 80-82 (Fig. 1). The results of the first trial year showed that depending on the variety the highest silage yield can be obtained by harvesting the biomass either at GS 70-72 ('Mulan') or GS 80-82 ('Skalmeje' and '99-115'). At the conditions prevalent during 2010, the variety 'Mulan' produced a lower organic

Table 1

Dry matter yield of winter wheat biomass depending on the harvest timing, t ha^{-1}

Variety (A)	Growth stage at harvesting (B), $LSD_{AB0.05}$ = 1.217			Average of A $LSD_{A0.05}$=0.7
	GS 60-62	GS 70-72	GS 80-82	
Mulan	6.48	13.31	11.77	10.52
99-115	8.09	13.17	13.93	11.73
Skalmeje	8.57	13.82	14.76	12.38
Average of B $LSD_{B0.05}$= 0.7	7.71	13.43	13.48	

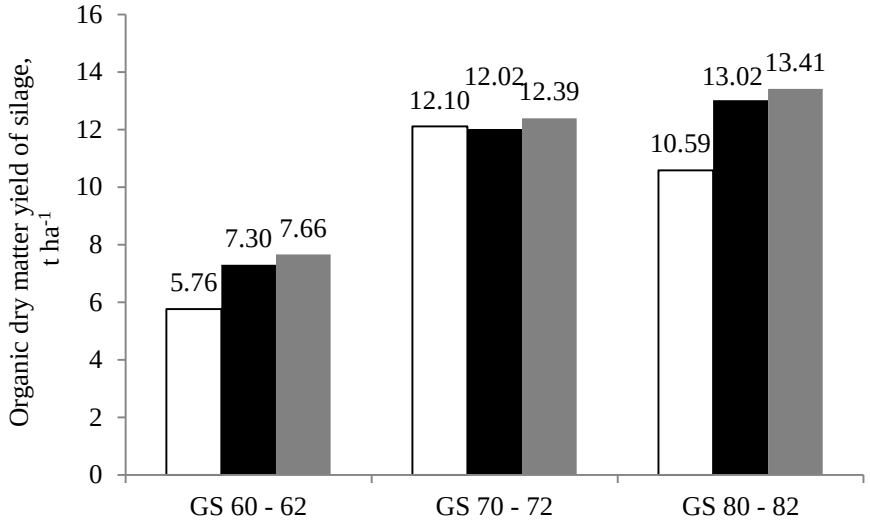

Figure 1. The organic dry matter yield of wheat silage depending on the winter wheat harvest timing, t ha^{-1}, $LSD_{AB0.05}$= 1.104: □ – 'Mulan'; ■ – '99-115'; ■ – 'Skalmeje'.

Table 2

The chemical composition of winter wheat silage at during harvesting times, g kg^{-1} of dry matter

Analysed indicators	The chemical composition of the silage depending on the harvest timing		
	GS 60-62	GS 70-72	GS 80-82
Crude ash	53.8	48.0	43.9
Crude protein	111.2	86.4	82.3
Crude fibre	354.0	300.6	286.4
Crude fat	19.8	21.8	15.8
Starch	5.0	174.7	274.5

dry matter yield (Table 1) and silage yield (Fig. 1) when being harvested at the early yellow ripeness stage (GS 80-82) in comparison to the yields obtained when the variety was harvested at the early milk stage.

Methane producing bacteria use only organic compounds during the biogas production process. The ODM content depends on the total dry matter content of winter wheat silage and on the ash content in the dry matter. The average ash content of the silage obtained from the biomass of all examined winter wheat varieties was higher in those samples that were harvested at GS 60-62. The ash content in the samples of silage made from the wheat that was harvested at later growth stages was slightly lower (Table 2). Whereas the total DM content of biomass at harvesting as well as that of silage had the tendency to grow when the wheat was harvested at later growth stages.

Although we discuss the production of biogas, the most important component of biogas is methane (CH_4). The methane outcome depends on the chemical composition of silage. According to the scientific data described in the research of Austrian scientists (Amon et al., 2007) the chemical components of silage, namely crude protein, crude fibre, crude fat and crude ash, affect the methane outcome.

Crude protein and crude fibre content in the wheat silage had the tendency to diminish if the silage was made from the biomass of wheat that was harvested at GS 70-72 and GS 80-82. Crude protein diminished in the range from 111.2 g kg^{-1} (GS 60-62) to 82.3 g kg^{-1} (GS 80-82) (Table 2). Canadian scientists (Khorasani et al., 1997) have also noted in their research that the crude protein content is lower in the silage that is made from plants harvested at later growth stages. The crude fibre content in the winter wheat silage diminished respectively by 53.4 and 14.2 g kg^{-1} at each succeeding plant growth stage. According to the data of other authors' research (Amon et al., 2007; Herrmann et al., 2011), the crude protein and crude fibre content of cereal silage significantly influences the methane outcome. The fact that starch content of the silage samples rose from 5.0 g kg^{-1} (GS 60-62) to 274.5 g kg^{-1} (GS 80-82) indicates that the cereals are ripening.

Examining other species of energy crops, Polish scientists have noted that an increased lignin content of silage reduces the methane outcome (Klimiuk et al., 2010).

Methane outcome and methane yield

Theoretical methane outcome from the ODM of the silage produced from the wheat that was harvested at different growth stages was affected by the changes in the chemical composition of the silage. Austrian researchers have noted that the biogas outcome is affected by the cultivated variety and the harvesting time of silage material because these two factors influence the chemical composition of the silage (Amon et al., 2007). The highest theoretical methane outcome from 1 t of ODM of silage was acquired by harvesting the wheat and preparing the silage at GS 60-62 – 262.04 Nm^3 (t ODM)$^{-1}$ on average. The methane outcome in Nm^3 (t ODM)$^{-1}$ decreased when the winter wheat was harvested and ensiled at later growth stages. The results obtained by German researchers show a similar tendency (Heiermann and Plöchl, 2004). The examined variety also slightly impacted the calculated methane outcome (Table 3). Researches on methane outcome Nm^3 (t ODM)$^{-1}$ that was obtained from silage prepared from winter rye and triticale provided similar results (Jansone and Gaile, 2011). The highest methane outcome from silage that was prepared at GS 60-62 was achieved by using the breeding line '99-115' and the variety 'Skalmeje'; the silage made from this variety provided the highest methane outcome from 1 t of ODM also at GS 70-72. However, the silage made from all varieties harvested at the beginning of the yellow ripeness stage (GS 80-82) provided similar results: 233.07 - 236.44 N m^3 (t ODM)$^{-1}$ (Table 3).

Only the wheat variety 'Skalmeje' harvested at all three growth stages was used to produce methane in the laboratory and to determine the methane outcome from 1 t of ODM (Table 4).

When the calculated (theoretical) methane outcome from the winter wheat silage was compared with that determined in laboratory, the theoretical methane outcome was naturally higher by 7.2 and 6.4% in comparison to the practical outcome determined in the laboratory, when the silage was made from wheat harvested at GS 60-62 and GS 80-82. However, if the silage was made from wheat harvested at GS 70-72, the practically determined methane outcome from 1 t of ODM was higher (+6%) than the theoretical outcome that was calculated using the chemical composition indicators of the silage (Table 4). It has to be examined further why the differences are so contradictory. Similar data can be found in scientific literature (Plöchl et al., 2009). Finally, both the theoretical and practical methane outcome from 1 t of ODM was at its lowest when the silage was made from wheat harvested at GS 80-82.

The most important indicator of energy crops is the methane yield from 1 ha. The variety, the harvest timing, and the silage losses during the ensiling process all left an impact on the methane yield from one hectare. Although the highest methane outcome from 1 t of the silage was achieved when the silage was harvested at GS 60-62 – 262.04 Nm^3 (t ODM)$^{-1}$ (Table 3), the ODM yield of the silage harvested at the same time was at its lowest – 6.90 t ha^{-1}. The highest theoretically calculated methane yield from the silage harvested at GS 60-62 was obtained using the winter wheat variety 'Skalmeje' – 2014 Nm^3 (ha ODM)$^{-1}$ (Fig. 2). The methane yield that was obtained from

Table 3

Theoretical methane outcome depending on the silage harvesting time, Nm^3 (t ODM)$^{-1}$

Variety, line	Methane outcome depending on the silage harvest timing		
	GS 60 - 62	GS 70 - 72	GS 80 - 82
Skalmeje	262.89	**242.54**	233.07
Mulan	258.42	234.40	**236.44**
99-115	**264.81**	237.81	233.39
Average per harvest	262.04	238.25	234.30

Table 4

Comparison of theoretical and practically in laboratory determined methane outcome from 1 t of silage made from winter wheat variety 'Skalmeje'

Stages of harvesting	Methane outcome, Nm^3 (t ODM)$^{-1}$		% of the calculated
	theoretically calculated	determined in laboratory	
GS 60-62	262.89	244.08	-7.2
GS 70-72	242.54	257.04	+6.0
GS 80-82	233.07	218.16	-6.4

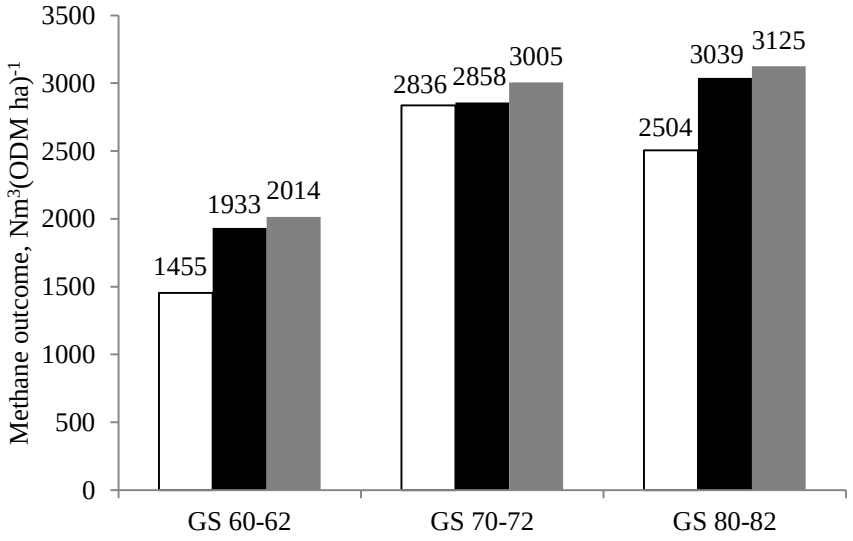

Figure 2. Theoretical methane yield from 1 ha depending on the silage harvesting time, Nm^3 (ha ODM)$^{-1}$: □ – 'Mulan'; ■ – '99-115'; ■ – 'Skalmeje'.

the silages of all examined winter wheat varieties harvested at GS 70-71 was similar, ranging from 2836 to 3005 Nm^3 (ha ODM)$^{-1}$. Although the methane outcome from 1 t of wheat silage ODM was at its lowest when the wheat was harvested at GS 80-82, the wheat harvested at this growth stage provided the highest silage yield. The silage yield from 1 ha leaves a greater impact on the methane yield than the methane outcome from 1 t. The compromise could be found by harvesting the wheat and preparing the silage at GS 70-72, when according to calculations the methane outcome is slightly higher than the methane outcome from the wheat silage harvested at GS 80-82, which is supported by the fact that the silage of the variety 'Skalmeje' harvested at this stage provided the highest methane outcome from 1 t of ODM in the laboratory (Tables 3 and 4). At the same time, the average silage yield (Fig. 1) at GS 70-72 was only slightly lower than that of the wheat harvested and ensiled at GS 80-82. Nevertheless, it has to be examined further whether this tendency will reoccur in next trial years.

Evaluating the results obtained from the silage of cereals harvested at all growth stages, it has to be noted that the highest methane yield was produced from the wheat variety 'Skalmeje', when it was harvested and ensiled at GS 80-82 (3125 Nm^3(ha ODM)$^{-1}$) (Fig. 2).

Conclusions

1. A significant impact ($p<0.05$) on the dry matter yield of the wheat biomass and on the silage yield was left by the growth stage of wheat at harvest and the examined variety. The highest wheat silage yield was obtained harvesting the wheat at GS 70-72 and GS 80-82.
2. The specific growth stage of wheat at which the wheat was ensiled also influenced the chemical composition of the silage. The highest crude protein, crude fibre and crude ash content was determined in the winter wheat silage prepared at GS 60-62, while at later stages when the wheat had ripened more, these indicators diminished.
3. The highest theoretically calculated methane outcome was obtained from the wheat ensiled at GS 60-62 (average 262.04 Nm^3 (t ODM)$^{-1}$); it decreased with each succeeding harvesting stage and reached the lowest level when the wheat was ensiled at GS 80-82 (average 234.30 Nm^3 (t ODM)$^{-1}$). The methane outcome Nm^3 (t ODM)$^{-1}$ obtained in the laboratory from the silage of variety 'Skalmeje' differed from the theoretical outcome by 6.0-7.2%; moreover, the differences were both positive and negative depending on the growth stage at which the wheat was ensiled.
4. It was noted during the trial that the highest methane yield (3125 Nm^3(ha ODM)$^{-1}$) was acquired from the silage of the wheat variety 'Skalmeje' that was harvested at GS 80-82, while the average methane yield was similar when the wheat was ensiled at GS 70-72 and GS 80-82. The research is being continued.

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CHANGES IN SUGAR CONTENT OF WINTER OILSEED RAPE PLANT DURING WINTER

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Abstract
Chemical composition of crops before winter is important for successful overwintering of plants. The aim of the research, carried out from 2007/2008 till 2009/2010 at the Research and Study farm 'Vecauce', was to investigate the influence of agricultural practices (sowing date, sowing rate, and fungicide application) and meteorological conditions during winter on the content of sugar in apical bud and root of two types of winter oilseed rape (*Brassica napus* L.) cultivars. The content of sugars (monosaccharides) in apical bud and root in autumn and following spring was analysed using the Luff–Schoorl method. Sugar content in apical bud and root of winter oilseed rape in autumn and spring differed depending on the trial year, because of different, even contrary (in season 2009/2010), meteorological conditions. Sugar content was higher in plant parts developed when rape was sown on earlier sowing dates, but it was substantially influenced by the sowing date only on some occasions. Sowing rate had no impact on sugar content in apical bud and root. Application of a fungicide as a growth regulator had no significant impact on the result, but sugar content decrease during winter 2009/2010 in the root of winter oilseed rape was smaller when fungicide as growth regulator was applied.
Key words: winter oilseed rape, apical bud, root, sugar content.

Introduction

The growing area of winter oilseed rape (*Brassica napus* L.) in Latvia has started to exceed that sown with spring rape only since the year 2006 in Latvia. Tendency is observed that winter oilseed rape growing area has been progressively increasing during the last ten years, reaching 64.4 thousand ha in 2010. The growers prefer winter oilseed rape to spring oilseed rape because of the possibility of obtaining higher seed yields. However, winter oilseed rape growing area was small if compared with area sown with spring rape in year 2003 (3.1 thousand ha) and in year 2011 (44.4 thousand ha). There are several reasons for such decreased sowing area for winter crop during the time when the tendency of total oilseed rape area increase is observed. From the experience obtained in oilseed rape growing it is clear that winterhardiness of winter oilseed rape is one of the key factors for successful growing of this crop in conditions of Latvia. Wintering of rape depends on the plant development stage in the autumn and plant chemical composition, which could be affected by the growing manner including used cultivar and agro-climatic factors. The extensive long-term research experience shows that periodical fluctuations in negative and positive temperatures during snowless winter and early spring, ice crust that is formed from melting snow, as well as thickness of frozen soil, which controls water removal from the soil, have negative influence on the wintering of crops, including rape.

Sowing date, autumn growth, and cold acclimatization period have significantly influenced biometric parameters of winter oilseed rape plants in several years (Velicka et al., 2005a; Velicka et al., 2010; Balodis and Gaile, 2011). Chemical composition of crops before winter also is important for successful overwintering. Cold acclimation and prehardening to frost for winter oilseed rape and other crops (rye (*Secale cereale*) (Griffith, Mclntyre, 1993), cabbage (*Brassica oleracea*) (Uemura and Steponkus, 2003) and rapa (*Brassica rapa*) (Sasaki et al., 1996)) have been studied because of importance of winterhardiness for successful growing of these plants. The role of sugar and other compounds such as soluble protein in relation with freezing resistance using mulberry tree (*Morus bombycis*), black locust tree (*Robinia pseudoacacia*), Canadian aspen tree (*Populus euramericana*), *Arabidopsis thaliana* and cabbage (*Brassica oleracea capilata*) has been explained already in sixties of the last century (Sakai and Yoshida, 1968). For several crops including oilseed rape, also different chemical compounds such as proline, as well as effect of light have been studied to understand and improve the plant performance in low temperatures and in other harmful conditions (Bates et al., 1973; Rapacz, 1998; Anisimoviene et al., 2004).

The impact of such parameters as temperature, length of day and night, and light intensity on plant freezing tolerance, which is determined by chemical composition of plants (e.g. soluble sugars content), mostly has been investigated under controlled laboratory conditions. Also chemical composition of winter oilseed rape plants has been investigated under controlled conditions (Rapacz and Janowiak, 1998; Rapacz, 1998; Maciejewska and Bogatek, 2002; Burbulis et al., 2008; Waalen, 2010). Field experiments are less common for such investigations, probably because of unpredictable conditions and necessity of long-term trials. However, Lithuanian researchers have used the field trials to explore chemical composition of different oilseed rape plant

parts and have explained the results in connection with oilseed rape growth and wintering. Nevertheless, determination of such compounds as proteins and proline in the apical buds and root column of oilseed rape is recognized to be more important for wintering of oilseed rape. At the same time, importance of sugar has not been denied (Anisimoviene et al., 2004; Velicka et al., 2005; Novickiene et al., 2010).

In agro-ecological conditions of Latvia, data on changes in sugar content in winter rape plant during winter is not documented. The aim of currently described section of our research was to investigate the influence of agricultural practices (sowing date, sowing rate, and fungicide application) and meteorological conditions during winter on the content of sugar in apical bud and root of two types of winter oilseed rape cultivars.

Materials and Methods

Three-year (from 2007/2008 until 2009/2010) experiments were carried out at the Research and Study farm 'Vecauce' (latitude: N 56º 28´, longitude: E 22º 53´) of Latvia University of Agriculture. A three-factor field trial using two type winter rape (*Brassica napus* ssp. oleifera) cultivars (line 'Californium' and hybrid 'Excalibur') was carried out. The paper is focused on the analyses of sugar content in the winter oilseed rape.

The following factors were investigated:

Factor A – sowing date:

1st – 1st August,

2nd – 10th August,

3rd – 20th August,

4th – 1st September,

5th – 10th September.

Factor B – sowing rate (100, 80, and 60 germinate able seeds per 1 m^2 – for 'Californium'; 80, 60, and 40 germinate able seeds per 1 m^2 – 'Excalibur').

Factor C – fungicide application (C1 – control, without fungicide; C2 – fungicide applied as growth regulator). Fungicide application scheme: a dose of 0.5 L ha^{-1} of fungicide Juventus 90 s.c. (metconazole, 90 g L^{-1}) was applied at the 4-6 leaves stage in plots of first three sowing dates.

Soil at the trial's site was strongly altered by cultivation in 2007/2008, and sod-gleyic in 2008/2009 and 2009/2010. Soil characterizing parameters were slightly different depending on year (Table 1).

Conventional soil tillage practices which included mould board ploughing and soil rototilling before sowing were applied. Pre-crop was cereal mixture for silage in all years. Plant fertilization was performed as follows: N – 12 to 28 kg ha^{-1}, P – 18 to 30 kg ha^{-1}, and K – 79 to 103 kg ha^{-1} in autumn prior to sowing, and N top-dressing in spring. After sowing, the rape was sprayed against weeds with Butisan Star s.c. (metasachlor, 333 g L^{-1} + kvinmerac, 83 g L^{-1}) 2.5 L ha^{-1}. Herbicide was applied when the rape was fully germinated in plots of first three sowing dates in 2007 and 2008. For plots of 4th and 5th sowing date, the herbicide was not used in autumn 2007 (Lontrel 300 s.c. (clopiralid, 300 g L^{-1}), 0.5 L ha^{-1}, was applied in spring 2008), but in autumn 2008 and in all plots in 2009 Butisan Star s.c. was used directly after sowing.

Table 1

Soil characterizing parameters at trial site depending on trial year

Parameter	Year		
	2007/ 2008	2008/ 2009	2009/ 2010
pH KCl	7.4	7.2	7.2
Available K, mg kg^{-1}	194	169	141
Available P, mg kg^{-1}	115	100	111
Humus content, g kg^{-1}	38	30	22

In autumn, the end of vegetation period was noted when the average day-and-night air temperature dropped below +5 ºC and stayed such for three successive days. At that time, ten rape plants were sampled randomly from each plot for measurement of biometric parameters and chemical analyses of apical bud and root. In spring, directly after renewal of vegetation period, again ten plants were sampled randomly from each plot only for chemical analyses of apical bud and root. Firstly, the samples were air-dried. The content of sugar was analysed in the Scientific Laboratory of Agronomy Research. Analyses were performed by the Luff–Schoorl method. The method is based on iodine titration of excess copper. Total sugars were determined by LVS 252:2000 method, and according to it the reducing sugars were expressed as invert sugar or glucose equivalent.

ANOVA two-factor analysis of variance was used for processing the experimental data of each separate year.

Meteorological data were collected from an automatically working meteorological station approximately 1 km from the trial site. Mean air temperatures during winter months (November to March) differed depending on years. Winter 2007/2008 was characterized with much higher mean air temperatures than the long-term data, and air temperature only for a short time in February 2008 dropped below the long-term average temperature. Temperature during the whole winter of 2008/2009 was higher than long-term average observations. In 2009, October, November and December were absolutely different if compared with the same months in both

previous trial years – mean air temperatures of these months per trial period were the lowest. Temperatures of January to March 2010, on the contrary, were closer to long-term average observations. Winter 2009/2010 was characterised by a thick long-lasting snow cover.

Vegetation (mean air temperature below +5 °C for at least 3 days) ended on 4th of November in 2007, also on 4th of November in 2008 (but it renewed for some short periods up to 4th December), and on 1st of November in 2009. In spring, growth of oilseed rape plants renewed (mean air temperature above +5 °C for at least 3 days) on 31st of March 2008, on 2nd of April in 2009, and on 28th of March in 2010.

Overall, meteorological conditions were rather different during the trial years. The winters of 2007/2008 and 2008/2009 were untypically mild compared to long-term winter observations. Winter conditions were hard for oilseed rape plant survival in 2009/2010 because of a thicker and long lasting snow cover during the winter months; also air temperature was lower than the long-term observations. Winter 2009/2010 was crucial for surviving of immature, not enough developed plants. Pools during snow melting killed even well-matured plants possibly by oxygen deficit under wet conditions.

Results and Discussion

Results obtained in season 2007/2008

The average sugar content for 'Californium' varied from 48.2 g kg^{-1} in apical bud of plants sown on fifth sowing date (10 September) to 312.3 g kg^{-1} in fungicide-treated root of plants sown on second sowing date (10 August), and for 'Excalibur' – from 44.1 g kg^{-1} in apical bud of plants sown on fifth sowing date to 301.6 g kg^{-1} in root of plants sown on third sowing date. Sugar content was mostly influenced by the sowing date (factor A) in autumn and in spring. A statistically significant ($p<0.05$) impact on sugar content in apical bud for both cultivars, 'Californium' and 'Excalibur', was noted, so sugar content showed tendency to decrease in later sowing dates. For example, sugar content was 48.2 g kg^{-1} in apical bud of plants sown on fifth sowing date and 214.8 g kg^{-1} in apical bud of 'Californium' sown on first sowing date. Sugar content in root was statistically significantly influenced by the sowing date only for 'Excalibur', and also the result decreased when rape was sown on later dates. No influence of the sowing date on sugar content in apical bud and root was observed for fungicide treated (factor C2) plants in autumn because first three sowing dates were early enough to accumulate similar amounts of sugars during autumn.

Average sugar content in spring (only plants from two sowing dates were analyzed – sown on 1 and 20 August) for 'Californium' varied from 71.3 g kg^{-1} in untreated-with-fungicide root of plants sown on 1 August to 309.3 g kg^{-1} in fungicide-treated root of plants sown on 20 August, and for 'Excalibur' – from 169.2 g kg^{-1} in untreated root of plants sown on 1 August to 309.8 g kg^{-1} in root of plants sown on 20 August. According to the results of analyses in spring, sugar content decreased significantly in plant parts when rape was sown on two sowing dates (1st of August and 20th of August) for line cultivar 'Californium', but for 'Excalibur' no statistically significant decrease was observed ($p>0.05$) (Fig. 1). Sugar content in spring even increased in roots of 'Excalibur' plants sown on 1 August (treated plants, Fig. 1) and on 20 August (untreated plants). The reason for such a result is still unknown, but might be explained by extremely mild winter when the temperatures were higher than the long term average. Winter conditions were beneficial for overwintering of plants sown late and poorly developed in autumn. Our

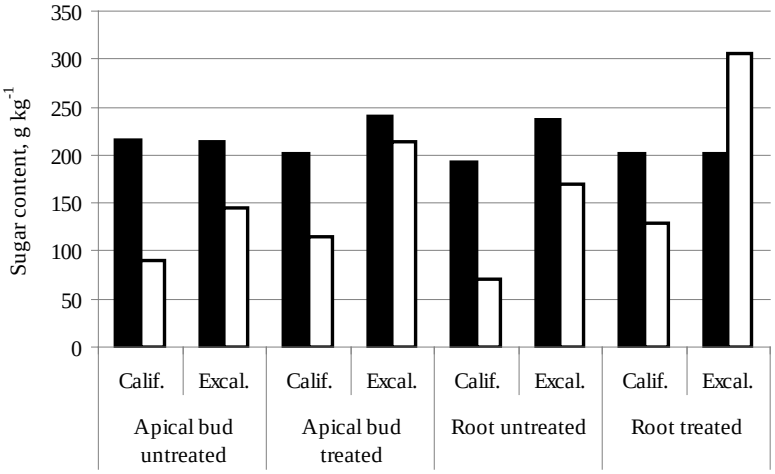

Figure 1. The content of sugars depending on cultivars and fungicide treatment (factor C) for plants sown on 1st of August (first sowing date) in season 2007/2008 (■ – autumn; □ – spring).

results are similar to the data obtained in the research on winter wheat where sugar content and its changes in the growing season mainly depended on wintering conditions (Ruza et al., 2011).

Sowing rate had no significant ($p>0.05$) influence on sugar content in oilseed rape apical bud and root neither in autumn nor in spring.

Results obtained in season 2008/2009

Performance of sugar content in analyzed plant parts of oilseed rape was quite similar to that obtained in the previous trial year. Average sugar content in autumn 2008 for 'Californium' varied from 54.8 g kg^{-1} in apical bud of plants sown on fourth sowing date (1st September) to 185.6 g kg^{-1} in root of plants sown on second sowing date (10th August), and for 'Excalibur' – from 29.6 g kg^{-1} in apical bud of plants sown on fifth sowing date to 168.3 g kg^{-1} in root of fungicide-treated plants sown on third sowing date (20 August). Similarly as in autumn 2007, there was a statistically significant ($p<0.05$) impact of the sowing date on sugar content in apical bud of plants of both cultivars: sugar content showed tendency to decrease with later sowing dates (Table 2). Sugar content in roots was significantly influenced by the sowing date only for 'Californium', and the result also decreased with later sowing dates the same as in apical bud. We observed a strong tendency ($p=0.058$) of a similar sugar content decrease in rape roots for 'Excalibur' when it was sown later. It may be explained by the possible increase in other chemical compounds in parts of plants sown later. Also R. Velicka (2010) has observed increased crude protein content and number of protein compounds in apical buds of winter oilseed rape plants from later sowing dates. In our research no significant influence of the sowing date on sugar content was observed in apical bud and root for fungicide-treated (Factor C2) plants in autumn. Average results from the first three sowing dates showed a tendency that fungicide-untreated apical buds and roots contained slightly more sugar than plant parts of 'Californium' treated with fungicide. Opposite were results for 'Excalibur' which fungicide-untreated apical buds and roots contained slightly less sugar than treated ones.

Trial year 2008/2009 confirmed tendencies observed in the previous year. Data showed that sugar content in plants sown on earlier sowing dates decreased more during winter than sugar content in plants sown on later sowing dates (Table 2). The winter was untypically mild if compared with winters 2007/2008 and 2009/2010, and with long-term data. Sugar content decreased during the winter despite the mild meteorological conditions (also a snow cover was observed for a short period in January). Sugar content in fungicide treated plant parts was similar to that of untreated plants, e.g., average sugar content was from 30.0 g kg^{-1} in untreated apical buds of plants from first three sowing dates to 33.3 g kg^{-1} in fungicide-treated apical buds for 'Californium', and from 31.5 g kg^{-1} in untreated apical buds to 29.0 g kg^{-1} in treated apical buds for 'Excalibur'. Also influence of the sowing date on sugar content was not observed when fungicide-treated plants were analysed in spring.

Like in the year 2007/2008, the sowing rate had no significant ($p>0.05$) influence on sugar content in apical bud and root neither in autumn nor in spring.

Results obtained in season 2009/2010

Results of sugar content in analyzed plant parts gave confirmation of tendencies in the changes in sugar content during winter. Average sugar content in autumn 2009 was from 63.0 g kg^{-1} in fungicide-treated apical bud of plants sown on third sowing date (20 August) to 191.4 g kg^{-1} in fungicide-treated root of plants sown on first sowing date (1 August) for 'Excalibur', and from 55.0 g kg^{-1} in apical bud of plants sown on third sowing date to 196.0 g kg^{-1} in fungicide-treated root of plants sown on first sowing date for 'Californium'. It is clear that sugar content in autumn in apical bud was lower than that in root for both cultivars (Fig. 2), exception was very high (182.3 g kg^{-1}) sugar content in apical bud of 'Excalibur' plants sown on first sowing date. Still, plant parts developed in plots sown on later sowing dates showed lower sugar content (Fig. 2). The results were lower but similar to those obtained in Lithuania

Table 2

Sugar content (g kg^{-1}) in apical bud and root depending on sowing date: plants untreated with fungicide in autumn 2008 and in spring 2009

Sowing date	Apical bud				Root			
	Californium		Excalibur		Californium		Excalibur	
	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
1st August	136.2	29.0	112.3	27.3	148.3	47.0	142.6	43.8
10th August	89.2	26.7	103.4	36.2	185.6	32.6	118.2	34.5
20th August	56.4	34.3	54.6	31.0	149.4	45.5	139.1	55.9
1st September	54.8	34.9	45.7	40.4	82.0	40.7	78.3	39.1
10th September	55.3	33.7	29.6	30.6	88.6	37.8	77.9	33.6

where sugar content reached 158.0 g kg^{-1} in apical bud of plants sown in the middle of August (Velicka et al., 2005). Sugar content in apical bud was not significantly ($p>0.05$) influenced by sowing dates, but sugar content in root was significantly ($p<0.05$) influenced by sowing dates for 'Californium', while for 'Excalibur' there were observed significant ($p<0.05$) differences in sugar content in apical bud and root depending on sowing date. Tendency of sugar content decrease in plant parts developed in later sowing dates was not as clearly demonstrated as in previous years (Table 2; Fig. 2). Sowing date did not significantly ($p>0.05$) influence the sugar content in analysed plant parts of 'Californium' when plots were treated with fungicide in autumn. A significant ($p<0.05$) effect of fungicide application on sugar content in apical bud and root of 'Excalibur' was observed. Comparison of the average sugar contents in untreated and treated plant parts showed that fungicide treatment had a positive but statistically insignificant effect.

Analyzes of sugar content in spring 2010 showed different results, because of the considerably harder winter conditions with a thick snow cower (up to 35 cm, authors' measurement) in February and pools in early spring (at the end of March) combined with lower air temperatures if compared with previous trial years. It should be taken into account that it was not possible to take plant samples in spring from plots sown on fifth sowing date (10 September) because of too small amount of plants of 'Excalibur' left in plots; besides, plants of 'Californium' did not survive at all after winter. A tendency was observed that sugar content in autumn before the end of vegetation was the highest for early sown plants but slightly decreased with each succeeding date of sowing, which was very similar to the results obtained in winter wheat (Ruza et al., 2011) in Latvia. In spring, a higher sugar content was noted in plant parts obtained from plots sown on second sowing date both for 'Californium and 'Excalibur': 84.0 g kg^{-1} and 98.0 g kg^{-1} in apical bud, and 95.0 g kg^{-1} and 107.0 g kg^{-1} in root column, respectively. The results showed that an important decrease in sugar content during winter was observed in apical bud of plants sown on 1 August for both cultivars, and in root column for 'Californium' in the same conditions (Fig. 2). An important decrease in sugar content was also observed in root of 'Californium' sown on third sowing date (Fig. 2). Overall, the research suggests that sowing date had a significant effect on sugar content in apical bud and root for both cultivars, which stresses the importance of sowing date for successful winter oilseed rape growing.

Fungicide treatment had a positive effect on sugar content in root of winter oilseed rape in autumn and spring. For 'Excalibur', a higher sugar content in autumn was noted in treated plants sown on first sowing date (191.0 g kg^{-1}), but in spring it was higher for treated plants sown on second sowing date (120.0 g kg^{-1}) (Fig. 3). The lowest sugar content decrease in root during winter was observed in treated plants sown on second sowing date, and the same tendency was observed also for sugar content in apical bud (Fig. 3). For 'Californium', the same tendency was observed if root was analysed, whereas sugar content in apical bud was higher in fungicide-treated plants on all sowing dates (reached 146.0 g kg^{-1} in plants sown on first sowing date in autumn, and 91.0 g kg^{-1} in plants sown on second sowing date in spring).

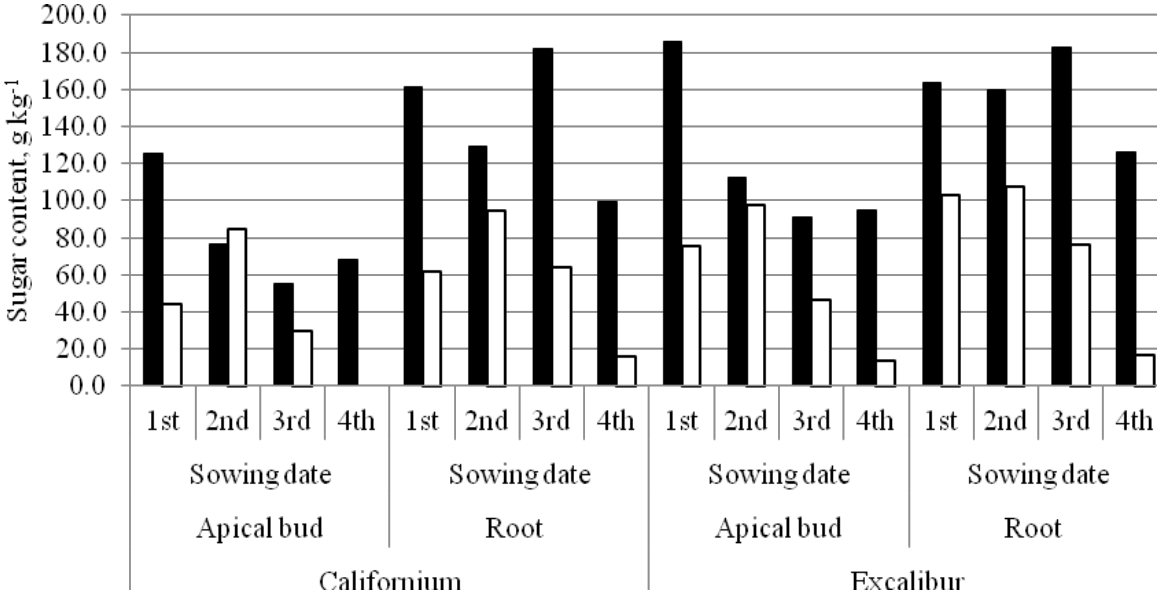

Figure 2. The content of sugars in fungicide-untreated plant parts depending on cultivar and sowing date (factor A) in season 2009/2010 (■ – autumn; □ – spring).

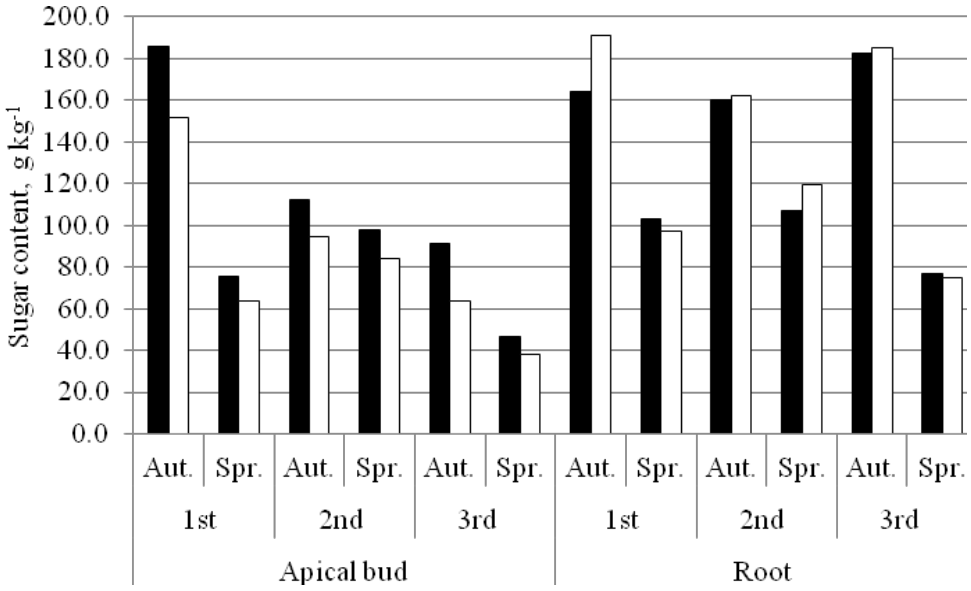

Figure 3. The content of sugars in autumn and spring for 'Excalibur' depending on fungicide treatment in different sowing dates in season 2009/2010 (■ – fungicide-untreated; □ – fungicide-treated; Aut. – in autumn; Spr. – in spring; 1st, 2nd, 3rd – sowing dates).

The same result was observed in the third trial year: the sowing rate had no significant ($p>0.05$) influence on the sugar content in analyzed parts of oilseed rape plants.

Summarizing the results of all trial years it is easy to conclude that sugar content in autumn was higher in early sown oilseed rape plants almost in all cases. Also the significant role of meteorological conditions for sugar accumulation during autumn can be emphasized in all three trial years (sugar content in analysed plant parts was different depending on trial years). Sugar content should be analyzed in combination with plant development in autumn and winter conditions. It is clear after three-year results that sugar content performance in winter oilseed rape plant parts under field conditions (when autumn and winter conditions are changeable) is variable. To find out more specific results on the effect of sowing date, sowing rate and growth regulators on plant chemical compounds and their changes under winter conditions, experiments should be performed in controlled conditions in laboratory, like in researches done by M. Rapacz (1998), M. Rapacz and F. Janowiak (1998), N. Burbulis et al. (2008), and D. Maciejewska and R. Bogatek (2002).

Conclusion

Sugar contents in apical bud and root of winter oilseed rape in autumn and spring differed considerably depending on trial year, because of different, even contrary (in season 2009/2010), meteorological conditions. Sugar content in autumn mostly was higher in plant parts developed when rape was sown on earlier sowing dates. Sowing rate had no impact on sugar content in apical bud and root. Fungicide as a growth regulator had a positive impact on the result. Decrease in sugar content during winter 2009/2010 in root was smaller on some occasions when fungicide as growth regulator was applied in autumn. In future, similar researches should be done in laboratory under controlled environment to find more accurate results of the effect of the investigated factors on plant chemical compounds.

Acknowledgements

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PERENNIAL GRASSES FOR BIOENERGY PRODUCTION: CHARACTERIZATION OF THE EXPERIMENTAL SITE

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Abstract
To promote the future of abandoned lands management and the reduction of fossil energy consumption in Latvia, the establishment of energy crops plantation facilities, including perennial grasses, was investigated. The objective – suitability of several perennial grasses for bio-energy production under condition of Latvia. The aim of the current research to evaluate the experimental field conditions for the cultivation of perennial grasses.
The perennial grasses are modest in terms of soil conditions, they are environmentally friendly, as well as provide high yields of biomass with adequate quality for bio-energy production without large investments. With increasing amounts of bio-energy production the amounts of various by-products which are profitable to utilize as energy crops fertiliser will also increase. It is essential that plant nutrients return back into circulation by creating a complete cycle. In order to test in practice the possibility of creating this complete cycle of growing perennial grasses, an experimental field was chosen at the Research Institute of Agriculture in Skriveri. In the summer of 2011, before trials establishment, the conditions of soil were examined at four depths: 0 – 20 cm; 20 – 40 cm; 40 – 60 cm and 60 – 80 cm. The analyses showed that the experimental field conditions were appropriate for growing of perennial grasses. The results of the soil agrochemical analysis will be a base for future studies of usage efficiency of different fertiliser types on perennial grass productivity and nutrient recycling opportunities in energy crop plantations.
Key words: bio-energy, digestate, perennial grasses, waste water sludge, wood ash.

Introduction

Bio-energy is important in the entire world, even at the moment it is the dominating source of renewable energy in Europe, and it is predicted that its production will grow significantly in the following decades. The European Union (EU) is committed to combat climate change and to increase the security of its energy supply. In 2009, the EU adopted Directive 2009/28/EC establishing the guidelines for the promotion of renewable energy. EU directives are binding for Latvia – we are bound by their commitment to the partial substitution of fossil fuels with renewable energy (Directive, 2009). Last year in the statement of Commission to the European Parliament on renewable energy sources **(**COM/2011/0031), a mandatory objective was suggested to ensure that 20% of the energy used in Europe will be provided by renewable sources of energy, including bio-energy, by the year 2020 (Agriculture and bioenergy, 2012).

Bio-energy is one form of renewable energy among many other sources (wind, solar, hydraulic, geothermal, etc.) which reduces greenhouse gas emissions. Bio-energy accounts for more than two thirds of total renewable energy in the EU. The share of agriculture – although still modest – but is growing fast (Donner and Kucharik, 2008; Agriculture and bioenergy, 2012).

Replacing fossil fuels by biomass reduces the accumulation of carbon dioxide into the atmosphere and a significant reducing of 'greenhouse effect' could be realized, thus preventing global climate change as by using biomass for energy production, the carbon cycle is closed. Plants during their growth cycle accumulate solar energy, a large amount of carbon dioxide (CO_2) is consumed during photosynthesis and a huge amount of oxygen is produced. Burning new biomass contributes no new carbon dioxide to the atmosphere, because replanting harvested biomass ensures that CO_2 is absorbed and returned for a cycle of new growth (McKendry, 2002).

Different raw materials can be used for the production of bio-energy: waste, woodchips, plant biomass, etc. Ways how to ensure the output of materials are searched for in the entire world; the possibilities of arranging the energetic plant plantations, including the growing of grasses, which have many advantages over other cultivated plants, are being studied. In the bio-based economy, renewable herbaceous biomass such as perennial grasses will become an important cellulosic feedstock for conversion to bio-fuels, electricity and heat (Bakker and Elbersen, 2005).

Perennial grasses are great for energy production, they are environmentally friendly, provide biomass of high energy and appropriate quality without large investments. There are suitable soil and climatic conditions in Latvia for the growing of perennial grasses. The amount of rainfall in Latvia's climatic zone is sufficient, and it is distributed so that high-quality biomass harvests from perennial grasses can be obtained during their vegetation period. Swards are not demanding in terms of soil, therefore for energy purposes it is expedient to establish them on less productive or abandoned land (Poiša et al., 2011; Kryževičiene, 2006). One of the opportunities

for farmers is conversion from traditional farming and cultivation of fast growing plants for bio-fuel purposes, especially in the regions where cultivation of traditional crops is not profitable (Šiaudinis, 2010).

Grasses are relatively modest in terms of the soil condition, compared to other cultivated plants. Their strong root system provides the plants with necessary nutrients even from the deeper layers of the soil. Reed canary grass (*Phalaris arundinacea* L.) and tall fescue (*Festuca arundinacea* Schrab.) tolerate short flooding which occurs frequently in the countryside of Latvia in early spring or as a result of heavy rainstorms.

Perennial grasses are not endangered by diseases and pests as it is with other cultivated plants, and that is why the production of bio-energy from these plants is much 'greener'. There is no need for additional protection measurements saving resources and the environment, which is a significant argument in the context of climate change.

Bio-energy production from perennial herbaceous energy crops is promising because it alleviates the conflict of using food crops for bio-energy and because high biomass yields are produced annually for several years in succession before replanting (Tilvikiene et al., 2011). Perennial grasses are high yielding; moreover, they can produce for 10 or more years without reseeding, protect soils on slopes from erosion and maintain soil fertility. Unlike other cultivated plants, grasses can be grown in monoculture without any problems.

Research results in several countries confirm that native perennial rhizomatous grasses, including reed canary grass, show the greatest potential as bio-energy crops (McLaughlin et al., 1998; Saijonkari–Pahkala, 2001; Lewandowski et al., 2003). Reed canary grass likes wet soils, although it does not tolerate stagnant ground water in the upper layers. It grows well in humus and nutrient rich soils. Similarly to other runner top-grasses reed canary grass likes well aerated loose soils. In suitable conditions it ensures over 9 t ha^{-1} of dry matter annually. Acidic soils are not suitable for growing reed canary grass.

The tall fescue can be successfully used for the production of bio-energy, mainly of solid fuel. It is perennial and can grow for 8 – 15 years without reseeding, and can produce high dry matter harvests (12 – 14 t ha^{-1}). The tall fescue is resistant to frost, thus perspective for latitudes of Latvia. Due to its strong root system this species of grass tolerates drought. Soils with low fertility and newly cultivated soils are suitable for growing tall fescue, as it is a relatively modest grass crop (Adamovičs, 2007).

Whereas the fodder galega (*Galega orientalis* Lam.) possesses all the best characteristics of grasses – the ability to grow in one place without reseeding and ability to fix the atmospheric nitrogen. It has a high harvesting rate and can ensure dry matter harvests for many years without reseeding and nitrogen fertilising (9 – 16 t ha^{-1}). The optimal conditions of soil for growing galega are: organic matter content 20 – 25 g kg^{-1}, soil reaction pH – 6 – 7, plant available P – 55 – 90, and K – 150 – 200 mg kg^{-1} soil. The fodder galega biomass is suitable for the production of biogas, as well as dry fuel – pellets and briquettes (Adamovičs, 2007).

The perennial lupine (*Lupinus polyphyllus* L.) has a highly developed root system that reaches deep into the soil. For the cultivation of this species the most suitable are sandy loam and light loam soils with acid soil reaction (pH KCl about 4 to 5). It grows well in sandy soil as well, if during the first year the plants are sufficiently provided with humidity. The perennial lupine is not demanding in the terms of nutrition, because it has possibility of fixing nitrogen from the atmosphere with the help of the N-fixing bacteria, but it extracts potassium and phosphorus from the deeper layers of the soil and uses the less soluble compounds by help of its strong root system. The lupine grows intensely for 3 to 5 years, but afterwards the formation of N-fixing nodules decreases gradually. It grows rapidly after cutting, although frequent mowing causes the thinning of the sowings. The potential harvest for the lupine is about 12 t ha^{-1} dry matter (Jansone and Rancāne, 2011).

The perennial grasses used for bio-energy production do not require a large initial investment, and the future of this area of management costs is relatively low. Perennial grasses have been identified as the lowest cost dedicated agricultural feedstock for energy and agro-fibre markets. Lithuanian research evidence suggests that the energy potential of perennial grasses was up to 153 GJ ha^{-1}, and it was up to 19 times higher than the energy input for bio-fuel production (Kryževičiene, 2006; Navickas et al., 2003).

Several studies have shown that grass biomass qualitative features significantly affect the harvest time and soil conditions. Many previous studies (Burvall, 1997; Finell et al., 2002; Xiong et al., 2008) have confirmed that the delayed harvest system for grass crops in which the harvest of the previous year's crop is undertaken after it has over-wintered in the field significantly improves the fuel quality for both combustion and gasification.

A significant fraction – up to one-fifth – of herbaceous biomass consists of inorganic constituents, commonly referred to as ash that cannot be converted to energy. The quantity and quality of ash in herbaceous biomass depends on many factors including the plant type, growing conditions, fertilisation, choice of harvest date, etc. However, it is of paramount importance that the ash content of these

feed-stocks should be reduced so as to facilitate their commercialization (Samson and Mehdi, 1998).

The critical elements that cause fouling and corrosion problems in boilers are alkali compounds and chlorine which are released during combustion. The concentrations of these are reduced by the delayed harvest system rather than harvesting at the end of the growing season (Xiong et al., 2008).

The loss of some leaf material and leaching of alkali compounds also contributes to an increase in the ash fusion temperature from 1070 °C to 1400 °C. Swedish research results indicate that ash content is influenced by the soil type as well. The most extreme variations were found between reed canary grass grown on clayey soils and those produced on very wet soils, with ash contents in grass of 101 g kg^{-1} and 22 g kg^{-1}, respectively (Burvall, 1997). Efforts should be made to integrate the approach with beneficial uses of ash derived from biomass, including the potential for recycling of nutrients to the field (Samson and Mehdi, 1998; Bakker and Elbersen, 2005).

In order to obtain high quality harvest, the grasses must be provided with all necessary nutrients. One of the most important elements that ensures the growth of harvest, increases winter-resistance and promotes sustained preservation in the sward is potassium. On average, with 1 t of grass dry matter, 21 kg of N, 2.3 – 2.8 kg of P and 20 kg of K are taken up from the soil (Kārkliņš and Līpenīte, 2011). Ash contains a lot of potassium (50 – 60 g kg^{-1}), thus it can be successfully used to fertilise the grass.

As a result of the anaerobic processing of the biomass, the by-product of the obtained gas is digestate, which contains all necessary plant nutrients. The presence of the nutrients in digestate varies depending on the contents of the fermentable biomass; usually digestate is a good source of phosphorus, potassium and nitrogen as well. Thus it can be successfully used as energy plants fertiliser, stimulating recirculation of the nutrients.

In Latvia and worldwide, research has been done about the usage of sewage sludge for the fertilisation of energetic plants, because often the sludge that comes from areas with bigger industrial centres contains more heavy metals, thus it is discussable whether it can be used to fertilise crops used as food or feed.

To promote the non-used and low-value land management and production of energy crops, growing conditions for perennial grasses were investigated. The objective – suitability of several perennial grasses for bio-energy production under condition of Latvia. The aim of the current research was to evaluate the experimental field conditions including soil parameters important for the cultivation of selected species of perennial grasses.

Materials and Methods

The experimental site is located at the LLU Research Institute of Agriculture in Skriveri. Four species of grasses are planned to be included in the test: reed canary grass (*Phalaris arundinacea* L.), tall fescue (*Festuca arundinacea* Schrab.), goats grass (*Galega orientalis* Lam.), and perennial poor-alkaloid lupine (*Lupinus polyphyllus* L.). Above mentioned crops will receive different kinds of fertilisers. The efficiency of these factors on grass productivity, yield quality, as well as time of grass harvesting will be studied. The proposed experimental scheme includes following treatments: 1) control (not fertilised); 2) mineral fertilisers; 3) wood ash; 4) digestate; 5) waste water sludge. The total area of the experimental field – 2 ha. The size of one experimental plot – 200 m^2. The variants of fertilisers will be arranged randomly in 4 replications.

Before the start of experiment (3rd decade of July, 2011), soil samples were taken from 20 places of the experimental field – 4 times for each plot at four different depths: 0 – 20 cm, 20 – 40 cm, 40 – 60 cm, and 60 – 80 cm. Two types of soil samples were taken: for agrochemical analysis and for bulk density determination.

The soil samples were prepared for the physical–chemical analyses in accordance with LVS ISO 11464 Standard (drying, crushing, sieving).

The following measurements were performed: bulk density, soil texture, pH $CaCl_2$, total carbon and sulphur, total nitrogen, and plant available P and K of the soil.

The bulk density of the soil (kg m^{-3}) was tested in accordance with LVS ISO 11272:1998, by placing a sample in a 100 cm^3 cylinder and drying it to an oven-dry condition in a laboratory at a 105 °C temperature. The exchangeable soil acidity was measured in a 0.01*M* $CaCl_2$ suspension potentiometrically (LVS ISO 10390/NAC). The total carbon and sulphur was analysed using an ELTRA CS 530 elementanalyzer. The soil texture was determined by dry sieving (LVS ISO 11277, 2000). The plant available phosphorus was extracted by 0.2*M* HCl, and phosphorous concentration was measured spectrophotometrically (LVS 398). Extraction of potassium was performed using 1.0*M* CH_3COONH_4, and atomic-absorption spectrometer was used for determination of potassium concentration in the extract. For determination of mineral nitrogen in soil (content of nitrate and ammonium ions), extraction using 0.1*M* NaCl was done, and a spectrophotometer for concentration measurements was employed. Statistical analysis of the obtained data was carried out – correlation analysis, and border differences and standard-deviations were calculated.

Results and Discussion

The test field is located in Skriveri – in the south-west part of Madliena tilt in Mid-Latvian lowlands. It is located on the border between the relatively cold north-west Vidzeme and the relatively warmer East-Latvian lowland. Consequently, the sum of annual average air temperatures is from 1800 to 2000 °C, and the sum of average soil temperatures ranges from 2000 to 2200 °C. It shows that the temperature conditions for the growth of the grasses are good. Also the average amount of rainfall is appropriate. The dominating humid sea air masses in Latvia provide a great amount of rainfall. In Latvia, and in Skriveri as well, the annual average rainfall is 600 – 700 mm (Nikodemus, 2009).

Topography of the experimental field is a slightly undulating plain. Soil cover is developed on glaciogenic sediments. The field is relatively well cultivated; for the past 10 – 15 years it was used for field crop rotation and is dominated by forage legumes and perennial grasses. The sampling scheme used for soil fertility evaluation enables the spatial (vertical and horizontal) variability of soil properties to be studied.

By evaluating the soil homogeneity in the top layer (at the 0 – 20 cm depth), it can be concluded that soil agrochemical indicators differed in different parts of the field. The bulk density of the topsoil layer was 1528 – 1618 kg m^{-3}, thus the topsoil layer can be evaluated as being medium loose, which will not restrict the primary development of the grass roots. After growing the perennial grasses for a longer period of time, their strong root system would have a favourable impact on the soil structure.

The soil reaction was weakly acidic – around neutral pH $CaCl_2$ 6.1 – 6.6, which is completely appropriate for the growing demands of the perennial grasses planned to be grown in the trial.

Carbon (C) content in the top layer of the soil ranged from 21.3 to 25.4 g kg^{-1}. This indicator is also sufficiently good for cultivation of these grasses for bio-energy production, as long-term studies show that in general grasses have a positive effect on the formation of soil organic matter.

In general, the experimental field has a good status of plant available phosphorus, its content in the top layer of the soil ranged from 106.73 to 142.35 mg kg^{-1} P_2O_5. The soil had less plant available potassium – from 85.66 to 129.02 mg kg^{-1} K_2O, which indicates that in this soil grasses could respond well to potassium fertiliser, including wood ash fertiliser, which has a high content of potassium.

As the area of the experimental field is 2 ha, the results in the topsoil varied quite a lot, but after carrying out a mathematical data processing it is clear that there are no significant differences between the soil agrochemical indicators of the planned test variants, which could not influence the experimental results.

After analysing the soil agrochemical indicators at different depths, several regularities were confirmed: by increasing the depth, also the soil bulk density increased, and there was a difference between the 1st and 2nd layer, but deeper – at 40 – 80 cm depth the difference practically disappeared. Although the correlation between the first and second depth was not observed, the intra-class correlation between the depths 2, 3 and 4 was significant $r=0.42>r_{0.05}=0.22$.

The average soil bulk density at the depth of 0 – 20 cm was 1570 kg m^{-3}, which means that the soil was sufficiently loose for the development of grasses in the soil top layer, which in its turn is important for the beginning of the growth. It allows grasses to develop rapidly, to create a dense sward and to compete with different weeds. The average soil bulk density at the

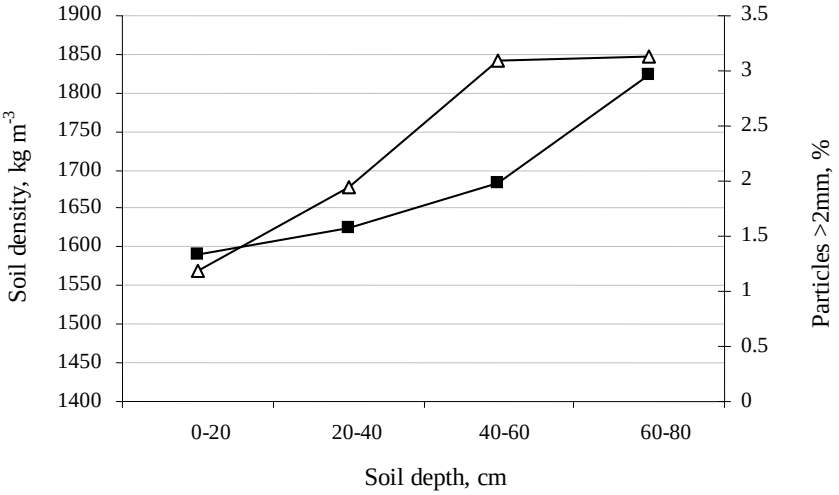

Figure 1. Soil physical properties of the four depths, cm: Δ - soil bulk density, kg m^{-3}; ■ - soil particles >2 mm, %.

20 – 40 cm depth was 1679 kg m^{-3}, and deeper the bulk density increased rapidly, thus the development of root system will be obstructed at the depth of more than 40 cm. However when the depth is more than 40 cm. Although these soil conditions will not delay the development of the grasses, because the main root mass occupies the soil volume up to the depth of 30 – 40 cm (Figure 1).

When the depth increases, the proportion of the fine particles of the soil also increases – at up to 60 cm for every layer of 20 cm thickness it increased by 20% on average, but at the depth of 60 – 80 cm, the proportion of the fine particles increased by 50% compared to the adjacent depth of 40 – 60 cm. There was a closer correlation in the deeper layers of the soil: between depths 1 and 2, and between depths 1, 2, and 3 the correlation was not significant – respectively $r=0.04<r_{0.05}=0.36$ and $r=0.22\leq r_{0.05}=0.22$; when excluding the 0 – 20 cm depth, the correlation between the deeper layers increased to $r=0.49>r_{0.05}=0.22$.

The soil acidity in the deeper layers slightly decreases – the soil turns more neutral; however but those are not essential changes and there is a closer pair and intra-class correlation.

After observing the content of plant nutrients, it is obvious that their quantity increases when the depth increases. The content of carbon, consequently the organic matter as well, is concentrated in the soil upper layer. Even at the depth of 20 – 40 cm it decreases by half compared to the amount of C found in the plough layer, but in the deeper layer it increased even more rapidly (Table 1). There was a significant correlation between C content at the depths 1 and 2 ($r=0.44>r_{0.05}=0.36$).

Similar tendencies were observed also for the total sulphur content – its decrease by the depth was not so rapid, but it is obvious that the soil upper layers had more sulphur. The closest correlation of sulphur content was between the depths 1 and 2 ($r=0.42>r_{0.05}=0.36$), whereas the potassium content, which similarly decreases with depth, significantly correlated between the three deepest depths ($r=0.42>r_{0.05}=0.22$).

The above mentioned tendencies were not observed for phosphorus, which content was practically the same at all the depths. The amount of plant available P decreased only at the depth of 20 – 40 cm, but at the other three depths it was at equivalent amounts (within P_2O_5 127 – 143 mg kg^{-1}), and this indicator had a significant pair and intra-class correlation.

One of the most important elements for the growth of grasses, particularly for cereal grasses, is nitrogen.

Table 1

Agrochemical characteristics of four soil depths on average

Parameters	Soil depth, cm			
	0 – 20	20 – 40	40 – 60	60 – 80
Soil pH $CaCl_2$	6.40±0.38	6.50±0.36	6.60±0.35	6.80±0.32
C total, g kg^{-1}	23.95±4.40	12.40±8.27	4,40±3.18	6.95±6.04
S total, mg kg^{-1}	17.80±9.82	22.87±16.80	13.32±14.00	14.28±11.10
P, mg kg^{-1}	121.85±29.78	86.58±39.98	129.84±51.38	136.91±51.49
K, mg kg^{-1}	114.96±25.54	70.96±17.80	53.74±11.66	48.32±9.71
$N\text{-}NO_3$, mg kg^{-1}	14.74±6.32	4.38±2.09	2.48±0.77	2.41±0.94
$N\text{-}NH_4$, mg kg^{-1}	3.81±2.40	3.29±2.21	3.75±2.05	4.35±2.49

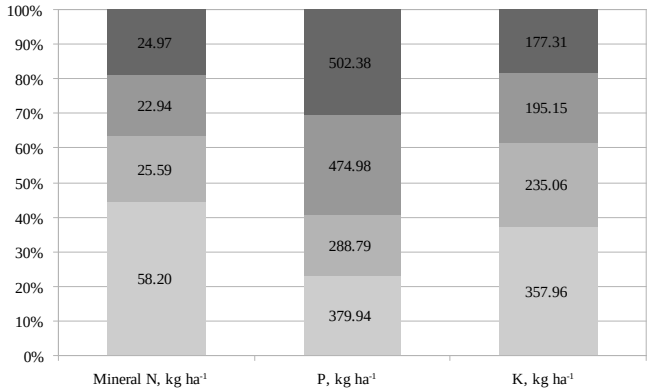

Figure 2. NPK content at different soil depths: 0-20 20-40 40-60 60-80.

$N–NO_3$ content significantly correlated between the three deeper depths ($r=0.50>r_{0.05}=0.22$), but nitrogen in the ammonium form $N\text{-}NH_4$ mostly remained at the same amount at all depths (Table 1).

The amount (kg ha^{-1}) of the main plant nutrients (NPK) in every soil layer was calculated. The amount of nitrogen in summer period, when all of microbiological processes actively took place, was sufficient – 58.2 kg ha^{-1} at the upper layer. At the depth of 20 – 40 cm, the N amount decreased approximately by half and remained practically the same up to the depth of 80 cm – on average 25 kg ha^{-1} (Figure 2).

The amount of plant available P was relatively high at all depths ranging from 288 to 502 kg ha^{-1}, and, unlike other elements, the highest P content was at the deepest soil layer. The average amount of potassium in the top layer was 357.96 kg ha^{-1}, and deeper it decreased sharply.

In general, it can be concluded that all the determined soil agrochemical indicators had a relatively close intra-class correlation between the deepest (2, 3 and 4) depths but, if we look at the all four depths, including the soil upper layer (0 – 20 cm) agrochemical indicators, the correlation decreased.

All the plant available nutrients, except phosphorus, were more concentrated in the soil upper layer because the microbiological processes occur most intensively there. At this soil depth, the plant roots have the easiest access to these nutrients, and can use them for root development, as the main grass root mass is located in the topsoil.

Conclusions

1. The perennial grasses are a perspective crop for the production of bio-energy in Latvia conditions, as they provide not only the production of biomass of high and appropriate quality but are modest in terms of growing conditions, their cultivation does not require large investments and they are environmentally friendly.
2. The soil agrochemical parameters of the experimental field were relatively good for the growth of the perennial grasses. The indicators were good not only in the soil upper layer, but at the depths of 20 – 40 cm as well, which allows grasses to form a deep root system.
3. The results of agrochemical analyses indicated that there was a certain spatial variability, but the statistical data processing demonstrated that the differences between the indicators of variants were not significant, which will allow obtaining objective results in the following research process.

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IMPACT OF SLURRY APPLICATION METHOD ON SWARD YIELD AND N AND K LEACHING FROM GRASSLAND

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Abstract
Studies comparing slurry injection versus spreading have reported inconsistent results so far. The objective of the study was to compare two slurry application methods, injecting and spreading, in terms of influence on sward yields and leaching of nitrogen (N) and potassium (K) from grassland. The experiment was conducted from 2008 to 2011 at the Eerika Experimental Station, Estonian University of Life Sciences. Mini-lysimeters filled with loamy sand soil embedded in three swards (three-species grass mixture (*Phleum pratense*, *Lolium perenne* and *Poa pratensis)*, grasses mixture with white clover (*Trifolium repens)*, and grasses mixture with lucerne (*Medicago sativa)*) were used. Three annual nitrogen rates (60, 120, 180 kg ha^{-1}) in conjunction with the two slurry application methods (injecting and spreading) were applied. Percolated water quantities, N and K content in leachate and the sward yield were measured. N leaching was significantly less with slurry injection while K leaching did not depend on slurry application method. N and K leaching was more affected by sward botanical composition and applied N rate and N:K ratio n slurry than by slurry application method. Sward yields did not depend significantly on slurry application method. Slurry injection is rational only in grasses sward when used N rates are above 120 kg ha^{-1} yr^{-1}. Slurry injection may have negative influence on the distribution of white clover in the grasses + white clover sward and thus lead to sward yield decrease.
Key words: Grassland, leaching, nitrogen, potassium, sward yield.

Introduction

Slurry is an organic and environmentally friendly source of plant nutrition. Since the increasing animal production also increases the slurry amounts, it is important to study it. Depending on the method of application, slurry remains either on the surface (spreading) or within the sward (injecting). The method of injecting slurry into the soil can efficiently prevent NH_3 volatilization (Frost, 1994) and surface runoff losses of NH_4 - N (Turtola and Kemppainen, 1998). However, large quantities of nutrients that are brought into the soil via slurry organic matter can mineralize when plants do not need them and can, therefore, lead to nutrient leaching (Bergström and Kirchmann, 1999). Interestingly, it has been found that nitrogen (N) in injected slurry is better utilized by plants than with surface application (Schils and Kok, 2003).

Unlike N and phosphorus (P), there has been no concern raised about potassium (K) as a potential pollutant. Losses of K can be considered to be of economic importance (McGechan and Wu, 1998). It has been found that although K leaching on grassland is usually low, high levels of available K in the soil, and large quantities of fertilizers may increase K losses significantly (Kayser and Isselstein, 2005).

The effect of slurry application method on sward is unclear, as opposing results have been presented. R.L.M. Schils and L. Kok (2003) found that yields are higher on the plots with injected slurry rather than on plots were slurry is surface - applied. Injection increases herbage N content due to the higher N recovery of injected slurry (Kemppainen, 1989; Rees et al., 1993). Other results indicate that injection produces significantly lower yield than surface application (Rodhe et al., 2006) or there is no effect on yield from injection technique compared with surface spreading (Mattila et al., 2003). It has been proposed that the mechanical damage by injection could be the underlying cause (Halling and Rodhe, 2010).

My hypotheses were that (i) sward yield is higher with slurry injection when compared to spreading and (ii) N and K leaching is less when slurry is injected.

The aim of the study was to examine whether injecting or spreading of slurry significantly influenced sward yields with different botanical composition and N and K leaching from those grassland swards.

Materials and Methods

The experiment was conducted from May 2008 to January 2011 at the Eerika Experimental Station, Estonian University of Life Sciences (58°23'32" N, 26°41'31" E; elevation 60 m). Plastic mini-lysimeters (surface area 0.0706 m^2; depth 30 cm) filled with loamy sand soil (sand 64%, silt 29%, clay 7%, specific surface area of 30.6 m^2 g^{-1}) were dug into the ground, so that soil surface of the lysimeters was at the same level as the surrounding soil. At the beginning of the experiment the soil organic matter (OM) was 170 000 – 190 000 mg kg^{-1}, total N content was 11 000 mg kg^{-1}, available P was 94 - 102 mg kg^{-1} and K was 165 - 180 mg kg^{-1} (Egner – Rhiem – Domingo).

The study involved the injecting or spreading of slurry equating to annual N rates of 60, 120 and 180 kg ha^{-1} onto plots of three types of sward (i) grasses mixture with *Phleum pratense*, *Lolium perenne* and *Poa pratensis*; (ii) grasses mixture with added *Trifolium repens* and (iii) grasses mixture with *Medicago sativa*. Slurry was applied manually. The application rate was calculated based on NH_4 – N

content. When injecting, holes (3 cm diameter, 5 cm depth) were made into the sward with a soil auger. The holes were filled with slurry and closed immediately with soil and a turf cap. Slurry was applied to the plots in one to three split applications, depending on the N rate, as follows: (1) 1 week after grass started to grow in spring, (2) in June/July (after the second cut) and (3) in August (after the third cut). N and K leaching was studied during three years only on grasses + white clover and grasses sward. Experiment conditions did not fit for lucerne and because of its small percentage in the grasses + lucerne sward in the second experimental year measurements took place only in the first and second year.

The quantities of leachate water and the total N and K content in the water were measured on a monthly basis throughout the vegetation period and beyond vegetation period until the soil became frozen and in April after soil thawed. The yield was harvested five times during the growing season.

VarioMax elemental analyser was used to measure the N content in the leachate and a flame photometer to measure the K content. The relationship between water percolation, sward yield and nutrient leaching was tested with correlation and multiple regression analysis. All calculations were carried out using the statistical package Statistica 9.0 (StatSoft.Inc) with the probability level set at 0.05.

Throughout the experimental period the field micrometeorological conditions were monitored with Metos Model MCR300 weather stations (Pessl Instruments GmbH, Weiz, Austria); the sensors were positioned 2 m above ground.

Weather Conditions

Sum of precipitation during the experiment was higher than usual (Tabel 1). When compared to annual precipitation from 1991-2009, precipitation during first year was higher by 141.8 mm, second year 159.2 mm and third year 69.6 mm. This is due to the higher rainfall in vegetation period. Vegetation period was longer than usual in Estonia (175-190 days): 216 days in 2008, 215 days in 2010 and 206 days in 2011. In year 2009 vegetation lasted for 188 days. When compared to average annual temperature in 1991-2009 (6.2 °C), average temperatures in our experiment were 5.6, 4.1 and 7.4 °C in the first, second and third year respectively. In the first and second year average temperatures were lower than average in the period from 1991 to 2009, in the third year it was higher. Average temperatures of winter periods (from December to February) were -2.0, -7.7 and -6.8 °C in the first, second and third year respectively. Average winter temperature of the period from 1961-2011 was -4.2 °C. Although average winter temperatures of last 50 years has become higher, winter temperatures of the second and third year in our experiment were lower than average of 1961-2011.

Table 1

Meteorological data in the experimental period from May 2008 to January 2011

Month	Sum of precipitation, mm				Average temperature, t °C			
	1. year	2. year	3. year	1991-2009	1. year	2. year	3. year	1991-2009
May	30.6	18.4	97.4	37.6	10.4	11.3	12.2	10.1
June	108.2	151.0	98.0	63.2	14.2	13.6	14.3	14.5
July	59.6	97.4	38.4	59.5	15.9	16.8	21.7	17.5
August	216.6	85.0	148.4	68.0	15.4	14.9	17.8	16.6
September	67.6	57.6	99.4	48.1	9.5	12.4	10.7	11.8
October	96.4	132.4	59.2	67.3	7.9	3.7	3.8	6.5
November	27.8	77.8	72.4	55.0	1.8	1.9	-0.2	1.3
December	42.6	57.0	0.0	44.0	-1.6	-5.5	-8.6	-1.5
January	24.0	0.0	40.4	45.6	-4.0	-14.7	-5.2	-2.5
February	16.8	8.6	0*	34.2	-5.4	-8.3	-12.0*	-4.1
March	33.0	33.6	0*	32.6	-2.2	-2.6	-2.5*	-0.8
April	3.2	25.0	0.6*	29.3	5.3	5.7	5.7*	4.9
Average temperatures or sum of precipitation:								
(a) in vegetation period	579.0	560.8	541.4		12.0	11.3	12.7	
(b) beyond vegetation period	147.4	183.0	112.8		-1.3	-5.8	-4.2	
Year	726.4	743.8	654.2	584.6	5.6	4.1	7.4	6.2

* – measurements did not take place

Results and Discussion

Sward yields

Average sward yields were similar (p>0.05) for both slurry application methods, resulting in 0.66 kg DM m^{-2} and 0.67 kg DM m^{-2} for slurry spreading and injecting, accordingly (average results of three years). Average yield of grasses + white clover sward was 0.81 and 0.79 kg m^{-2}, grasses sward 0.45 and 0.49 kg m^{-2} and grasses + lucerne sward 0.75 and 0.75 kg m^{-2} when spreading and injecting, respectively.

The differences in sward yields within one sward when comparing different slurry application methods at different nitrogen rates used were not statistically significant (Figure 1), which is in accordance with results presented by P.K. Mattila et al., (2003). In grasses sward yields were 9.0% (p>0.05) higher when slurry was injected suggesting that slurry injection has small advantage only in grasses sward.

Previous study has noted sward damaging during slurry injection, which results in reduction of sward yield (Rodhe et al., 2006; Halling and Rodhe, 2010). Our results indicate that slurry injection may have negative influence on the distribution of leguminous plants in sward, which is a consequence of sward damage during application. It appeared most clearly in the case of white clover and the phenomenon was evident in all experimental years, where grasses + white clover sward yields were 20.3% (p>0.05) higher when slurry was spread at N60 rate. The same was true also in the first year in grasses + lucerne sward with N rates 60 and 120 kg ha^{-1} yr^{-1}, where yields were slightly (p>0.05) higher with spreading. By the following year lucerne was predominated by grasses. Slurry application method impact on yield was similar to one in grasses sward, where yields were higher when slurry was injected. M.A. Halling and L. Rodhe (2010) presented a negative effect of injection on grasses distribution, but our results did not confirm that. In our study the result was rather reverse.

Leaching of N

Average N leaching across three swards was 2.23 g m^{-2} yr^{-1} and 1.95 g m^{-2} yr^{-1}, accoringly when slurry was spread or injected. Those results were statistically different (p<0.05). Average leaching from grasses + white clover sward was 1.94 and 1.57 g m^{-2}, from grasses sward 2.39 and 2.16 g m^{-2} and from grasses + lucerne sward 2.13 and 1.82 g m^{-2} for spreading and injecting, accordingly (Figure 2). N leaching was significantly less when injecting in grasses + white clover sward and in grasses + lucerne sward (average of N rates used). In grasses sward N leaching did not depend on slurry application method. The differences in N leaching within one sward when comparing slurry application methods at different nitrogen rates used were not statistically significant (Figure 2), except in grasses sward were N leaching was significantly reduced with slurry injection with applied N rate 180 kg ha^{-1} yr^{-1} (p<0.05). From previous results it can be presumed that slurry spreading decreases N leaching, because spreading promotes N volatilization (Mengel and Kirkby, 2001). In this

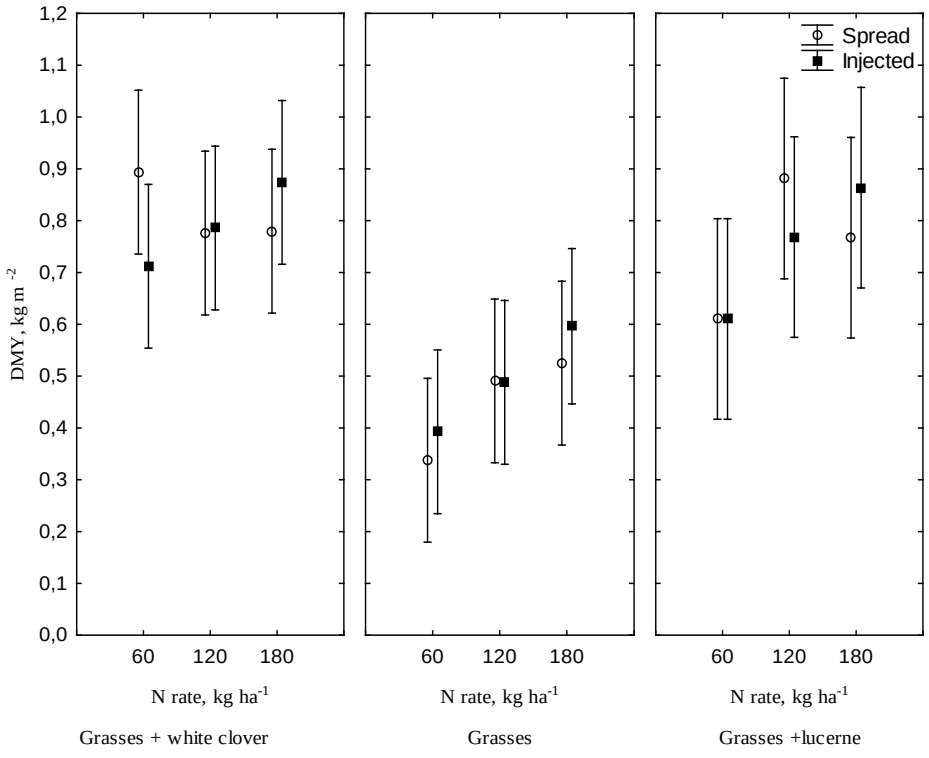

Figure 1. DMY depending on slurry application method at different N rates (average results of three years).

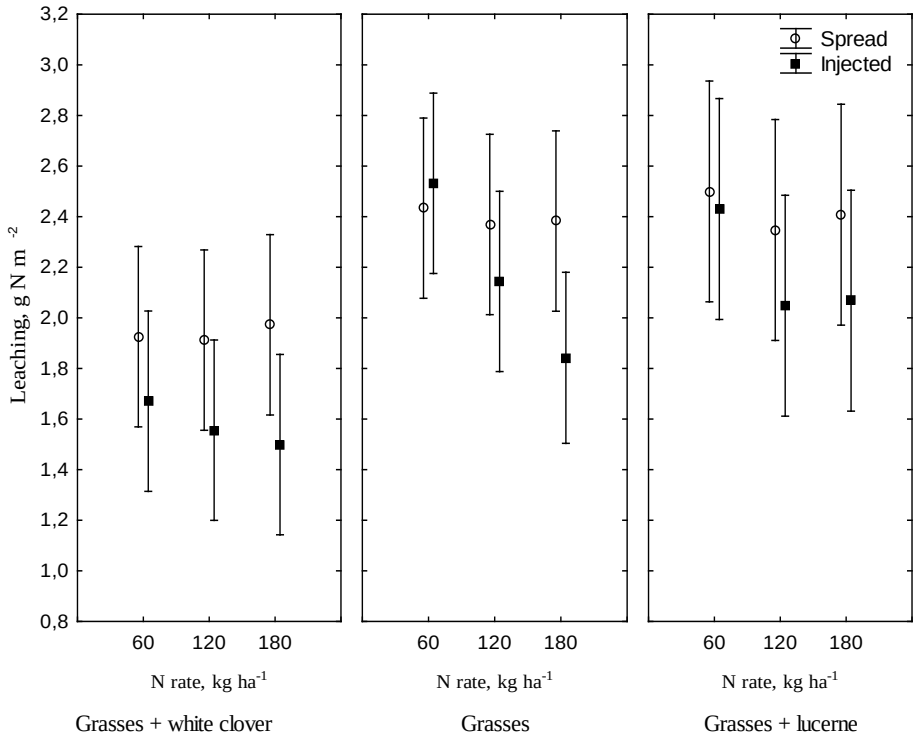

Figure 2. Average N leaching during vegetation period in two slurry application methods (average results of three years).

case the amount of N that reaches the soil is lower compared to injection. In my experiment this theory was not confirmed. N leaching was slightly ($p>0.05$) higher with slurry spreading, probably because N in the slurry was more accessible for plants when it was injected.

When comparing N leaching in different swards it appeared that leaching did not depend on applied N rate when slurry was spread. When injecting leaching from grasses sward decreased with increasing N rate, in grasses + lucerne sward leaching decreased until applied N rate 120 kg ha^{-1} yr^{-1}, but N leaching from grasses + white clover sward did not depend on the N rate used. The reason why N leaching did not differ in grasses + lucerne sward at applied N rates 120 and 180 kg ha^{-1} yr^{-1} is currently unclear and needs further investigation. With increasing N rate yields of grasses and grasses lucerne swards increased, which in turn decreased N leaching as less water percolated through the sward (Tampere et al., 2011). The yield of grasses + white clover did not depend on used N rate, because increasing N rate decreased the partial yield of white clover and increased grasses yield. As a result, the sward yield did not change (results have not presented). N leaching depended almost solely on sward yield; this is in accordance with the result presented before by M.L. Decau et al. (2004). With increasing yield less water percolates the sward and for this reason N leaching decreases (Jarvis et al., 1989; Saarman and Viiralt, 1982; Tampere et al., 2011).

Leaching of K

Average K leaching when slurry was spread was 1.57 g m^{-2} yr^{-1} and 1.36 g m^{-2} yr^{-1} when slurry was injected. Average leaching from grasses + white clover sward was 0.56 and 0.55 g m^{-2}, from grasses sward 2.20 and 1.90 g m^{-2} and for grasses + lucerne sward 2.06 and 1.72 g m^{-2} when spreading and injecting, accordingly.

My experiment showed that K leaching was not significantly affected by slurry application method. Still K leaching was 14.6% ($p>0.05$) higher in all swards with slurry spreading, except in grasses + white clover sward with applied N rate 60 kg ha^{-1} yr^{-1}, when 23.8% ($p>0.05$) more K leached through the sward when slurry was injected. When slurry was injected the partial yield of white clover in the sward was smaller when compared to spreading: due to the less plant available N in the soil uptake of K decreased.

K leaching depended more on sward botanical composition (especially on the partial yield of legumes) and applied N rate than by slurry application method. K leaching was significatly less from grasses + white clover sward ($p<0.05$). K leaching decreased in grasses sward when N rate used increased only when slurry was injected. In earlier experiments it has been found that increasing N supply enhances growth, and consequently, increases the demand for K, and for this reason K leaching decreases (Taube et al., 1995; Wilkinson et al., 2000; Alfaro et al., 2003). Used N rate had the greatest influence on K leaching

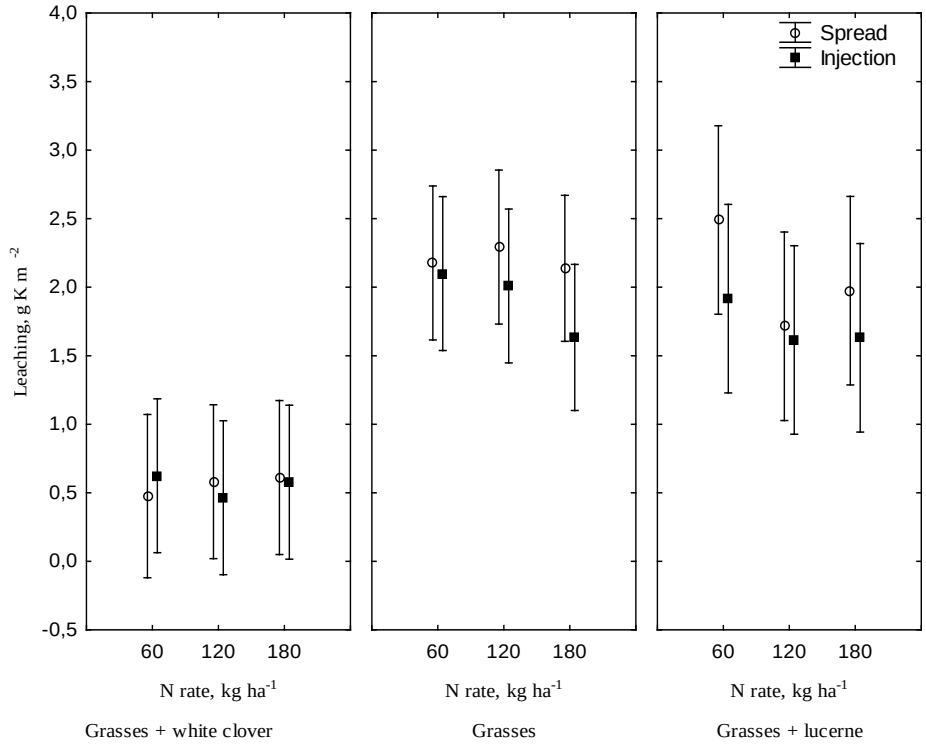

Figure 3. Average K leaching during vegetation period in two slurry application methods (average results of three years).

in grasses sward, the influence was less in grasses + lucerne sward and it was absent in grasses + white clover sward. Legumes do not tolerate high N rates. When used N rates are high, the importance of legumes in the sward declines remarkably.

The reason why K leaching in grasses + white clover sward remained constant with increased N rate could be that K leaching was very small already at the lowest rate N60. Increased N rate brought down the importance of white clover in the sward and its N supply. The amount of N brought in the soil by slurry was not quantitatively higher than the amount of N brought to soil by white clover.

K leaching may also be affected by the nutrient content of the slurry, which does not correspond to plants needs. In our experiment N:P:K ratio was 1:0.20-0.22:0.62-0.75, while the optimal nutrient ratio recommended in the literature for grasses is 1:0.22:0.62 (Viiralt, 2007). It has to be considered that when applying slurry in the year of fertilization, the sward yield is mainly influenced by the ammonium nitrogen in the slurry. In our study the importance of the ammonium nitrogen in the slurry was 39.5-58.2%. Therefore, when giving N rate based on ammonium nitrogen, plants may receive more K than plants are able to consume.

Conclusions

1. Our results support the hypothesis that slurry injection results in less N leaching. N leaching was significantly smaller with slurry injection in grasses + white clover and grasses + lucerne sward. Our results indicate that N and K leaching depends on sward yield. It was most noticeable in grasses sward where with higher yield leaching decreased.
2. From our results it can be concluded that sward yields and K leaching did not depend statistically on slurry application method. According to our results slurry injection is potentially beneficial only in grasses sward when used N rates are above 120 kg ha^{-1} yr^{-1}. Slurry injection may have negative influence on the distribution of white clover in the sward, thus resulting in lower sward yield.
3. We consider that K leaching may also be influenced by the N: K ratio in the slurry. In our experiment it could have been too narrow as plants did not have enough N to assimilate K.

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IMPACT OF ORGANIC PRODUCT EXTRACTS ON POTATO 'BORODJANSKIJ ROZOVIJ' TUBER YIELD IN ORGANIC CROP PRODUCTION SYSTEM

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Abstract
Products of organic origin used in agriculture to reduce the application of artificial pesticides and fertilizers are investigated world-wide now. Composting organic fertilizers, plant residue and household waste results in new products of organic origin. In several countries (USA, Russia, Belorussia, and some others), the role of earthworms in organic waste processing and the possible further use of this processed product have been investigated. In Latvia, similar investigations are few but they are needed. A goal of our investigation was to investigate the impact of extracts made from the products of organic origin on potato (*Solanum tuberosum*) tuber yield in biological crop production system. A field experiment was established at the State Stende Cereals Breeding Institute in 2011, using an early-maturing potato variety 'Borodjanskij Rozovij' suitable for growing both in conventional and organic production systems. Tubers or plants were treated with peat elixir and earthworm biohumus extract obtained at different temperatures: +45 °C and +95 °C, and their mixtures. In total, 18 treatments and untreated control variant were included in the experiment. Tubers were treated just prior to planting, but potato plants were treated three times in season. In the first production year, the obtained potato tuber yield ranged 26.16-45.38 t ha^{-1} in treated plots, and 35.27 t ha^{-1} in untreated control plot. Data were subjected to analysis of variance. In 2011, tuber yield was significantly dependent on organic extracts applied, which increased the yield significantly ($p<0.05$) in four treatments, but in the rest of them significantly decreased if compared to control.
Key words: potatoes, yield, extracts from the products of organic origin, peat elixir, humus extract.

Introduction

Potato (*Solanum tuberosum*) is a most important farm crop species because of its wide use (Skrabule, 2003). High and good quality potato yields are produced when crop requirements for cultivation operations, proper choise of soil and plant nutrition are met. Plant supply with nutrients occurring both in soil and applied fertilizers is essential for normal growth and development of potatoes. Recently, a tendency towards soil fertility decline is observed because of unconsidered application of chemical fertilizers and pesticides. Modern technological processes, plant growing technologies have almost destroyed soil microflora which is chiefly responsible for fertility. Nowadays, researchers world-wide are aware of the consequences caused by the reduced humus content in soil resulting in increasing soil toxicity, which is not conducive to crop yield production of good quality. Restoration and remedying soil fertility allow obtaining high yields of good quality (Köpke, 2007).

Soil fertility is the capacity of a soil to supply plants with nutrients and water (Nikodemus et al., 2008). Humus is of particular importance in soil fertility supply. It forms in the process of microbial actions occurring in soil resulting in organic matter decomposition. Complete decomposition of plant residue results in humus. The more intensive soil microbial action, the more humus is formed, the more fertile is arable layer (Ковлягин, 2001).

In concern for environment in Latvia, more attention is focused on thense of different organic fertilizers. In cattle breeding farms, manure is widely used for crop production thus providing an excellent source of organic matter. However, not all the farms have access to organic fertilizers of animal origin. The problem is solved in a different way.

Peat, brown coal or lignite, coal, leonardite, sapropel, sewage sludge, earthworm biohumus or vermicompost are the most widespread sources of humic substances world-wide (Chen et al., 2004; Theunisen et al., 2010). At present, organic products are investigated world-wide to reduce the use of pesticides and mineral fertilizers in agriculture. Composting organic fertilizers, plant residue and household waste, new organic products have been created (Ndegwa and Thompson, 2001). In several countries, such as USA, Russia, India, Belorussia, and some others, the use of earthworms in organic waste processing is studied (Aira et al., 2006; Pathasarathi et al., 2007). At present, there is no information in Latvia regarding investigations on the use of organic products and their impact on crop plants, though extracts from peat and earthworm biohumus are known and available. Latvia is sufficiently rich in peat resources and is utilizing about 6% of them now. Peat contains 17-61% of humic substances (Kuršs and Stinkule, 1997).

Organic product extracts – peat elixir and earthworm biohumus - obtained at different temperatures: (+45 °C and +95 °C) as well as their mixtures were used in this investigation. A goal of this investigation was to study the impact of extracts,

which were obtained from the products of organic origin, on potato 'Borodjanskij Rozovij' yield in organic production system.

Materials and Methods

A field experiment was conducted at the State Stende Cereals Breeding Institute in 2011 to investigate the impact of extracts from the organic products on potato yield in organic crop production system. The experiment was established in three replications with randomized treatment design. Plot size was 22.4 m^2, yield recorded area – 11.2 m^2. Planting rate was 46000 potato tubers per hectare. Early maturing potato variety 'Borodjanskij Rozovij', developed in the Ukraine, was investigated. Suitability to breeding both in conventional and organic crop production systems was determinant criterion in the variety selection. This variety is well-known to potato growers in Latvia, and is successfully grown in farms.

The experiment was established on sod podzolic gleysolic soil with pH KCL 6.48 suitable for potato growing, 38 g kg^{-1} of organic matter, high available for plants (146 mg kg^{-1}) P content, and medium high (112 mg kg^{-1}) K content. The soil was low in N, S, Zn, Cu and B. Plant food elements in soil were determined at the Laboratory of Plant Mineral Nutrition of the Institute of Biology of Latvian University. The soil in the field was levelled and cultivated using chisel cultivator KR – 4. Furrows with a 0.70-m row spacing were made on 23 May using self-made furrow plough. Potatoes were hand-planted on 24 May. Spacing between tubers was 0.30 m. Potato field was harrowed two times – on 1 June and 6 June using network harrow, and loosened four times in season (on 1 June, 7 June, 14 June, and 20 June) with ripper cultivator RKT-2.

Extracts from the organic products were applied corresponding to methodology, in total for 19 treatments (Table 1).

Treatment groups used in organic crop production system were as follows:

1) absolute control – untreated with organic product extracts;
2) tuber treatment with organic product extracts prior to planting;
3) plant treatment with organic product extracts three times in season: (1) after germination, at plant height of 10 cm, (2) before flowering, and (3) after flowering;
4) tuber treatment with organic product extracts prior to planting, and plant treatment with these products three times in season on the above mentioned dates.

Potatoes were treated with:

1) two types of peat elixir obtained at temperatures of 45 °C and 95 °C;
2) two types of earthworm biohumus extract obtained at the different temperatures: 45 °C and 95 °C;
3) mixtures of two extractions (mixture ratio 1:1 – peat elixir obtained at the temperature of 95 °C mixed with humus extraction obtained at a temperature of 45 °C;
4) mixture of earthworm biohumus extracts (humus extract obtained at the temperature of 95 °C mixed with humus extract obtained at the temperature of 45 °C).

Potato tubers were treated with extracts on the day of planting - on 24 May using hand-sprayer JACTO HD 300, dose of spraying - 2.5 L t^{-1}.

Plants were treated with organic product extracts for the first time on 22 June and repeatedly on 3 July and 20 July, dose of spraying - 1.5 L ha^{-1}. Organic product extracts were sprayed with the specific bicycle-type sprayer Birchmeier Spray-Matic 10 S used for experiments. This sprayer is equiped with flat-jet nozzles, pressure - 250 kPa, consumption of working solution - 250 L ha^{-1}. Extracts were sprayed late in the evening when air temperature was not high. Prior to harvest on 17 August, potato tops were cut using haulm cutter. Yield was harvested on 6 September using two-row potato digger KTN-2V.

Analysis of variance was used to analyze the data.

Meteorological conditions in season. The weather in May at potato planting time was moderately warm, and mean monthly temperature (+10.6 °C) was corresponding to the long-term average observations (thereafter – norm). In the third last ten days of May, mean air temperature was comparatively higher (+13 °C) than in the first twenty days. At planting time, soil temperature at the depth of 0.10 m was optimal: +10 °C. Precipitation (54.7 mm) in May was 22% above the norm.

In June, the first ten days was particularly warm. Mean air temperature was +20.1 °C, however, dry conditions with no rainfall prevailed. Potato germination started on 8 June. The second ten days of this month was characterized with lower temperature (+14.8 °C) and high rainfall (40.5 mm). In the third last ten days, the weather was warm with mean air temperature +15.5 °C and low rainfall (19.1 mm). In general, weather conditions in June were favourable for germination and growth of potatoes. Monthly air temperature averaged 2.6 °C greater than norm, and rainfall exceeded the month is norm by 4.6%.

The first ten days in July was warm with the average air temperature +20 °C and low rainfall (5.3 mm). Early flowering was observed on 5 July. Sum of precipitation reached 74.1 mm in the second ten days and 85.9 mm in the third last ten days of month. In total, month is norm of precipitation was exceeded by 90% resulting in excessive moisture due

to which conditions necessary for tuber development became worse. Warm and moist weather favoured the spread of potato late blight (*Phytophtora infestans*) in the potato plantation.

In August, warm weather with mean air temperature +16.8 °C and abundant rainfall (41.0 mm) occurred in the first ten days. Warm and particularly moist weather set in the second ten days of the month when sum of precipitation was 75.6 mm and mean air temperature was +15.6 °C. Because of air deficiency caused by excessive moisture, white pustules were observed on potato tubers in places which were higher in moisture. The third last ten days of August was characterized with warm and moist weather. In general, rainfall in August reached 155 mm or was 78% above the norm. Air temperature (15.5 °C) somewhat exceeded the norm (0.8 °C). In some lower places where puddles formed, rotting of tubers was observed.

Also the first ten days of September was characterized by frequent rainfall, which made harvest difficult. Rainfall of 16 mm and mean air temperature of 13.6 °C occurred in this period.

Potato was harvested on 6 September.

In general, vegetation period was not particularly favourable for the growth and development of potatoes because of frequent rainfall, which favoured potato rotting in soil and decreased potato yield in several plots.

Results and Discussion

Potato tuber yield obtained in the experiment ranged from 26.16 t ha^{-1} to 45.38 t ha^{-1} (Table 1). Results of variance analysis suggest that the use of organic product extracts significantly ($p<0.05$) influenced tuber yield of the potato variety 'Borodjanskij Rozovij**'.**

Tuber yield of 35.27 t ha^{-1} was obtained in control plot. Tuber treatment with organic product extracts resulted in a significant yield decline in five out of six treatments (Table 1, treatments 2-7) if compared to control. Tubers treated with peat elixir alone (made at +95 °C temperature) produced a significantly higher yield – 38.57 t ha^{-1} (+ 3.3 t ha^{-1} if compared to control). Plant treatment with organic product extracts three

Table 1

Effect of organic product extractions on potato tuber yield, t ha^{-1}

Treatment	Yield of tubers	Yield compared to control
1. Control	35.27	-
2. Peat elixir (45 °C) tuber treatment	31.52	-3.75
3. Peat elixir (95 °C) tuber treatment	38.57	3.30
4. Humus extract (45 °C) tuber treatment	29.41	-5.86
5. Humus extract (95 °C) tuber treatment	27.64	-7.63
6. Peat elixir (95 °C) + Humus extract (45 °C) tuber treatment	26.74	-8.53
7. Humus extract (95 °C) + Humus extract (45 °C) tuber treatment	33.11	-2.16
8. Peat elixir (45 °C) plant treatment 3 times	42.67	7.40
9. Peat elixir (95 °C) plant treatment 3 times	26.16	-9.11
10. Humus extract (45 °C) plant treatment 3 times	28.19	-7.08
11. Humus extract (95 °C) plant treatment 3 times	26.62	-8.65
12. Peat elixir (95 °C) + Humus extract (45 °C) plant treatment 3 times	32.84	-2.43
13. Humus extract (95 °C) + Humus extract (45 °C) plant treatment 3 times	28.61	-6.66
14. Peat elixir (45 °C) tuber treatment + plant treatment 3 times	30.32	-4.95
15. Peat elixir (95 °C) tuber treatment + plant treatment 3 times	26.71	-8.56
16. Humus extract (45 °C) tuber treatment + plant treatment 3 times	26.66	-8.61
17. Humus extract (95 °C) tuber treatment	29.46	-5.81
18. Peat elixir (95 °C) + Humus extract (45 °C) tuber treatment + plant treatment 3 times	45.38	10.11
19. Humus extract (95 °C) + Humus extract (45 °C) tuber treatment + plant treatment 3 times	41.40	6.13
$RS_{0.05}$	×	2.06

times in growth period – after germination, before and after flowering – resulted in a significantly higher yield when peat elixir (made at +45 °C temperature) was used. Yield obtained in this treatment was 42.67 t ha^{-1} (+7.40 t ha^{-1} if compared to control). Tuber yields in all the rest plant treatments were significantly lower if compared to control (Table 1).

Organic product extracts used for tuber treatment prior to planting and also treatment of plants performed three times in season resulted in a significantly higher tuber yield when mixtures of both products used were applied (Table 1, treatments 18 and 19). Mixture of peat elixir and humus extract in this treatment gave a significant ($p<0.05$) yield (45.38 t ha^{-1}) increase - by 10.11 t ha^{-1} (+29%) if compared to control. Mixture of humus extract made at different temperatures, in its turn, gave tuber yield of 41.40 t ha^{-1} or 6.11 t ha^{-1} (+17%) more if compared to control.

To clarify the impact of organic product extracts on tuber yield depending on temperature at which extracts were made, these products were grouped apart from others and analysis of variance was employed.

The impact of peat elixir, which was made at the temperature of 45 °C, on potato 'Borodjanskij Rozovij' yield was evaluated. It was stated that only plant treatment three times in vegetation period provided significant yield increase if compared to control. In the rest of treatments (only tuber treatment, and tuber treatment + plant spraying three times in season), significant yield decline, if compared to control, was observed. In general, peat elixir made at a temperature of 45 °C influenced potato tuber yield significantly ($p<0.05$).

Peat elixir, obtained at the temperature of 95 °C, reduced tuber yield significantly when only plants were treated three times in vegetation season, and when tuber treatment was combined with plant treatment. However, tuber treatment prior to planting resulted in a significant yield increase (Table 1). In this case as well it can be concluded that peat elixir obtained at the temperature of 95 °C affected tuber yield significantly ($p<0.05$). It was assumed that tuber treatment with peat elixir obtained at the temperature of 95 °C had a beneficial effect on yield, but in the rest of cases the impact was negative. It may occur that the applied dosage (1.5 L ha^{-1}) is not suited to plant treatment because of too high concentration. In England, for instance, R. Atiyeh together with his colleagues has found that organic extracts in high concentration (500 mg kg^{-1} substrate) reduce plant yield (Atiyeh et al., 2000; 2001; 2002). However, research results documented in the scientific literature give evidence that organic products and their extracts have a positive effect on the growth, development and productivity of plants (Cavender et al., 2003; Gamaley et al., 2001). In some investigations, however, both negative and positive effect is noted. It is noted that high doses of vermicompost extract, or vermicompost obtained from the pig (*Sus scrofa domestica*) manure have a negative impact on the growth of tomatoes (*Solanum lycopersicum)* and cucumbers (*Cucumis sativus)*. Sorghum (*Sorghum bicolor*) growth is slowed down with non-sterilized vermicompost worked into the soil (Cavender et al., 2003). Vermicompost obtained from the horse (*Equus caballus)*, sheep (*Ovis aries)* and goat (*Capra aegagrus hircus)* manure reduces tomato infection with phytophthora (*Phytophtora infestans*), but vermicompost produced from sewage sludge does not reduce the spread of the disease, and also has a negative impact on the growth and development of plants (Szczech and Smolinska, 2001). Results from the experiments conducted in Belorussia give evidence that bio-preparations have a positive effect on the yield of potatoes. Treatment with bio-preparations resulted in a 10-30% greater yield in comparison with control treatment. Spread of rhizoctonia (*Rhizoctonia solani*) reduced by 8-35% in potato plantation in vegetation season (Влияние биопрепаратов..., s.a.).

Hence, both the results from our investigation and those of literature findings suggest that the product of organic origin – earthworm compost or vermicompost and its extracts - affect the growth of plants, their productivity and spreading of the diseases differently depending on the product origin, type of application, and doses applied.

Our experimental evidence showed that the use of humus extract obtained both at the temperature of +45 °C and +95 °C resulted in a significant ($p<0.05$) tuber yield decrease in all treatments (Table 1).

Investigations will be continued to find out why our results differ from those obtained at similar investigations conducted in other countries. Possibly, specific environmental conditions (including meteorological conditions) influenced efficiency of extracts obtained from the products of organic origin.

Conclusions

1. Peat elixir, which was made at the temperature of 45 °C, substantially affected potato tuber 'Borodjanskij Rozovij' yield; however, a significant ($p<0.05$) yield increase, in comparison with control, was obtained when it was used for plant treatment three times in vegetation period; tuber yield of 42.67 t ha^{-1} was reached in this treatment. In the rest of treatments, yields significantly decreased.
2. Peat elixir, which was made at the temperature of 95 °C, also substantially affected potato tuber 'Borodjanskij Rozovij' yield, but a significant ($p<0.05$) yield increase also in this case was obtained only with tuber treatment prior to

planting without subsequent treatment of plants (38.57 t ha^{-1}). In the rest of treatments, yields significantly decreased.

3. Humus extracts, which were made at the temperature of 45 °C and 95 °C, significantly ($p<0.05$) reduced tuber 'Borodjanski Rozovij' yields in all treatments.
4. Mixtures of organic product extracts – peat elixir (+95 °C) + humus extract (+45 °C) and humus extract (+95 °C) + humus extract (+45 °C) - contributed to a significant yield increase in treatment where both treatments were made: tubers were treated prior to planting, and plants were treated three times in vegetation season.

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COMBUSTION ABILITY OF ENERGY CROP PELLETS

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Abstract
Future perspective of the research is the production of fuel pellets from energy plant (*Phalaris arundinacea* L., *Festuca arundinacea*, etc.) biomass, because it can be better burnt in granule burners and is more environmentally friendly, if compared to the fossil mineral resources (coal, oil, gas), moreover it has low moisture content (70 – 90 g kg^{-1}) and correspondingly it produces higher combustion energy. The research covered preparation of various content tablets from reed canary grass *(Phalaris arundinacea* L.*)* variety 'Marathon' (N fertilizer rate on the N-90 kg ha^{-1}), energy wood - osier *(Salix viminalis* L) and poplar (*Populus tremula* L.) with N fertilizer norms N-0 and N-120 kg ha^{-1}, and afterwards research of the combustion ability of and ash content in these tablets. Combustion ability of reed canary grass (*Phalaris arundinacea* L.) variety 'Marathon' reached 17.48 MJ kg^{-1}. The highest average combustion ability with different pellet content was found for the fast-growing poplar both with doses of N fertilizer - 18.55 MJ kg^{-1} and without N fertilizer - 18.49 MJ kg^{-1}. Optimum content of various component pellets for biomass was a mixture of components 1/3 (reed canary grass/osier or poplar). The lowest indicators in respect to the ash content were observed for osier (*Salix viminalis* L.) - 27.9 g kg^{-1}. The best ash content indicators for a mixture of granular composition was in a mixture of components one-fourth of the reed canary grass with three parts of osier - 34.3 g kg^{-1} or with poplar - 41.8 g kg^{-1}.
Key words: *Phalaris arundinacea* L., *Salix viminalis* L., *Populus tremula* L., ash content, combustion ability, nitrogen fertilizer.

Introduction

Along with the accession of the European Union countries to the Kyoto Protocol, which aims at restriction or even complete refusal from the fossil fuels, renewable energy resources should be reasonably used, amount of greenhouse gas emissions should be reduced, and, at the same time, environmentally friendly energy production from renewable energy sources should be enhanced (Enerģētisko …, 2007).

On a global scale, resulting from the reduction of fossil raw materials the need for renewable energy resources is growing. Although production of energy from forestry products is traditional, increase in the fossil energy prices has led also to the beneficial production of energy from agricultural produce – biomass (DIRECTIVE ..., 2009).

An overall negative trend for production of fuel pellets in Europe, including the Baltic States, that should be considered is the lack of traditional raw materials (saw-dust from conifers), obtained from the wood processing industry waste (Adamovičs et al., 2009).

Studies on this subject are topical, especially the ones devoted to search of alternative biofuel raw material sources, as well as discussed in the global forums in respect to the problems of biomass use for energy needs.

Currently, biomass use comprises approximately 14% of the total global balance of energy resources (50 EJ per year from the total 406 EJ per year). For the last 15 years, heat and power generation from biomass in the EU countries has increased by 2 – 9% per year, though it gives only about 5% of the total amount of energy produced (Irbins, 2009).

Pellets are environmentally friendly renewable biofuel, and they are CO_2 neutral, because biomass raw materials, used for the production thereof, during a growth attracts the same amount of CO_2 that is released during burning of the product. As biofuel wood pellets are environmentally friendly also due to the fact that they contain such a small amount of sulphur and nitrogen that, when correctly burnt, amounts of nitrogen oxide and sulphur dioxide practically cannot be detected in gases.

Pellets are biomass dried and pressed to the size that can be easily transported, stored, efficiently burnt and may ensure fully automatic burning process. Woodchip pellets or fuel pellets are solid biofuels, chemical transformation-combustion of which results in a conversion to heat. Pellets are made from dried woodworking residues and waste: sawdust, wood shavings, barks, twigs, branches, etc. One kilogram of wood pellets contains energy equal to 0.5L of liquid fuels (Irbins, 2009).

Pellets have high thermal efficiency - the new pellet burning technologies allow burning woods with very high efficiency (88 – 92%). Pellet heating capacity is only 10% lower than that of coal. Pure and natural raw materials used for the pellet production are sawdust, woodchips, and wood. For granulation, a variety of herbaceous species, their mixtures, natural meadow grass, and reeds can be used (Adamovičs et al., 2009; Enerģētisko …, 2007; Lazdiņa et al., 2008).

In addition, the biomass dried in the pellet manufacturing process is compacted in proportion from 1:7 to 1:10 of the initial biomass volume. If compared to wood, one pellet unit volume has two times more calories than the same volume of wood.

Even if perfectly dried, firewood contains 20-30% more moisture than a pellet. As a result, due to the fact that 'air' and 'water' are not transported, the transportation of pellets is much more effective than transportation of any other biomass.

The highest quality pellets have natural wood colour, they are clean, pleasantly fragrant and smooth. Pellets for heating are produced from 100% natural material-wood shavings. Pellets are cylindrical in shape and approximately as thick as a pencil (Irbins, 2009).

Pellets have several advantages, if compared to their raw material - wood shavings - because they are more compact, more easily transportable and, the most important, they can be obtained from wood residues and therefore pellets are produced from are renewable resources. Wood combustion process is less harmful: carbon dioxide released to the atmosphere does no shift balance in nature, and does not increase the greenhouse effect, unlike black fuel oil or fossil fuels (Tardenaka and Spince, 2006).

The studies show that, if residential houses are heated with pellets instead of black fuel oil, the released CO_2 pollution is reduced by about 4.8 tons per year, but substitution of gas-fired central heating leads to the reduction of the emitted CO_2 by approximately 2.5 t per year. Transportation and storage of pellets does not pose risk for the environment. Ash generally can be used as chemical fertilizers, since chemical elements in its content usually do not exceed the specified limits (Enerģētisko …, 2007).

Increase of biomass on the Earth every year is valued as 200 billion tonnes. Although biomass energy potential is 10 times higher than possibilities of fossil fuels, still the use of biomass is very complicated. In comparison with the fossil fuel, the natural fuel has lower heat output. For fresh carved wood it is 2.9, for dry - 4.28, for cardboard - 4.39, for black fuel oil - 11.73, for coal from - 6.5 to 9, and for natural liquefied gas - 14.33 kWh kg^{-1}. Technologies for the use of biomass are constantly improved, but fossil fuel resources are running short, so in future we can expect faster price rises. For the past 15 years, the heat and power generation from biomass in the EU countries has been increasing by 2 - 9% per year, while currently biomass constitutes only about 5% of the total energy produced (Zaķe et al., 2010).

At present wood products (firewood, woodchips, and pellets) are the most popular renewable fuels in Latvia. However, also wood resource regeneration ability is limited in time and space. In many countries cultivation of various plants is recommended as an alternative to the thermal energy production (Adamovičs et al., 2009; Белосельский and Соляков, 1980).

One of the alternatives used for the biomass production is cultivation of grasses (*Phalaris arundinacea* L., *Festuca arundinacea* L., etc.). Evaluating the reed canary grass as a fuel, it should be noted that it is very suitable for the use in automatic boilers.

However, use of the reed canary grass for the heat production is characterized by major problems in burning process, such as the quantity of ashes, composition of flue gases and ash melting temperature (Boateng et al., 2006).

Production of thermal energy (from pellets) would need cultivated plants with high biomass yield, good combustibility, higher heat output and lower content of ashes. Finding the most appropriate reed canary grass composition (reed canary grass biomass together with biomass osier and poplar biomass) with the best pellet combustion ability will result in the best and the most efficient solution. And perhaps in future there will be economies specializing directly on cultivation of reed canary grass intended for heating and its use in the manufacture of pellets.

One of the most significant indicators of the fuel material quality is ash. However, larger quantities of ash are causing problems with automation of the combustion process (Tardenaka and Spince, 2006).

The most important indicator in the production of thermal energy is the quantity of ashes, which according to the standard (DIN 51731) rates up to 15 g kg^{-1}. Higher ash contents are causing problems with automation of the combustion process. In addition, heating capacity of such pellets is 600 – 1000 kJ kg^{-1} lower, for example, for the bark briquettes having ash content of 140 g kg^{-1} heating capacity comprises 16711 kJ kg^{-1} (by the standard DIN 51731, net calorific value must reach at least 17500 kJ kg^{-1}). Fuel combustion heat is a key performance indicator, which largely depends on the amount of moisture and ashes. At mean granule moisture of 67 – 78 g kg^{-1} it ranges from 18400 to 17700 kJ kg^{-1}.

New standards for the production of pellets-DIN Plus-indicate that ash content must not exceed 0.5% (Tardenaka and Spince, 2006).

Wood ashes have almost a full set of minerals required by the plants. They contain macro-elements (except nitrogen) and trace elements in the form of oxides and carbonates. Wood ash, depending on the tree specie, contain 40 – 200 g kg^{-1} of potassium, 180 – 300 g kg^{-1} of calcium, and 5 – 10 g kg^{-1} of phosphorus. The effect left by the ash is lasting for three to four years; it can be used as a fertiliser annually, on average providing 300 - 400 g per square meter. Wood ash is a valuable source of minerals and high-quality alkali, containing more than 30 nutrients (potassium, calcium, magnesium, iron, phosphorus, sulphur, and

other elements) necessary for plants, and in a very short time it can reduce the soil acidity (Irbins, 2009).

Combustion ability is the primary characteristic of a fuel that determines its effectiveness (Friedl et al., 2005) - the maximum amount of energy that can be produced during the substance combustion. The highest combustion heat is the enthalpy of complete fuel combustion, that is, to achieve the maximum degree of oxidation. The highest combustion heat is determined by burning a sample in the calorimetric ball. The highest heat of combustion includes also the heat that is released at the water vapour condensation (Obenberger and Thek, 2010).

One of the most important indicators in the heat production is the amount of ashes. In compliance with the DIN 51731 standard for the assessment of pellets and briquettes elaborated in Germany, the norm has been specified up to 1.5%. Larger quantities of ashes are causing problems with the combustion process automation (Tardenaka and Spince, 2006).

Reed canary grass *(Phalaris arundinacea L.)* according to its characteristics and composition is similar to the wood, while when burning generates more ashes. Therefore, in the production of pellets it should be mixed with the shavings and chips.

Objectives of the research:

1. to examine and assess combustion ability of and ash content in the energy plant pellets (*Phalaris arundinacea* L./ *Salix viminalis* L. and *Phalaris arundinacea* L./ *Populus tremula* L.) with various proportions of components (1/3;1/1;3/1);
2. to assess the impact of nitrogen chemical fertilizers on the quality of pellets;
3. to determine the best combinations and proportions of the energy plant components.

The aim: to determine the burning capability of energy plant biomass pellets.

Materials and Methods

Research objects: reed canary grass (*Phalaris arundinacea* L.) - (RCG), energy plants: osier (*Salix viminalis* L.) and poplar *(Populus tremula* L.*)*.

In the territory of Latvia, reed canary grass (*Phalaris arundinacea* L.) biomass is regarded as one of the alternative sources of raw materials for the production of pellets in the Baltic States and Northern Europe. This grass is characterized by its stability under local climatic conditions and high biomass yield.

Samples (reed canary grass variety '*Marathon'* at N-90 kg ha^{-1} dose of chemical fertilizer) for the study were taken at Latgale Centre of Agriculture Science (*SIA Latgales lauksaimniecības zinātnes centrs*) on 06.10.2010.

Whereas cultivated energetic plants: osier (*Salix viminalis* L.) variety '*Tordis*' ((*Salix schwerinii* × *S. viminalis*) × S. *viminalis*) and poplar *(Populus tremula* L.*)* (N chemical fertilizer dose N-0 and N-120 kg ha^{-1}) were collected in the Vežaiči Agricultural Research Institute Centre (Lithuania) on 15.10.2010.

Pellets were made of single components and two components varied as follows:

Single-component pellets:

1. *Salix viminalis* L.,
2. *Populus tremula* L.,
3. *Phalaris arundinacea* L.

Two-component pellets in following proportions:

1/3 - 1 part RCG + 3 parts *Salix viminalis* L. or *Populus tremula* L.,

1/1 - half RCG + half *Salix viminalis* L. or *Populus tremula* L.,

3/1 - 3 parts RCG + 1 part *Salix viminalis* L. or *Populus tremula* L.

In the pellet manufacturing process the energy plant biomass is chopped and ground in the laboratory mill ЭМ-3А УХЛ 4.2, and afterwards powder produced in a mill is formed into a pellet with the hand press 'IKA WERKE'.

Energy plant biomass pellets were made from 100% natural ingredients – chopped wood (*Salix viminalis* L. or *Populus tremula* L.) and chopped RCG biomass. Pellets have cylindrical shape and they are approximately as thick as a pencil.

Combustion heat of the energy plant biomass pellet samples was determined calorimetrically with a calorimetric capsule IKA C 5003 in Klaipėda University scientific -research laboratory, in compliance with the LST CEN/TS 14918:2006 standards.

Ash content in different composition samples was found out in the agricultural scientific laboratory for agronomic analyses of the University of Latvia in compliance with the ISO 5984: 2002/Cor 1: 2005 standard. For each sample three parallel experiments were carried out.

The mathematical evaluation was performed with the help of three experiments taking place simultaneously.

Results and Discussion

Evaluation of the medium calorific capacity of the sample with the ingredient proportion 1/3 (RCG/ *Populus tremula* L.) show that it was significantly higher ($p<0.05$) (19.01%), if compared to (*Salix viminalis* L.) ($RS_{0.05A}=0.08$).

Comparison of the obtained results show that significant ($p<0.05$) is ratio $RS_{0.05B}=0.14$. But considering the fertilizer effect on combustion, it appears that there are significant differences ($p<0.05$) ($RS_{0.05A}=0.08$).

The highest combustion heat indicators were observed for peat: 28.8 MJ kg^{-1}, moreover the number is very close to the coal index. The straw biomass had the highest combustion heat indicators - 17.3÷18.5 MJ kg^{-1}, wood (various tree species) combustion heat comprised 18.5÷19.5 MJ kg^{-1}, wood bark - 18.5÷19.5 MJ kg^{-1}, and canola - 18.6÷19.6 MJ kg^{-1} (Zaķe et al., 2010).

When analyzing combustion ability of different proportion reed canary grass and osier pellets, depending on the dose of nitrogen chemical fertilizers, we came to the conclusion that best combustion ability was indicated in samples without the dose of nitrogen chemical fertilizers.

Best combustion ability indicators were found in the combination 1/3 (RCG /*Populus tremula* L.) - 18.59 MJ kg^{-1} with nitrogen chemical fertilizers and 18.83 MJ kg^{-1} without the dose of nitrogen chemical fertilizers (Fig. 1).When analyzing combustion ability of different composition reed canary grass and osier pellets, depending on the dose of nitrogen chemical fertilizers, we found out that the best combustion ability was in samples without the dose of nitrogen chemical fertilizers. The best combustion ability is indicated at 1/3 (/RCG/*Salix viminalis* L.) with a nitrogen fertilizer - 18.57 MJ kg^{-1}, but without a dose of nitrogen chemical fertilizers - 18.69 MJ kg^{-1} (Fig. 2).

Consequently, nitrogen chemical fertilizers are not of vital importance, therefore for clearness the average results of combustion capacities in pellets with different compositions were displayed in Figure 3.

The research shows that 100% RCG biomass is having lower combustion ability than pellets with osier and poplar. The best reed canary grass biomass combustion ability indicators are presented on samples with ratio 1/3 (/RCG/*Salix viminalis* L.) - 18.71 MJ kg^{-1}, 1/3 (RCG/*Populus tremula* L.) - 18.63 MJ kg^{-1}.

Heating with lower ash content allows operating with economically higher quality heating systems. In Latvia amount of ashes from wood chip pellets and briquettes ranges within limits from 2.5 to 10 g kg^{-1}.

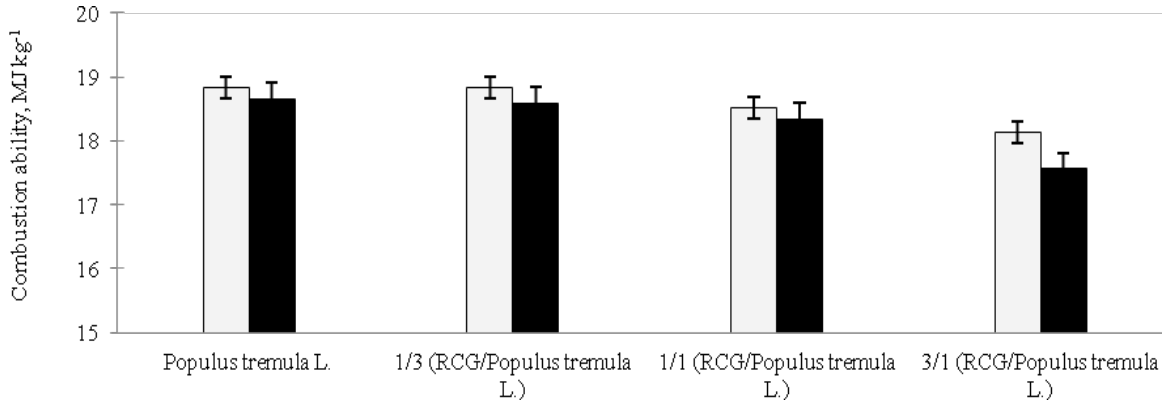

Figure 1. Combustion ability of osier and reed canary grass pellets depending on the nitrogen chemical fertilizers: □ -without mineral nitrogen fertilizer; ■ - with mineral nitrogen fertilizer.

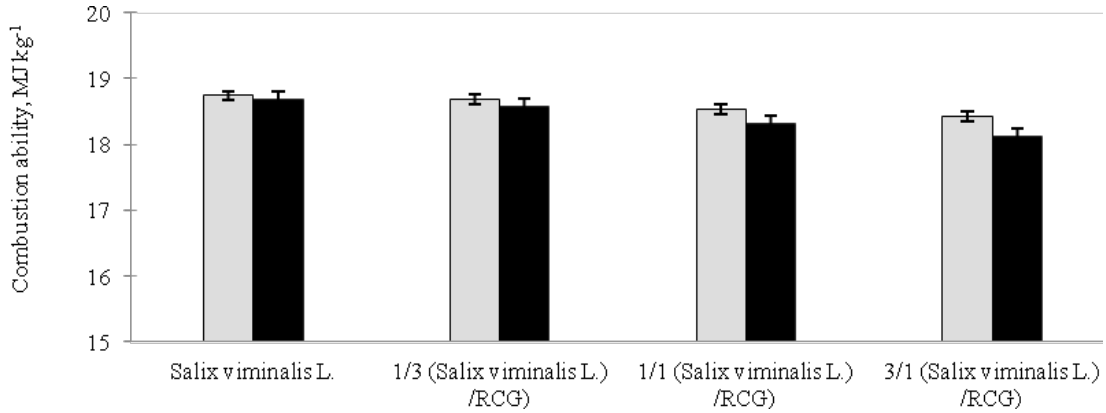

Figure 2. Combustion ability of poplar and reed canary grass pellets depending on nitrogen chemical fertilizers**:**
□ - without mineral nitrogen fertilizer; ■ - with mineral nitrogen fertilizer.

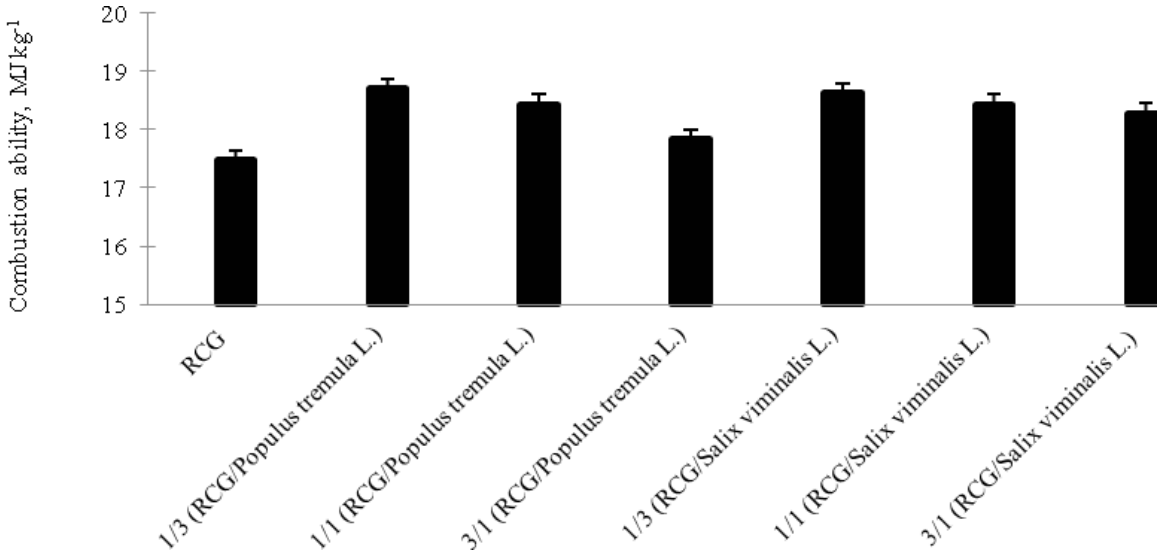

Figure 3. Combustion ability in pellets with different component proportions.

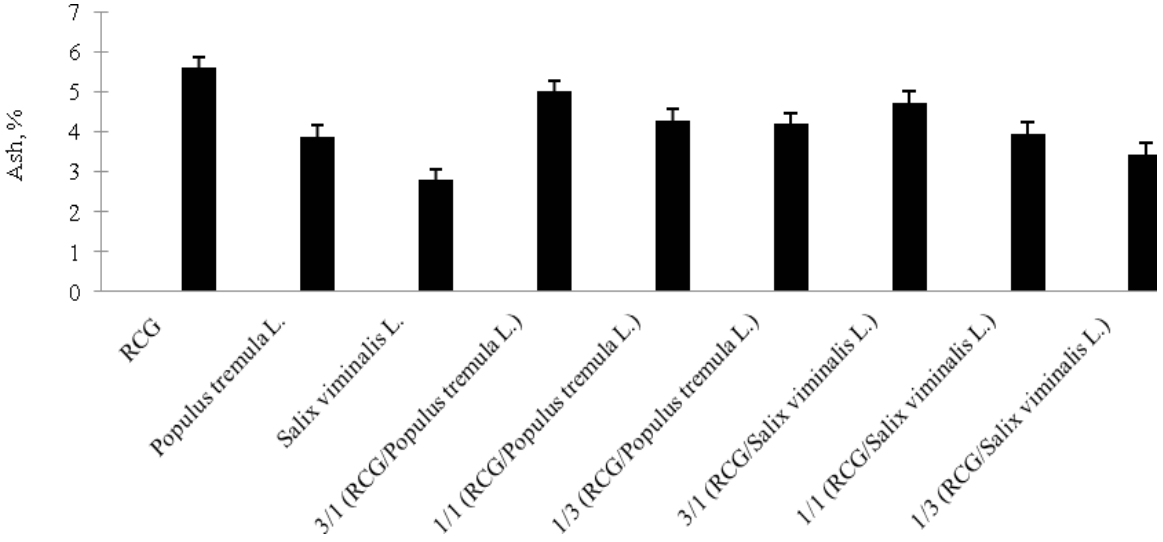

Figure 4. Ash content for different content of pellets.

In addition, the heating capacity of such pellets is 600–1000 kJ kg^{-1} lower, e. g. heating capacity for bark briquettes with ash content of 140 g kg^{-1} comprises 16.554 kJ kg^{-1} (according to the standard DIN 51731 heating capacity should reach at least 17.500 kJ kg^{-1}). Fuel combustion heat is an essential quality indicator, which largely depends on the amount of moisture and ashes. With the average pellet moisture comprising 67–78 g kg^{-1} it ranges from 18.400 to 17.700 kJ kg^{-1} (Obenberger and Thek, 2010).

Available data suggest that ash content produced by the autumn reed canary grass (*Phalaris arundinacea* L.) biomass is large - 55.9 g kg^{-1} (Fig. 4), therefore it should be appropriate to produce pellets from the reed canary grass biomass with the wood raw material supplement.

Thus one of the main problems during burning process in the heating system - the ash content - will be reduced. Therewith economic production and use of the reed canary grass in the heat production would be increased.

During the interpretation of the ash content indicators in pellets with different component proportions, we have identified that in part of the reed canary grass biomass the ash content is the lowest - respectively in the ones where indicators are proportional to the component combination 1/3 (RCG + wood) with both osier (34.3 g kg^{-1}) and poplar (41.8 g kg^{-1}).

Conclusions

Two variance analysis results showed that combustion can significantly influence ($p<0.05$) interaction of both factors (proportion and fertilizer) ($RS_{0.05AB}=0.20$), ($\eta^2=0.2026$), still the fertilizer impact was essential.

The largest average combustion ability of the reed canary grass biomass was for pellets with component ratio 1/3 (RCG/wood), reaching 18.76 MJ kg^{-1}, therefore it is appropriate to grow and use reed canary grass as an alternative energy plant cultivated for the production of biofuel pellets in Latvia.

The best component composition used for the production of pellets is the ratio 1/3 (RCG + wood). The lowest ash content was for a mixture of various granules with the average ratio 1/3 (RCG/*Salix viminal* L) - 34.9 g kg^{-1} and also 1/3 (RCG/*Populus tremula* L.) - 41.8 g kg^{-1}.

Nitrogen fertilizers do not leave significant effect on the quality of pellets and their combustion ability. Average combustion ability of pellets of different sizes without nitrogen fertilizers comprised 18.53 MJ kg^{-1}, but with nitrogen fertilizer - 18.34 MJ kg^{-1}.

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RESEARCH OF OREGANO (*ORIGANUM VULGARE* L.) INFLORESCENCE`S PARAMETERS

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Abstract
Oregano (*Origanum vulgare* L.) is one of the most popular spice and medicinal plants of untraditional horticulture in Latvia. Wild populations of this plant are too few. That is why it is necessary to cultivate oregano for keeping the biodiversity of Latvian nature. It is important to use local oregano genetic resources in agrocenosis as well as to get as rich and qualitative yield as possible. The aim of this research was to explore the parameters of oregano inflorescence in Latvia and to recommend the most productive clones for cultivation. In summer 2011, a total of 45 oregano clones from an *ex situ* collection of spice and medicinal plants of the Laboratory of Cultivated Plants and Apilogy (Jelgava, Strazdu Street 1) were analysed. Such inflorescence parameters as length and width were explored. The average length of inflorescence of all clones was 17.99 cm, and the average width was 5.74 cm. The results showed that the clone No 26 had the largest width of inflorescence (9.6 cm), but the clone No. 2 had the largest length of inflorescence (31.1 cm). Using oregano Draft Descriptor List, the inflorescence was characterized as short, medium or long. The variability between clones was significant ($p<0.05$), but between samples of each clone - non-significant ($p>0.05$). It is recommended to grow oregano clones No. 2, 5 and 26 in agrocenosis as the most productive.
Key words: length of inflorescence, width of inflorescence, plant height.

Introduction

Oregano (*Origanum vulgare* L.) is classified as a medicinal, spice and ornamental plant (Hammer and Spahillari, 2000). It is one of the most popular plants of untraditional horticulture in Latvia. Besides, it is a paramount medicinal and aromatic plant in Europe (Asdal et al., 2009). Oregano is used in production of essential oil, in medicine, perfumery, culinary, food and beverage production, aromatherapy, for attracting bees. Historically the Latvians used oregano as an ingredient in production of sausages (Spice- and..., 2006). Oregano has been utilized in folk medicine for thousands of years in the Baltic countries. In Latvia, oregano tea is traditionally used against hangovers. Oregano has also been used in sauna switches composition and its extract - for bathing.

'Herba origani' is the inflorescence (flowering top of the herb). If oregano is used in medicine, plants should be cut at the date of full flowering. If it is used as a spice, plants should be cut at the beginning of flowering or in the flower bud stage.

Wild populations of oregano in Latvia are too few (Baricevic, 2010). It is important to cultivate oregano for keeping the biodiversity of Latvian nature. The local genetic resources are adapted to concrete climate conditions and possible stress situations in a specific environment, which is why it is necessary to use Latvian oregano clones in agrocenosis (The international…, 2001). Oregano cultivation needs to get as rich and qualitative yield as possible. Oregano local genetic resources have to be explored with the aim to select the most valuable clones.

The inflorescence is the productive part of oregano plants. The evaluation of inflorescence`s parameters is the basis for oregano selection. The aim of this research was to explore the parameters of oregano inflorescence in Latvia and to recommend the most productive clones for cultivation.

Materials and Methods

Plant Material and Growing Conditions

The samples for experiment were selected from an *ex situ* collection of spice and medicinal plants (latitude: N 56°39′47″; longitude: E 23°45′13″). It is a fundamental collection in Latvia, attached to the Laboratory of Cultivated Plants and Apilogy (Jelgava, Strazdu Street 1).

There are 120 clones of 13 species of spice and medicinal plants in this collection. In 2001-2006, thanks to various international projects, the genetic resources of oregano from different places of Latvia were added to this collection. The plants had been collected from nature using the method of professor E. Muižarāja (Žukauska, 2008). The main point of this method is the initial visual division of an area into squares and zigzag passing through these squares, as well as the random gathering of accessions. The oregano collection was planted in 2008 and reconstructed in 2009.

For the year 2012, in the *ex situ* collection there are 45 clones of oregano, planted in three rows, each clone in three repetitions. The clones are in random order. In the process of selection of wild clones, the latitude and longitude was registered, the topographic description of places as well as the morphological description of plants was made. All these data are registered in the system of Nordic Gene Bank.

Soil at the trial site was strongly altered by cultivation loam with organic matter content of 2.7 g kg^{-1}, soil reaction was slightly acidic (pH KCl - 6.3), P content was 102 mg kg^{-1} and K content was 207 mg kg^{-1}. Plant care (weeding,

watering, fertilizing) was provided for the *ex situ* collection.

For experiment in summer 2011, a total of 10 stems per each of 45 oregano clones (in total 450 samples) were cut from the ground level to the tip of the plant at the beginning of flowering. The samples were dried at +26 °C temperature in a special drying cabinet with ventilation. After 3 weeks the length and the width of inflorescence were measured. According to Draft Descriptor of oregano, the inflorescence can be short (less than ½ of plant heigt), medium (½ of plant height) or long (more than ½ of plant height). That is why for better analysing of the length of inflorescence the data on plant height were recorded (Žukauska and Sivicka, 2010). Figure 1 shows some parameters of oregano: plant height and the length and width of inflorescence.

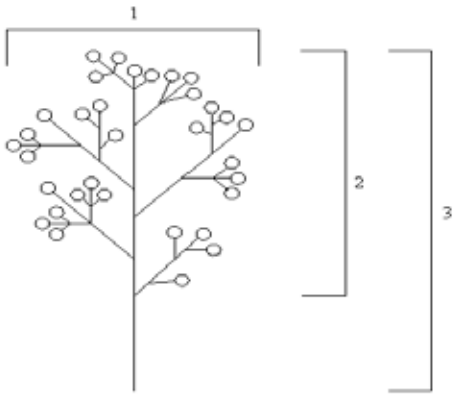

Figure 1. Oregano plant parameters:
1- width of inflorescence, 2- length of inflorescence,
3 - plant height.

Meteorological conditions

According to data of the Latvian Environment, Geology and Meteorology Centre, the average air temperature in 2011 was +7.3 °C (1.6 degrees above long-term average observations), and the quantity of rainfall was 690 mm (4% above long-term average observations). The snow cover during all winter months was thick that year. After close to normal spring, June and July were the second warmest months in the last 88 years in Latvia, but according to the data of observation stations, the rainfall in summer was more than 4-5 times above long-term average observations for this period. From 31 May to 12 June (after plant cutting), the average air temperature was 8-9 degrees above long-term average observations. The average air temperature in summer was +18 °C. In the period of plant cutting, weather conditions were stable warm, about 6-8 degrees above long-term average observations. Twelve days in the period from June to August were very hot, and the air temperature exceeded +30 °C (Weather..., 2011).

In scientific literature, it was proved that during the vegetation period the influence of air temperature from +20 to +30 °C and of the quantity of rainfall of about 600 mm on oregano yield components is positive (Caliskan et al., 2010; Rzekanowski et al., 2008). In total, in 2011 the meteorological conditions were appropriate for oregano cultivation and plant biomass creation.

Results and Discussion

The length and the width of inflorescence are the most important parameters of oregano productivity. The data about the length of inflorescence of samples from the *ex situ* collection are presented in Table 1.

The results showed that the average length of inflorescence of 2 clones was more than 30 cm (the most productive clones), it was less than 10 cm for 3 clones (the less productive clones), 13 clones had the inflorescence from 20 to 30 cm, 27 clones - from 10 to 20 cm. The average length of inflorescence of all clones was more than 15 cm (i.e. 17.99 cm) which is good result. The clone No. 2 had the largest average length of inflorescence (31.1 cm). The data statistical analysis showed that the variability between clones was significant ($p<0.05$), but between samples of each clone it was non-significant ($p>0.05$).

In 2006, oregano clones from Latvia, Lithuania, Estonia and Norway were described using Descriptor list for *Origanum vulgare* L. (Spice- and..., 2006). The average length of inflorescence for all countries was more than 15 cm (i.e. 23.2 cm in Estonia, 22.5 cm in

Table 1

Oregano length of inflorescence on average, cm

Interval	Minimum	Maximum	Clone number
Less than 10	1.5	20.5	16, 33, 39
10 - 20	2.2	48.1	1-4, 6, 7, 9, 11, 13-15, 17, 19, 20, 22, 24, 27, 29, 31, 32, 34-36, 38, 40, 41, 43, 44
20 - 30	4.0	54.0	8, 10, 12, 18, 21, 23, 25, 26, 28, 30, 37, 42, 45
More than 30	13.0	52.2	2, 5

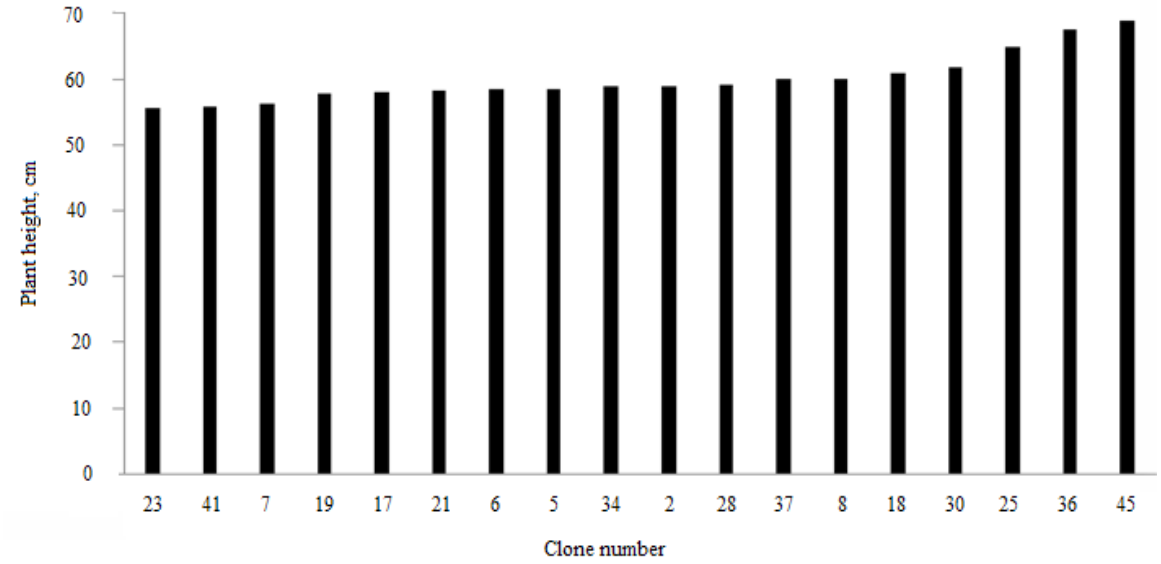

Figure 2. Plant height of most productive oregano clones on average, cm.

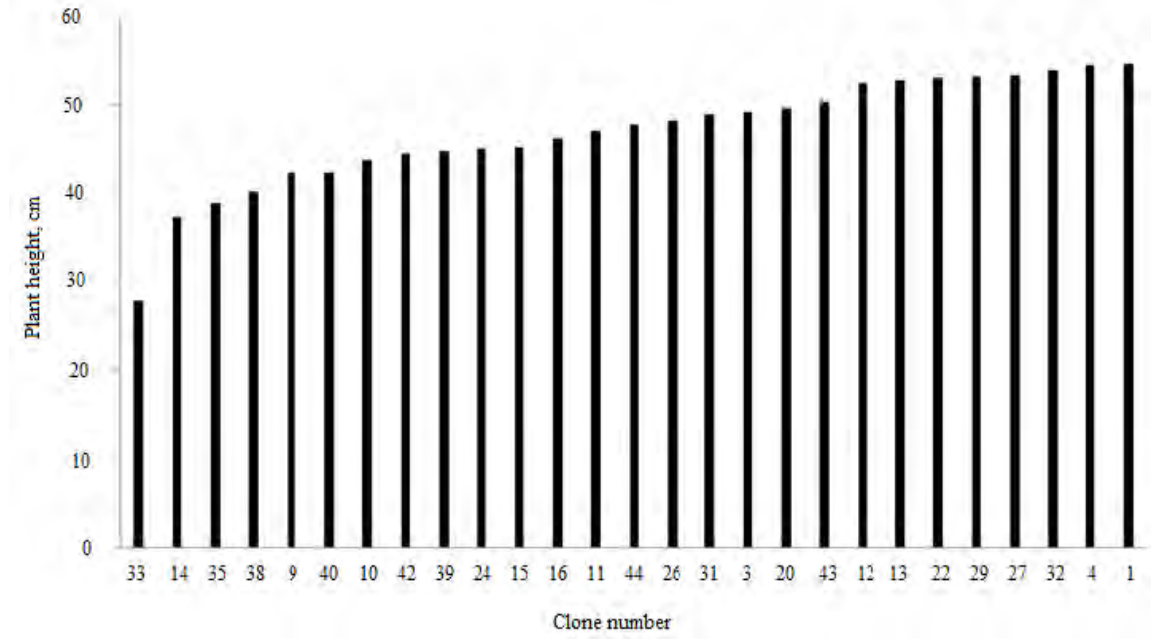

Figure 3. Plant height of less productive oregano clones on average, cm.

Latvia, 19.8 cm in Lithuania, and 15.7 cm in Norway). Besides, the result in Latvia in 2006 was better than in 2011 (17.99 cm).

For better analysis, the data about plant height were recorded, and the results showed that it varied from 27.9 to 68.9 cm. The most productive were clones with the plant height from 55 cm and higher (Figure 2).

The less productive were clones with the plant height from 27.87 cm to 55 cm (Figure 3).

The length of inflorescence was compared with the plant height. Using oregano Draft Descriptor List, it was characterized as short, medium or long (Table 2).

Table 2

The characteristics of length of inflorescence

The length of inflorescence	Clone number
Short (less than ½ of plant height)	1, 3, 4, 6-25, 27-45
Medium (½ of plant height)	None
Long (more than ½ of plant height)	2, 5, 26

The results demonstrated that only 3 clones had long inflorescence, but all the others had short inflorescence. In agrocenosis it is necessary to

cultivate plants with long inflorescence in order to obtain a higher oregano yield. Table 3 presents data about the width of inflorescence of oregano clones.

Table 3

The width of inflorescence on average, cm

Interval	Minimum	Maximum	Clone number
Less than 5	1.2	8.9	15, 16, 20, 34, 38-40, 44
5 - 7	1.0	16.2	1, 4, 6-12, 14, 15, 17-19, 21-25, 27-33, 35-37, 41-43
More than 7	1.5	14.5	2, 3, 5, 13, 26, 45

The results showed that the average width of inflorescence of 6 clones was more than 7 cm (the most productive clones), 31 clone had the width from 5 to 7 cm, but for 8 clones it was less than 5 cm (the less productive clones). The average width of inflorescence of all clones was less than 10 cm (i.e. 5.74 cm), that is bad result. The data statistical analysis revealed that variability between the clones was significant ($p<0.05$), but between samples of each clone - non-significant ($p>0.05$).

In 2006, the average width of inflorescence was 13.6 cm in Estonia and Latvia, 12.1 cm in Lithuania and 6.1 cm in Norway (Spice- and…, 2006). In 2011, the result of Latvia was worse (5.74 cm).

To sum up all the results, the most valuable were the clones Nos. 2, 5 and 26. Their productive part (inflorescence) had the most optimal parameters of length and width. It is necessary to continue this research with the aim to explore the changes in oregano inflorescence`s parameters through the years.

In future, an additional task is to study the changes in the content and composition of essential oil in the vegetation period (Nurzynska-Wierdak, 2009; Radušiene et al., 2005). To evaluate the importance of these clones as a source of essential oils with culinary and medicinal value, it is necessary to investigate more thoroughly the actual ingredients in each example (Lukas et al., 2011; Иванов, 2011). It is important to continue the research of oregano morphology and biochemistry. In this way, it will be possible to cultivate oregano clones with optimal parameters of inflorescence and with the richest biochemical content.

Conclusions

Such parameters as the length and width of inflorescence, as well as characteristics of the length of inflorescence of 45 oregano clones had been explored in Latvia. It is recommended to grow oregano clones Nos. 2, 5 and 26 in agrocenosis as the most productive ones.

Acknowledgements

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INFLUENCE OF SOIL MODIFICATION ON CHANGE IN ITS PROPERTIES AND MINERAL NUTRITION OF HIGHBUSH BLUEBERRIES

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Abstract
Blueberry cultivation is becoming more and more popular in Latvia, and several commercial plantations have been established recently. Highbush blueberries (*Vaccinium corymbosum* L.) are perennial and can grow without replanting for 50 years, therefore a choice of soil and its preparation have a great role. This article summarizes results of research carried out in commercial blueberry plantation established in 2004 on loamy Haplic Cambisol. Soil properties, especially reaction and organic matter content, initially were not suitable for blueberry cultivation, therefore deep tillage and application of soil conditioner (acid sphagnum peat) were done before planting and similar peat mulch was applied every second year. Berry yield of 4 blueberry cultivars, soil properties and the nitrogen, phosphorous and potassium content in growing plant leaves were determined. The obtained results showed that sphagnum peat is an effective material for lowering of soil pH in plant root layer (0 – 40 cm). Data about plant nutrient content in topsoil and subsoil as well as in growing plant leaves will help to develop criteria for soil fertility assessment and diagnosis of plant nutrition.
Key words: blueberries; cultivation in Latvia; soil properties**.**

Introduction

Highbush blueberries (*Vaccinium corymbosum* L.) raised interest in Latvia's farmers around 1995 when first plantations were established. At the same time, research was started together with studies of experience as the countries where this crop has already been grown commercially. Interest about the use of berries, their processing and marketing increased. Will highbush blueberry cultivation become the success story for Latvia like it happened in Chile, it depends on many circumstances but it is very feasible that there is a chance for diversification of agricultural activities (Buģina and Reševskis, 2009).

The experience obtained from other countries as well as in Latvia shows that blueberries have specific soil requirements, therefore not always it is possible to find a place where soil properties naturally are appropriate. Potential growers shall consider that scrupulous investigation of soil properties as well as good knowledge about blueberries requirements are the basic factors for successful selection of place where plantation might be established. Modification of soil properties (if possible) and site preparation are activities which should be done before blueberries are planted. Importance of that is high because the highbush blueberries are perennial plants which will stay at the same place up to 50 years without replanting. Only the above-ground parts of the bush are renewed periodically. Therefore site preparation, soil amelioration, variety and plant density selection, methods of growing, etc., are items which should be considered very carefully for establishment of a high productive and long-lasting highbush blueberry plantation. The research hypothesis was that appropriate modification of soil properties gives possibility of expanding the possible highbush blueberry cultivation area and to help its expansion in Latvia.

In Latvia, there is not many research data about the influence of soil physical and chemical properties on the development of vegetative and reproductive parts of highbush blueberries as well as about the methods suitable for modification of soil properties. Therefore the aim of the study was to investigate the influence of soil modification methods on its main properties which have importance for highbush blueberry cultivation.

Materials and Methods

Research was carried out at „Bīsnes" farm Mazozoli parish Ogre district in 2011 on the basis of a high bush blueberry plantation established in 2004. The total acreage of the plantation is 3 hectares with planting density of 2000 plants per 1 ha. Predominant soil – Base-unsaturated brown soil (Latvijas augšņu …, 2009) or Haplic Cambisol (WRB – World Reference Base for soil Resources), sandy loam, developed on stony moraine (Augšņu diagnostika ..., 2008). Original topsoil's reaction pH H_2O – 6.14, pH KCl – 5.37, and organic matter content – 25 g kg^{-1}. Before planting of bushes, radical improvement (change) of soil properties was done. In 2007, fertigation system was constructed. Experimental plots were set up on a convex slope in different relief positions. In 2004, bushes were planted in 1.65 × 3 m rows, which were deep cultivated and mixed with acid (pH KCl 3.0 ± 0.3) sphagnum peat. The same kind of peat was used every second year as a mulch to cover a 5-cm layer on soil surface between bushes. A strip, 0.7 m wide, along the bushes was kept free of vegetation during summer, but grasses *Lolium perenne* L., *Phleum pratense* L., *Festuca pratenis* H. and *Festuca rubra* L. were sown and periodically mown in inter-row space.

Research was performed in 5 plots located in different positions on slope and representing different (4) varieties of highbush blueberries. Each plot consisted of 7 fully developed bushes located in one row. Soil sampling was done in April 19 in each plot and in two depths – 0-20 and 20-40 cm. Two soil profiles in different locations were prepared and described. The following analytical methods were used for soil analysis: pH – potentiometrically in 1*M* KCl suspension; organic matter (OV) for mineral soils – using Tyurin's method, for organic soil – by dry combustion; total nitrogen – by Kjeldahl method; plant available phosphorous and potassium in mineral soil – by Egner–Riehm method, but in organic soil – total concentrationa after dry ashing of sample. Soil physical properties were measured in June and repeated in August. Undisturbed soil samples using 50 mL stainless steel cylinders were collected for bulk density, porosity and field capacity measurements.

Two times per season, plant leaf samples from each plot were collected: in July 8 from previous year shoots, and in August 5 from new shoots. Total nitrogen was tested using Kjeldahl method, but after ashing total phosphorus (calorimetrically) and potassium (flame photometry) as well as total manganese (atom absorption) concentrations were analyzed.

Results and Discussion

Soil conditions (more important for highbush blueberry growth and development), which should be considered and monitored, are those, located within the strip where plant's roots are located. According to publications (Pormale et al., 2009), the main root distribution (around 90% by mass) of blueberries is within the 1-m strip horizontally and up to the depth of 40 cm vertically. Therefore, the main focus related to the soil conditioning, tillage and fertiliser application should be performed there.

Soil investigation (morphological observations, sampling and profile descriptions) showed that soil cover despite of different topography was comparatively homogenous without contrasting inclusions. Soils could be regarded as typical mineral soils belonging to the automorphic genetic class. In the lower part of hillside, the occurrence of free carbonates starts from the depth of 100 cm. These soils correspond to the Base unsaturated brown soil group (Latvijas augšņu ..., 2009). The soils located on the upper part of the morainic hill had a slightly different parent material. Firstly, at a shallow depth (40 cm), clayey material (lens) as well as fragments of dolomite were located. Therefore occurrence of carbonates started already within the depth of 30 cm. These soils according to the Latvia Soil classification correspond to the Eroded sod-calcareous group. According to the WRB 2006 classification, both of them are Haplic Cambisols.

Soil investigations showed that the plantation of highbush blueberries was established and it operates on a typical mineral soil formed on low calcaric till. Numerous researchers suggest that such soils are not suitable for highbush blueberries due to the high pH, low organic matter content, high soil density etc. Therefore, if other soils, more suitable for blubbery cultivation, are not available on the place, some modifications in soils' initial properties might be necessary. These activities were performed using deep (40 cm) ploughing in strips where bushes were planted, acid sphagnum peat was incorporated as well as bush strips were periodically covered with peat and sawdust mulch. Using such technique, soil conditions around the blueberries were transformed and together with the irrigation and fertilizer use provided good conditions for plant growth and productivity of berries within the range of 4 to 5 tons per ha annually.

Influence on soil's physical characteristics. One of the parameters important for any crop growth is bulk density of substratum. They survey showed that soil properties in the strips had changed dramatically, growth media is not more the real soil but rather substratum. As bulk density is dependent on moisture conditions, sampling was done twice – on June 9 and August 19. Parameters of bulk density measured on June 9, when vegetation was fully renewed and moisture conditions were typical for this time of year, are shown in Figure 1. Measurements were done in both sites – in lower part of plantation (soil profile) and in the upper part (additional site). Two sampling points were chosen in every site – unchanged soil in inter-row and modified substratum in the strip of bushes. Stepwise sampling using 5 cm increments from the soil surface up to 40 cm depth was applied.

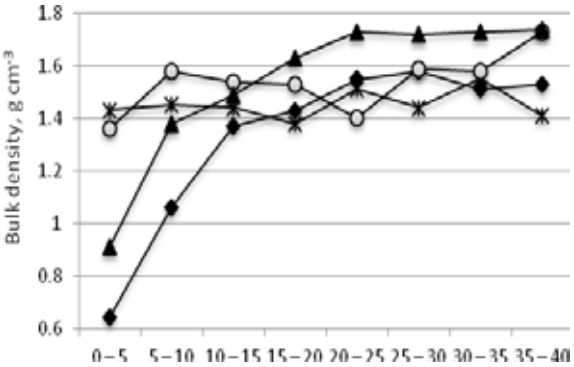

Figure 1. Soil bulk density in two experimental sites, cm:
soil profile — unchanged soil; modified
additional site — unchanged soil; modified

Bulk density in both locations for unchanged soil was very similar in all layers starting from the topsoil up to 40 cm depth. Density was rather high, about 1.50 g cm^{-3}, which points to the compaction effect – an usual occurrence in the places where soil tillage has not been used for several years. Modification effect was significant and affected the top layer up to 15 cm deep.

Field capacity is a parameter showing the volume of the water the soil is able to retain. This means that at higher field capacity soil or substratum can hold more plant available water in the periods when precipitations are absent. As highbush blueberry is a water consuming plant it is important to provide sufficient water supply all over the growing season including periods with high temperature and absence of precipitations. Influence of soil modification on field capacity of experimental sites is shown in Figure 2.

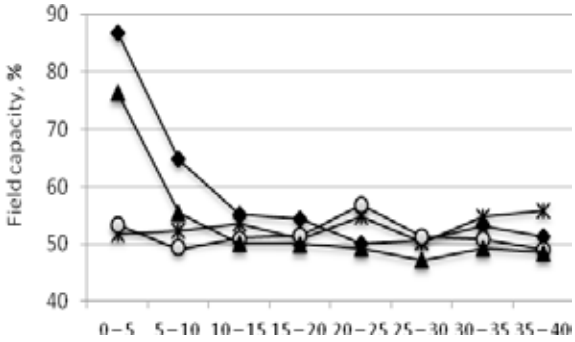

Figure 2. Field capacity depending on the depth of soil, cm:
soil profile: unchanged soil; modified
additional site: unchanged soil; modified

Field capacity has a close positive correlation with air porosity. Therefore upper layer of modified substratum has both higher field capacity (better water storage ability) and better aeration. The last one is important not only for providing good air exchange between soil and atmosphere but also for fast excess water infiltration during rainstorm or snow melting. In situation when drip irrigation system is operating and water supply can be adjusted according to the actual needs physical properties of substratum could be evaluated as adequate for good development of highbush blueberries.

Agrochemical properties of soil. Many researchers are discussing and pointing out the importance of soil (substratum) reaction for highbush blueberries cultivation. It is considered that this crop requires moderately acid growth media – pH KCl < 5.5. In many cases this is the limiting factor for establishment of plantations in mineral soils which are non-acid naturally or after liming. USA and Canada are countries where highbush blueberries are cultivated very widely, therefore advanced experience is accumulated here. Researchers from these countries suggest that for highbush blueberries the optimal soil reaction pH KCl is 4.5 – 4.8. In a very low pH KCl environment (lower than 3.4), plants are suffering, and if reaction becomes less acid (pH KCl 3.4 – 3.8), plants gradually recover but productivity (yield of berries) is very low. Therefore it is considered that pH KCl 3.8 is the lowest possible (reasonable) level for blueberry production (Haynes, Swift, 1985; Hancock, 2009). The similar research and findings were obtained in Latvia as well. A. Ripa reported that for highbush blueberry growth in Latvia, an optimal soil reaction is pH KCl 3.8 – 4.8 (excluding rabbiteye blueberries that require a higher pH KCl level – around 5.8) (Ripa, 1992). Similar recommendations are provided also by Polish researcher K. Smolarz (2009) who is also involved in the breeding of new varieties of blueberries. The high reaction of soil (pH KCl > 5.2) reduces mobility of plant available micronutrients Fe, Zn and Cu and relative deficient for blueberries could be a case (Haynes and Swift, 1985).

In 2011, the soil reaction in experimental plots for the upper 0 – 40 cm soil layer was acid (pH KCl 2.95 – 5.35). Topsoil was comparatively more acid than subsoil due to the periodical application of mulch – acid (pH KCl 3.0 ± 0.3) sphagnum peat plus sawdust. Therefore for variety 'Bluecrop' at the depth of 0 – 20 cm, soil reaction reached even pH KCl 2.95, which actually can be considered inadequate or too acid. In such situation calcium uptake for plants is very limited. In very acid environment calcium is simply lacking (absolute deficit). If the blueberries are receiving water soluble calcium without the raising of reaction, e.g. by fertilisation, the negative effect of low reaction could be minimised.

Other parameters of soil agrochemical properties are shown in Table 1. Experimental plots were located at the different positions in the landscape. Therefore plots located on the upper part of landscape are marked with P, and with N – plots which were located on the lower part of hillsides. Soil sampling was done directly in the strips around bushes approximately in the zone where plant roots are located.

Organic matter (OM) content in topsoil varies significantly due to the application of mulch. Soil mulching was done periodically every second year. Therefore some experimental plots received fresh mulch, but some plots – from previous year. The difference between topsoil and subsoil in terms of organic matter and plant nutrient content was significant. Nutrient accumulation in the upper part of substratum are provided by from the applied fertilisers.

Table 1

Soil agrochemical properties

Position on landscape and variety	0 – 20 cm topsoil				20 – 40 cm subsoil			
	OM, g kg^{-1}	N, g kg^{-1}	P, mg kg^{-1}	K, mg kg^{-1}	OM, g kg^{-1}	N, g kg^{-1}	P, mg kg^{-1}	K, mg kg^{-1}
1. P 'Bluecrop'	296.8	2.73	584	498	25.8	0.98	85	64
2. P 'Bluecrop'	38.2	1.43	116	106	13.2	0.67	31	65
3. P 'Northland'	43.9	7.96	124	80	22.6	0.88	74	75
4. N 'Duke'	214.3	3.72	536	398	26.3	1.09	140	105
5. N 'Patriot'	167.1	2.72	266	224	23.0	0.91	112	74

This layer could be regarded as a nutrient rich and probably stimulates development of blueberry roots close to the soil surface. But generally both the upper and lower part of substratum might be evaluated as containing an adequate amount of plant nutrients if the reference is made related to the other small berry bush trees grown in Latvia.

Evaluation of plant nutrient concentrations in soil (substratum) for highbush blueberries is rather complicated. Difficulties arise because of the small amount of research data which are obtained using one standardized analytical method and experiments performed in compatible conditions. Only then some correlation criteria could be developed. Currently, at least two kinds of analytical procedures for soil fertility evaluation are used by highbush blueberry growers in Latvia. The State Plant Protection Service offers the same analytical methods as for field crops (also used in this research) but they lack any interpretation criteria relevant for highbush blueberries. Officially, all growers who are involved in the Integrated horticulture scheme in Latvia should test the soil in their plantations using these methods periodically. Another laboratory which offers the soil testing for horticultural crops uses completely different analytical methods (soil extraction with 1*M* HCl for determination of all nutrients) and data are not compatible with data, obtained by methods recommended by Integrated horticulture scheme. But, at the same time, research data for interpretation of analytical results are more advanced for 1*M* HCl procedure (Nollendorfs et al., 2007). Therefore it is an open question to decide about the common scheme for soil fertility evaluation and to accumulate a sufficient amount of research data to be able to develop criteria for their assessment. Therefore any data and their interpretation are important.

One of essential plant nutrients having importance for highbush blueberry growth is nitrogen (Smolarz, 2009). Therefore, in this study, the attempt was made to evaluate which soil properties are influencing total nitrogen concentration in substratum. A method of multiple correlation was performed (Figure 3).

In the topsoil rich with organic matter mulch mineralization gradually occurs and mineral nitrogen in substratum releases. This is also reflected in the obtained coefficients of correlation. In general, it can be evaluated positively because according to other researchers (Ripa, 1992; Nolendorfs, 2004), for productive growth of highbush blueberries the content of organic matter in substratum should be more than 70 g kg^{-1}. The high content of nitrogen not only stimulates the blueberry growth but also promotes mineralisation of mulch. To keep the soil physical properties (bulk density, porosity etc.) on an adequate level, the fresh material should be applied periodically.

Plant nutrient diagnosis. One of the methods to evaluate the crop nutrition status is to analyse parts of a growing plant. Compared with soil (substratum) analysis this method has some advantages. Plant nutrient concentration in growing plant tissues shows that the crop was (or was not) able to absorb the ions from the soil solution and they were already in the plant ready for all metabolic processes. Chemical procedure used to find this concentration is not important and compatibility problems are more evident. The only things which should be harmonised are selection of the plant part used for analysis and preparation of the sample. Therefore comparison of the obtained data with data from the literature is more possible and applicable. In Table 2, data obtained are presented. Two types of plant leaves were taken for analysis: leaves from the new shots produced in 2011, and leaves from shots which the plants produced a year before. The results were compared with data obtained by Nolendorfs et al. (2007). According to their research, the optimal concentration of plant nutrients in leaves varied within 17 – 20 g kg^{-1} of N, 2.0 – 3.0 g kg^{-1} of P, and 4.5 – 7.0 g kg^{-1} of K. If this criteria is considered as reference point then plant nutrient concentration in plants grown in experimental plots should be evaluated as inadequate.

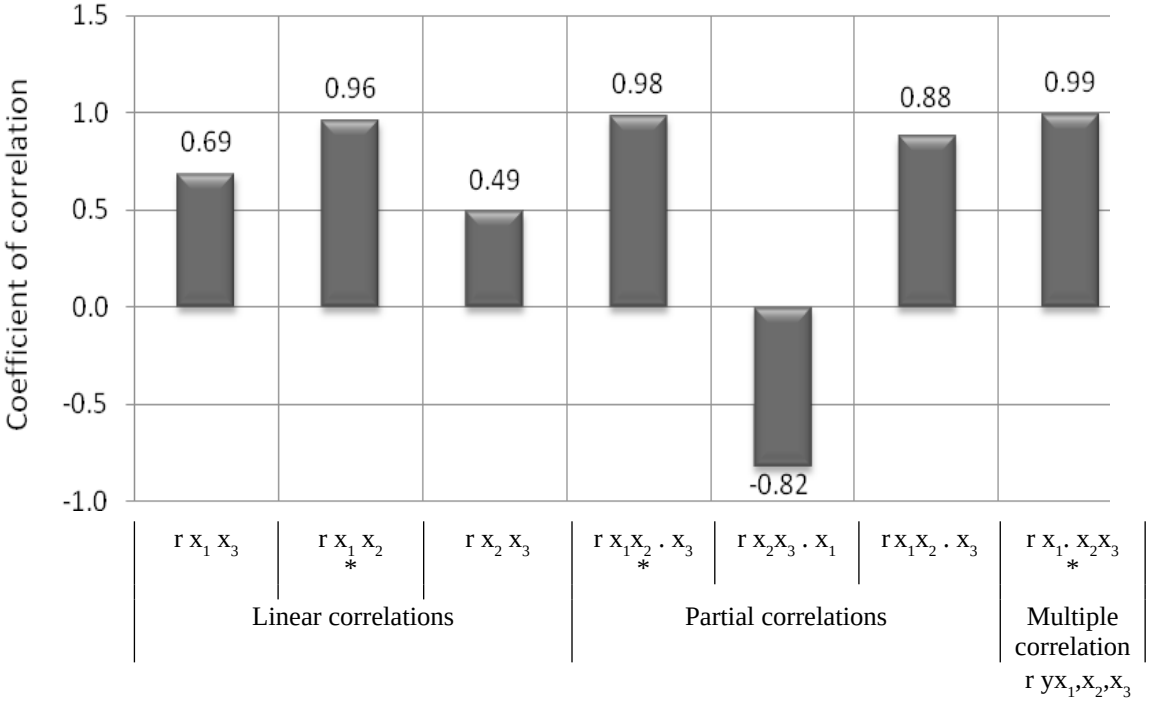

Figure 3. Evaluation of nitrogen concentration (y) and possible influencing factors (x_1, x_2, x_3) for subsoil, r yx_1,x_2,x_3.

Terms: * correlation is significant ($p<0.05$)
r yx – correlation in absolute values
x_1 – N concentration in subsoil, g kg^{-1}
x_2 – OM concentration in subsoil, g kg^{-1}
x_3 – K concentration in subsoil, mg kg^{-1}

Table 2

Plant nutrient concentration in dry matter of leaves, g kg^{-1}

Position on landscape, and variety	New shoots			Previous year shoots		
	N	P	K	N	P	K
1. P 'Bluecrop'	10.28	1.49	3.99	9.64	0.99	3.69
2. P 'Bluecrop'	11.05	1.51	3.87	11.33	1.02	4.06
3. P 'Northland'	9.78	1.20	2.83	8.59	0.80	2.21
4. N 'Duke'	10.33	1.47	3.34	9.78	1.08	3.62
5. N 'Patriot'	9.94	1.19	3.76	9.95	1.63	3.85

On the other hand, full scheme of blueberry fertilisation was done in 2011, including all necessary materials and applications. This scheme is already successfully maintained in the plantation for several years, therefore it is difficult to agree that plant nutrient inputs were too small. Probably some more literature should be read and field research should be done to find the correct criteria relevant for a similar soil and climate situation.

Manganese is one of chemical elements raising interest in highbush blueberry growers. Low concentration of manganese could be the limiting factor for high productivity of blueberries, but too high concentration can be harmful. Therefore concentration of manganese in plant leaves was performed (Table 3).

Table 3

Manganese (Mn) concentration in dry matter of leaves, mg kg^{-1}

Position on landscape, and variety	Previous year shoots	New shoots
1. P 'Bluecrop'	163	132
2. P 'Bluecrop'	240	154
3. P 'Northland'	203	229
4. N 'Duke'	271	190
5. N 'Patriot'	311	303

Generally, in both types of leaves samples, the concentration of manganese was high. It is reported that the optimal level of manganese in leaves for blueberry growth is 40 – 100 mg kg^{-1} (Nolendorfs et al., 2007). Also data are found that toxic influence of manganese for blueberries occurs when Mn concentration in leaves exceeds 450 mg kg^{-1}. It is not possible decrease the total Mn concentration in the soil. Better soil aeration can only stimulate oxidation of Mn compounds and make them less available for plants. Another way how to reduce Mn availability is to increase the concentration of plant available iron, copper, zinc and molybdenum in soil as well as to provide the plants sufficiently with water soluble calcium in both ways – through roots as well as through foliage by means of foliar application (Pormale et al., 2009). Additional aspect is an adequate nitrogen supply which stimulates growth and development of foliage part of bushes and 'dilutes' the manganese already absorbed by plants. The use of complex fertilisers containing more than 0.02% Mn in such a situation should be avoided (Nollendorfs, 2004).

Conclusions

The use of sphagnum peat significantly changed physical properties of soil. Soil bulk density was decreased but soil porosity and aeration increased. It positively affected growth of highbush blueberries because stimulation of air exchanges, increase in water infiltration and, as a result, facilitation of microbiological processes provide better environment for crop development. Rapid drainage of excess water was compensated by soil surface irrigation simultaneously with plant nutrient application. Irrigation installation is an important component for modified soil because the use of peat decreases soil capillary porosity and subsequently water supply from the deepest layers. The use of acid (pH KCl 3.0 ±0.3) sphagnum peat is an efficient way to decrease soil reaction in blueberry root zone as well as to raise the organic matter content in soil and partly to serve as a tool for depression of weeds growth between bushes. In many cases, soil is not a limiting factor for establishment of highbush blueberry plantations in Latvia. It is possible to select the methods for modification of its properties in the rows of plants, and plantations could be established also in typical mineral soils formed on low calcareous moraine. A few researches have been done in Latvia concerning optimal nutrient concentration in soil and in vegetative parts of highbush blueberries. The research in this direction is topical, because these data has high importance for fertilization planning.

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INCIDENCE OF POSTHARVEST ROT OF CRANBERRY (*VACCINIUM MACROCARPON* AIT.) IN LATVIA

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Abstract
The American cranberry (*Vaccinium macrocarpon* Ait.) is a perspective and marketable crop both in Latvia and foreign markets, but berries are affected by rot in storage. The aim of the study was to detect the incidence level of cranberry fruit rot at the beginning of storage period in different places in Latvia. In 2007 - 2011, two hundred sound berries (out of 1000) were randomly collected by hand along a diagonal from five different cranberry plantations from locations all over Latvia. Berries were kept in plastic bags for a month and refrigerated at +7 °C. At the end of November, berries were sorted and rotten berries were separated from the sound ones. Over the period of 2007 - 2011, the incidence of storage rot reached 12 - 15% at the end of November, with an upwards trend observed every year, but the hot and rainy summer of 2010 significantly reduced the quality of fruit in storage, peaking on the average 33% of decayed berries. The incidence of fruit rot varied among the inspected cranberry plantations, but a tendency was observed that older plantations produced more rotting fruit and incidence of the disease was 12 - 50% after a month's storage in the oldest plantation. The application of fungicides during the vegetation season did not affect development of post-harvest rot. Storage rot was a problem in the cranberry samples from all inspected plantations in Latvia, and in future the incidence of fruit rot is expected to increase.
Key words: cranberry, storage rot, shelf-life.

Introduction

In Latvia, the American cranberry (*Vaccinium macrocarpon* Ait.) is a perspective and marketable crop. The same as in North America, the climate in Latvia is appropriate for cranberry growing. American cranberry has been cultivated for fifteen years in Latvia and alongside with plantations enlarging every year, the spreading of diseases, including fruit rot, turns into one of the most important risk factors in cranberry cultivation. In Latvia the first investigations of fruit rot were started in 2006 (Vilka et al., 2009b). Cranberry rot is a disease complex caused by several pathogens in Latvia: *Botrytis cinerea, Allantophomopsis cytisporea, Fusiccocum putrefaciens, Phomopsis vaccinii, Coleophoma empetri, Phyllosticta elongata, Physalospora vaccinii, Pestalotia vaccinii, Gloeosporium minus,* and *Discosia artocreas* (Vilka et al., 2009a).

The harvested cranberry fruit is used in three ways: frozen, processed into juice or other products, and stored as fresh fruit. At storage the storing expenses grow and quality of fruit gets reduced, but it offers an opportunity to use fresh fruit for some months after harvest. Fresh fruit account for about 10% of total North American sales and only some growers store berries until November or December (Charles, 2003; Olatinwo et al., 2004).

The fruit rot is one of the most important problems in cranberry plantations in North America, because it reduces the quality of yield. If in North America cranberries were cultivated without fungicide application, losses due to field rot could reach 50 - 100% (Cranberry diseases, 1995; Johnson - Cicales et al., 2009). Even with application of fungicides, incidence of fruit rot in New Jersey and Massachusetts has ranged from less than 1% up to 15% (Stiles and Oudemans, 1999; Polashock et al., 2009). A lower incidence level of fruit rot was observed in Wisconsin where field rot reached 10 - 40% and fungicides usually were not used to control fruit rot (McManus et al., 2003). The incidence of pre-harvest and post-harvest fruit rot in most of cranberry growing regions is very high, therefore storage time of fruit is short (Charles, 2003).

Incidence of berry rot during storage has been investigated by researchers in different places and different periods of time. In New Jersey in 1976 - 1978, after 4 weeks in storage, incidence of fruit rot was 4.3% if berries were water-harvested (wet method) and immediately removed from field, but for berries harvested by hand (dry method) incidence level reached only 3.2% (Stretch and Ceponis, 1983). In recent years, R.O. Olatinwo et al (Olatinwo et al., 2004) have obtained different results: in Michigan after a month in storage (5 °C), incidence of fruit rot of 'Stevens' varied between 5 - 85% (in 2000) and 3 - 40% (in 2001) in different growing sites. Development of berry rot is influenced by temperature during storage. In Wisconsin, in 1999 – 2000, when berries were harvested by hand and stored at 3.5 °C, after 7.5 weeks the incidence of fruit rot was 16 - 52%, but after storing berries at 6 °C for 6 weeks, the incidence was only 4-6% (Blodgett et al., 2002). The incidence of storage rot depends on water immersion time at harvest. Increased water immersion time (up to 24 hours) has significantly increased the fungal rots (about 6.9%). Hand-picking of berries is an expensive method, but it decreases the possibility that berries might become infected by fungi (Stretch and Ceponis, 1983).

Researchers from Wisconsin have proved the importance of ripening fruit at harvest for prolonging shelf-life in storage. The red berries have a thicker and stronger cuticle and are better covered with wax formation than unripe (white) berries. During wet harvest, the calyx of the red berries may retard the entry of fungi better, so it is important for severity of storage rot. The ripening berries are unsusceptible to infection with causal agent of fruit rot, so these berries will store better (Ozgen et al., 2002).

Incidence of field rot and storage rot can vary among years and locations. Scientists from North America have investigated fruit rot of cranberries for many years, but the reason for variances in the rot incidence level is still unknown (Charles, 2003).

For the competition with American imported berries, Latvian growers have to store berries by fresh (without frozen), how long is possible and profitable. Therefore the present investigation is necessary to detect incidence level of cranberry fruit rot after a month's storage in conditions of the customer's refrigerator.

The study aim was to detect the incidence level of cranberry fruit rot at the beginning of storage in different places in Latvia.

Materials and Methods

Five cranberry plantations from different locations (Talsi, Babite, Alsunga, Rucava and Ape municipality) in Latvia were inspected at harvest (Table). The samples were taken from cultivar 'Stevens'. The trials were carried out in 2007, 2009, 2010, and 2011.

Berries were harvested from 10 th to 17 th of October in 2007, 19 th - 27 th of October in 2009, 4 th - 10 th of October in 2010, and 1 st - 06 th of October in 2011 depending on the ripening time at five different cranberry plantations from all over Latvia.

Two hundred sound berries (out of the total of 1000) were randomly collected by hand along a diagonal in each plantation.

Berries were kept in plastic bags for a month and refrigerated at +7 °C (Forney, 2009). At the end of November (time depended on harvesting), berries were sorted and rotten berries were separated from the

Table

Description of inspected cranberry plantations

Cranberry growing site	Structure of bog	Year of plantation set up	Acreage of plantation (2011), ha	Fungicide use	Provenance of seedling
1. Rucava municipality, farm Purva dzērvenīte	established after peat extraction	1998	13	used since 2009*	USA, Mena
2. Alsunga municipality, farm Stīgas	established after peat extraction	1995	1.5	not used	Belarus (previously USA, Wisconsin)
3. Ape municipality, farm Lienama - Alūksne	established after peat extraction	1997	20	used**	USA, Wisconsin
4. Talsi municipality, farm Piesaule	established after peat extraction	1998	11	not used	National Botanic Garden of Latvia in Salaspils (previously USA)
5. Babīte municipality, farm Strēlnieki	Established on a field (1.5-m layer with sawdust, 30-cm upper layer with peat)	2002	3	not used	Belarus (previously USA, Wisconsin)

* application of copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) at the dosage of 4 kg ha^{-1} was carried out at the beginning of bloom (10%) in 2009; copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) at the dosage of 4 kg ha^{-1}, was applied at the beginning of bloom (10%), and mixture (1 kg ha^{-1}) of pyraclostrobin, 67 g kg^{-1}, and boscalid, 267 g kg^{-1},- after bloom in 2010 and 2011.

** application of copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) at the dosage of 3.5 kg ha^{-1} was carried out at bud elongation in 2007; in 2009, fungicides were not used; copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) at the dosage of 3.5 kg ha^{-1} was applied at bud break in 2010; copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) at the dosage of 3.5 kg ha^{-1}- at bud elongation in 2011.

sound ones. The rotten berries were kept and used for identification of the causal agent of the storage rot and for further investigations.

The surface of rotted berries was disinfested in 95% ethanol solution (761 g L^{-1}) for 1 - 2 minutes. The piece of berries was put down onto potato-dextrose agar (PDA) for causal agent detection. Plates were incubated at 20 - 25 °C for 3 to 4 weeks (Waller et al., 1998; McManus et al., 2003; Olatinwo et al., 2004). Fungi were identified directly on the isolation plates by comparing morphological characteristics of the spores and spore bearing structures with descriptions in the literature (Cranberry diseases, 1995).

Analysis of the weather conditions in the inspected years showed that in 2011 an excess precipitation level persisted for several months (May to September) in comparison with other years. Weather conditions of the vegetation seasons in 2007, 2009 and 2011 were usual. A considerably higher amount of precipitation was observed in August 2010, when precipitation level exceeded 190% of the average over longterm observations, reaching 147 mm. Specifically high precipitation level was observed in Rucava municipality, where a downpour produced 82 mm (93% from the norm of the month).

The results of the trials were statistically processed with MS Excel for windows and SPSS package. The obtained data were analyzed using descriptive statistics, and significance ($p<0.05$) of the differences between the samples was assessed using ANOVA Single factor.

Results and Discussion

In Latvia, for marketing, berries are stored at the temperature of 2-4 °C in a refrigerator, whereas most of the customers berries store at +7 °C in fridge for consumption of fresh fruit. The incidence of fruit rot in the first month of storage varied by years of inspection. The overall incidence level varied from 35 to 57% at the end of November (Figure 1). Cranberry rot is a disease complex that could can be coused by several pathogens. During our investigations, *Fusiccocum putrefaciens, Coleophoma empetri, Phomopsis vaccinii, Botrytis cinerea, Allantophomopsis cytisporea, Phyllosticta elongate* and *Physalospora vaccinii* were isolated from the rotted berries in storage.

The harvesting time did not influence the level of berry rot by years (differences were not statistically significant), except in 2010, when a substantially higher incidence of the disease was established, reaching 33% ($p = 0.008$).

Nevertheless, a slight increase in the tendency to rot over the trial period was observed. At the end of November 2007 the disease incidence was 12%, and in different cranberry plantations the rot level ranged from 8 to 16% (Fig. 1). After two years (in 2009), the average amount of decayed berries had slightly increased and differences among the incidence level (3 - 32%) in different farms had also increased. The high level of disease incidence in 2010 probably was caused by the weather conditions. In August, when the majority of berries go through an active growing phase, raindrops can easy wound their skin therefore weather is favourable for the development of the causal agent of the fruit rot.

The incidence of cranberry postharvest diseases in Latvia is less to compare with North America. Probably growing conditions are different. Density of upright shoots affects incidence level of fruit rot. Farmers in North America have target to get a high yield and good incomes, therefore berries were taken away unripe and it is the reason for high level of fruit rot in storage in North America.

Usually, harvesting time of cranberries in Latvia is in the middle of October, but in USA the berries are harvested in September or in early October (Olatinwo et al., 2004).

Influence of plantations in Latvia of postharvest diseases is shown in Figure 2. In general, significant differences between the incidence of fruit rot in different cranberry plantations were not observed ($p = 0.643$). In 2010, in comparison with other farms, the tendency for higher incidence of fruit rot (43 - 57%)

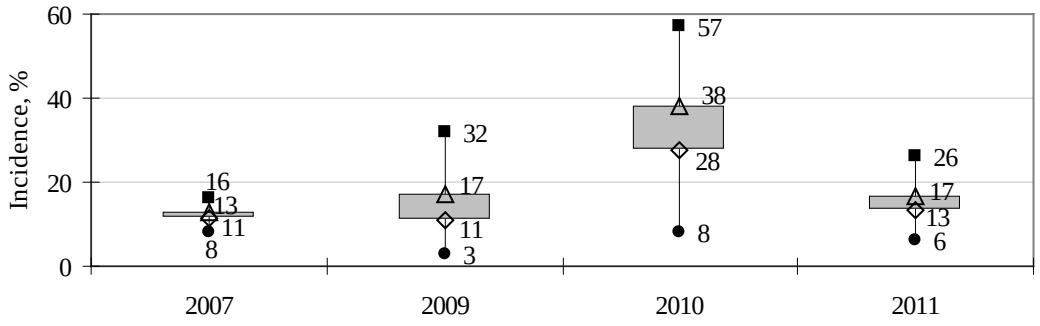

Figure 1. The incidence of fruit rot in storage at the end of November by years.
(◇ $\bar{x}$ - Sx; ■ max.; ● min.; △ $\bar{x}$ + Sx; Sx - standard error).

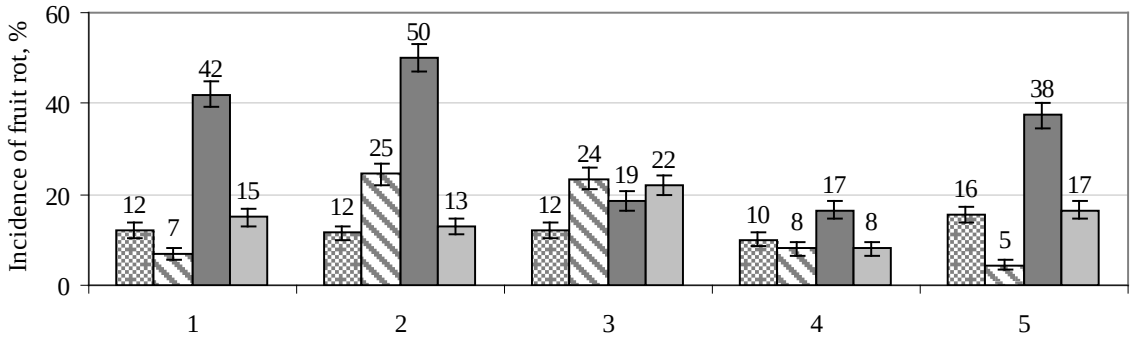

Figure 2. The incidence of fruit rot after a month's (end of November) storage depending on growing site, 2007 – 2011, % (2007; 2009; 2010; 2011; 1- Rucava municipality; 2- Alsunga municipality; 3- Ape municipality; 4-Talsi municipality; 5- Babīte municipality).

was observed in the farm in Alsunga municipality (Number 2) having the oldest cranberry plantations (Fig. 2). The younger farm (in Babīte municipality) will have had to be opposite results. The amount of decayed berries was highly variable by years: from 5% in 2009 to 38% in 2010. The lowest prevalence of what at the end of November was observed in plantations of Talsi municipality (Number 4) reaching an average of 11% in the course of the trial years. It is difficult to explain the prevalence differences among the farms, because the disease development depended on varying, usually unknown factors. Similar results have been obtained in North America. The age of a cranberry plantation in North America can reach up to 100 years, and most of them are use fungicides for fruit rot control, but the level of fruit rot after a month's storage in different growing sites varies (5 - 85% in 2000) (Olatinwo et al., 2004).

The most of inspected cranberry plantations' growers were not applied fungicides for fruit rot control, but only two growers of them take for necessary to use fungicides. In cranberry plantations of Rucava municipality fungicides were applied starting from 2009. For comparison, in 2007, the incidence of fruit rot after a month's storage was 12% in Rucava municipality, but in 2009, after applying copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) at the dosage of 4 kg ha^{-1} at beginning of bloom (10%), incidence reached 7 %.

Although the fungicides were used the next years as well, incidence of fruit rot did not decrease any more. In cranberry plantations of Ape municipality fungicides were used for many years, except for 2009, when copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) was not applied and the amount of decayed berries in storage increased for times compared to the years 2007 and 2009. Although in the most of inspected plantations weather conditions increased the incidence of fruit rot in storage, but only berries from Ape municipality stored better. Probably copper hydroxide (770 g kg^{-1}; copper equivalent: 500 g kg^{-1}) applied (dosage 3.5 kg ha^{-1}) at bud break reduced incidence of fruit rot in storage 2010. American cranberry growers and researchers recommend copper hydroxide better for upright dieback control (Cranberry diseases, 1995). Although in cranberry plantations of Rucava (Number 1) and Ape (Number 3) municipalities fungicides were applied (n=6), the incidence of fruit rot was at the same level as in plantations where fungicides were not used (Fig. 3). It means that application of fungicides was not effective to reduce the incidence of fruit rot in storage. Researcher S.N. Jeffers (Jeffers, 1991) from North America has verified that fungicides application in bloom did not affect incidence of fruit rot in storage, for the severest infection by fungi occurred at harvest. Many times of applications with fungicides in bloom affect incidence level of fruit rot on field. That is the most important problem in North America (Cranberry diseases, 1995; Stiles and Oudemans, 1999; Charles, 2003; Johnson - Cicales et al., 2009; Polashock et al., 2009).

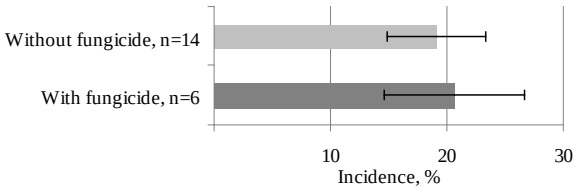

Figure 3. Incidence of fruit rot after a month's storage depending on fungicide application in Latvia.

In general, the average incidence level of fruit rot in storage in Latvia is lower than in North America, wich means that local berries are competitive on local and foreign markets. Customers have to freeze the fresh cranberries as far as possible after berries purchase.

Conclusions

1. In 2007 – 2011, the incidence of fruit rot after a month's storage reached 12 - 15% and, an upwards trend was observed with every successive year.
2. The high level of precipitation (compared to the norm) during the summer and autumn of 2010, significantly reduced the quality of berries in storage, reaching an average of 33% of decayed berries
3. The incidence of fruit rot among the inspected cranberry plantations was highly variable and trend was observed that older plantations had higher levels of fruit rot, for example, the oldest plantation in Alsunga municipality, had 12 - 50% of decayed berries**.**
4. Fungicide applications did not decrease the cranberry post-harvest diseases in storage during our investigations.

Acknowledgements

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PERSPECTIVES ON TRUFFLE CULTIVATION IN LATVIA

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Abstract
This paper discusses some aspects of introducing a new agricultural crop in Latvia – truffles *Tuber* spp. Truffles are mycorrhiza–forming mushrooms with edible fruit bodies developing in the upper layer of soil. In Latvia, there is a growing interest among farmers to start cultivate truffles. From several cultivated truffle species, the Burgundy truffle *Tuber aestivum* syn. *T. uncinatum* is the most suitable to cultivate in Latvia. There are both autochthonous and introduced tree species in Latvia, which are suitable for the Burgundy truffle cultivation and it is suggested to use *Quercus robur, Corylus avellana, Tilia cordata* and *Fagus sylvatica*. In order to find the regions in Latvia with the highest potential for successful Burgundy truffle cultivation, climate and soil characteristics had been co-analysed. By superimposing soil and climate maps of Latvia, the most suitable regions for truffle cultivation are situated in the central and southern part of the country. Considering the variability in soil composition even within regions in Latvia, it is thereafter also of great importance to investigate site-specific soil characteristics in order to find the most favourable grounds for truffle cultivation. Other truffle species which might be suitable for cultivation in the future are also discussed, as well as some general recommendations in establishing truffle orchards.
Key words: *Tuber aestivum, Tuber uncinatum, Tuber* spp., climate, soil, Baltic States.

Introduction

Searching for and consuming truffles has a long tradition in the Mediterranean region as well as Northern Africa. Records of consuming the Burgundy truffle (sometimes also referred to as Summer truffle or Common truffle) *Tuber aestivum* Vitt. syn. *T. uncinatum* Chat. are known already from ancient Greece. A form of truffle cultivation was exercised already in the 19th century by planting acorns in the naturally truffle producing areas of Southern France. When the trees developed, they formed mycorrhiza with the truffle spores of various species present in the ground, which eventually could lead to truffle fruit body production. In the late 1960s, intense research efforts started in Italy and France in order to produce greenhouse tree seedlings harbouring truffle mycorrhiza (Chevalier and Palenzona, 2008), for efficient truffle cultivation. These efforts were a direct effect of the strongly declining natural harvests of the Périgord black truffle *Tuber melanosporum* Vitt. After ensuring proper truffle colonisation of the root system, the seedlings could be planted in orchards. This also meant that truffles could be potentially grown in areas, or even continents, where there were no previously existing natural populations of these truffle species. Today truffle cultivation has expanded and is established also outside of the traditionally truffle producing countries of Southern Europe. Truffle orchards on a commercial or experimental scale have been established in Central and Northern Europe (Hungary, Sweden, Poland, Finland), Americas (Canada, USA, Argentina, Chili), North Africa (Morocco), Asia (China), New Zealand and Australia (Chevalier, 2010). As yet, only a few of the gastronomically and hence economically important truffle species are commercially available for truffle cultivation.

An interest in truffle cultivation has arisen also in Latvia. Despite that Latvia is a relatively small country, with a total area of 64,000 km^2, there are marked differences in climate and geology within the country, which may affect the success of truffle cultivation. Latvia is close to the northern border or even outside the distribution area of most commercial truffle species. Occurrence of edible truffle species in Latvia or adjacent areas would be a very important indication for choosing the right species for successful cultivation. So far, there are no confirmed finds of edible truffle fruitbodies from Latvia. Findings of black truffles in Latvia were reported by several authors at the end of 18th and beginning of the 19th century. Famous mycologist, specialist of hypogeous fungi of the beginning of 20th century Fedor Bucholtz (Бухгольц, 1902) attributed the old 19th century reports from Latvia to the Burgundy truffle *T. aestivum*. He cited literature data and reported black truffle finds from Russia and adjacent areas, including St Petersburg, Estonia, Lithuania, Ukraine and Moscow region. Which truffle species were found, remains to be confirmed until finding of the old herbaria collections or making new collections from these areas. Today, the known localities of the wild Burgundy truffle closest to Latvia are from the Swedish islands of Gotland and Öland (Wedén et al., 2004**),** the southern part of Poland (Ławrynowicz et al., 2008**)** and Belarus (Гапиенко, 2006). The edible and closely related Bagnoli truffle *Tuber mesentericum* Vitt. is also known from Sweden (Gotland) and Poland (Ławrynovicz, 1988). The Bianchetto truffle *Tuber borchii* Vitt. has been reported from Denmark and the UK (Pegler et al., 1993), Lithuania (Kataržytė, 2009), Poland and even southern Finland (Ławrynovicz, 1990). From neighboring Lithuania, which has very

similar climatic and physiogeographic characteristics, another edible truffle fungus is also known – *Choiromyces venosus* Vitt.

Even truffle species within the *Tuber* genus may differ markedly in biology and ecology. Practically this is evident in the different cultivation techniques required by the different species and in for example their different harvest seasons, when the fruitbodies ripen and could be collected. Harvest season for the highly prized *T. melanosporum* and also for the slightly less prized *T. brumale* is restricted to the winter months, it starts from late November and finishes at the end of March. Both species are commercially available for truffle cultivation. In Latvia the ground starts to freeze permanently from the 20th of November (Kalniņa, 1995) and stays frozen at least until March 31st, which makes maturing of the above mentioned species unlikely in the Latvian climate. During the last years, cultivation techniques have been developed also for a white truffle *T. borchii*. Harvest season for this species starts in January and ends in April, although findings of fruitbodies has been reported from late summer till autumn from several countries of Northern Europe, mentioned above.

For four species of edible hypogeous fungi the main maturing season is within the time allowed by climatic conditions in Latvia: *T. aestivum* (September 15th - January 15th), *T. mesentericum* Vitt. (September 15th - January 15th), *Tuber macrosporum* Vitt. (Septemer 1st - December 31st) and *Choiromyces venosus* (July 1st – September 30th) (Wedén, 2008). Among these, only the Burgundy truffle *T. aestivum* is cultivated on a wider scale and cultivation techniques are well known. Adding the fact that the known natural distribution area of *T. aestivum* is very close to Latvia and possible finds within the Baltic States are not unlikely, makes *T. aestivum* the most suitable truffle species for the first experiments with truffle cultivation in the Baltic States. The following assessment of suitable soil and climate regions in Latvia is therefore based on conditions necessary for *T. aestivum*.

Therefore, the objective of this study was to justify the choice of truffle species suitable for cultivation in Latvia, make a list of the most appropriate host tree species, make assessment of soil and climate conditions in Latvia from the point of view of truffle cultivation and compose a map of areas favourable for truffle cultivation in Latvia, using geological and climate data.

Materials and Methods

In order to find the regions of Latvia most suitable for the cultivation of the Burgundy truffle, soil and climatic data were consulted. Maps used for the evaluation were: soil map of Latvia (Nikodemus et al., 2008), map of quaternary deposits of Latvia (Juškevičs and Krūmiņš, 1998), climatic map of Latvia (Pastors and Krūmiņš, 1998), maps with description of the main soil regions of Latvia (Nikodemus et al., 2008; Āva, 1994), map of climate regions of Latvia with descriptions (Kalniņa, 1995). A map of potential truffle cultivation regions in Latvia was composed based on the administrative regions of Latvia, adding geological information and climate data. Soils in Latvia are very variable, and different soil types could be found within a small territory, so the proposed regions favourable for truffle cultivation are general recommendations, and detailed assessment should be made on spot when choosing the site for truffle orchard establishment.

Results and Discussion

Truffle species suitable for cultivation in Latvia

The Burgundy truffle has been chosen as the most suitable species for development of truffle cultivation in Latvia because of its fruiting season, wide natural distribution, including neighboring countries of Latvia and thus also the probability of finding natural population of this species in Latvia. Preliminary results from molecular analyses indicate *T. aestivum* mycorrhiza in root samples from a forest in Latvia (Meiere, unpublished data). There are several other *Tuber* species with high gastronomic value, for which cultivation techniques presently are in different stages of development. One of these is the most highly priced truffle, the Alba truffle *T. magnatum* Pico, which previously has failed to produce in orchards. On a small scale, *T. macrosporum, T. mesentericum* and *T. brumale* are cultivated in Italy and France and are proposed for cultivation in Hungary (Vezzola, 2008; Gogán, 2011). Of the mentioned species, only *T. mesentericum* has been found close to Latvia – in Sweden. Another edible hypogeous mushroom *Choiromyces venosus* has been found in Lithuania (Kataržytė, 2009) and Sweden. It has been suggested as a potential species for cultivation experiments in Sweden (Wedén, 2007). Until cultivation techniques have been developed and more insight is gained into the ecology and distribution patterns, it can however not be recommended for commercial truffle cultivation.

Soils of Latvia

Soils suitable for *T. aestivum* are well-drained, well-aerated, neutral or alkaline (pH KCl between 7.1 and 8.0) (Chevalier and Frochot, 1997). Soil texture could be variable, in natural *T. aestivum* habitats on Gotland (Sweden) the range of clay content is 10.4-32.6 g kg^{-1}, silt – 9.8-64.7 g kg^{-1}, and sand – 12.9-79.8 g kg^{-1} (Wedén et al., 2009). To find the areas with the highest proportion of suitable soils, several factors could be considered: soil type, soil texture as well as soil parent material. The

physiography of Latvia and its neighboring areas was formed, to a large degree, during the Quaternary period and the Pleistocene ice age. The parent material for Latvian soil formation are the Quaternary deposits, among them glacial tills (moraines) are prevailing (Kārkliņš et al., 2009) and glaciolimnic deposits are the main source of calcareous soils. The National soil classification system used in Latvia is not always easily comparable to the international systems. According to the international soil classification WRB (World Reference Base for soil resources), Latvian soils belong to *Phaeozems* and *Stagnosols* (Zemgale Plain), soils in uplands usually are *Luvisols, Cambisols, Gleysols* as well *Histosols.* In the forests soils are *Arenosols, Podzol, Umbrisols, Stagnosols, Albeluvisols* and *Cambisols* (Nikodemus et al., 2008). In the national classification system there are several types of soil with high content of carbonates, which means that soils are neutral or slightly alkaline and are favorable for the truffle development. Such soils (Sod calcareous soils in national classification system) cover approximately 5.5–6.0% of the total area of agricultural land; in several parts of Latvia (central part of Latvia in vicinity of Jelgava, Bauska, Dobele and to a lesser extent – around Tukums) they comprise about 50% of the total area of agricultural land (Kārkliņš et al., 2009). There are smaller territories of such soils in Rietumkursas Upland (western part of Latvia) as well as in other parts of Latvia where limnoglacial deposits are found. In the vicinity of Kandava and Sigulda, very shallow carbonatic soils of Rendzina type are found.

Climate in Latvia and its suitability for the truffle cultivation

Evaluation of the climatic characteristics is very important in finding the most favorable areas for the truffle cultivation in Latvia. It is believed that the climate is the most important limiting factor for the development of *T. aestivum* cultivation in Europe, especially in the northern countries (Chevalier, 2010). A long and mild autumn is crucial for the proper maturation of *T. aestivum* fruitbodies throughout the harvest season. If the soil freezes early, the truffle harvest also ends for that season.

The Latvian climate is humid European continental with the maritime influence of the Baltic Sea. Summers are warm, and the weather is mild in spring and autumn, however, winters could be fairly cold. The mean temperature of the year is 4.3–6.6 °C. Precipitation is distributed throughout the year with the heaviest rainfall in July. Annual precipitation ranges from 500 to 837 mm with the maximum rainfall during the summer and autumn months (70% from April till October), which is favorable for the development of most truffle species (Table 1). The mean daily temperature in January is from -2.6 °C (coastal areas of the Baltic Sea) to -7.5 °C (more continental parts of the country); in July from +16.5 °C to +17.6 °C which is equivalent or even a bit higher than for *T. aestivum* and *T. borchii* producing areas in Sweden, Denmark and the United Kingdom (Hall et al., 2007). Accumulated degree days show the length of the vegetation period for plants, and since truffles are closely connected with their host plants, good conditions for the host trees might also mean good development of truffles. Despite that Latvia is a northern country, the number of annual sunshine hours is relatively high. As seen in the summary of the climatic data given in Table 1, the only parameter which differs significantly between Latvia and edible truffle-producing areas is a comparatively low mean air temperature in January. It could be a limiting factor for the development of truffle species with a more southern distribution, but a stable snow cover might also reduce the impact of the cold.

Suitable host tree species

Truffles are mycorrhiza-forming fungi, and grow in a symbiotic association with, usually arboreal, plant. Most truffle species can form symbiosis and grow with several tree species, although some of them are considered better host trees than others. For the cultivation purposes, choosing tree seeds from species well adapted to the local climate is important.

Table 1

Comparison of climatic data of edible truffle *Tuber* spp. – producing areas and Latvia

Truffle species	Annual precipitation (mm)	Mean daily temperature in summer, July (°C)	Mean daily temperature in winter, January (°C)	Accumulated degree days (>10 °C)	Annual sunshine hours
Tuber melanosporum[1]	563-1443	19.2-24.6	1.6-9.2	1115-2341	1704-2837
T. magnatum[1]	589-1545	21.3-24.6	2.4-5.0	1349-2009	1989-2388
T. aestivum[1]	514-1045	15.2-26.2	–1.1-12.5	489-2009	1375-2837
T. borchii[1]	514-1045	15.2-26.2	0.1-12.5	489-3125	1375-2388
Latvia[2]	500-837	16.5-17.6	**–7.5- –2.6**	1700-2100	1680-1900

[1] Data from Hall et al., 2007
[2] Data from Kalniņa, 1995

We believe that in the best case both tree and truffle inoculum should be of local origin in order to have the best conditions for local production success. In Latvia there is no lack of tree species which can form mycorrhiza with *T. aestivum.* However, not all of them could be recommended for the establishment of truffle orchards. Only some of the introduced tree species (Table 2) grow in Latvia well enough to be suitable for truffle orchards. On the other hand, not all local mycorrhiza-forming tree species are good symbionts of truffles (e.g. coniferous trees).

According to Table 2, the local tree species which could accommodate *T. aestivum* and could be recommended for cultivation are common hazel *Corylus avellana* L., pedunculate oak *Quercus robur* L., and small-leaved lime *Tilia cordata* Mill. Of the introduced tree species, beech *Fagus sylvatica* L. and *Pinus nigra* Arnold could be used for several truffle species (mostly in the western part of Latvia).

Regions of Latvia potentially suitable for the truffle cultivation

Soil (pH, chemical properties, and texture) and climate (rainfall, temperature) are the factors which in combination may suggest the regions with the highest potential for truffle cultivation. On the map (Figure 1) soil, climate and also shallow deposits of calcareous minerals have been superimposed. Although calcareous soils suitable for truffles could be found almost in every region of Latvia, the highest probability is within the marked areas.

Table 2

Tree species growing in Latvia with which different truffle species have been known to form mycorrhizal associations

Truffle species	Autochthonous arboreal species[1]	Introduced arboreal species[1]
Tuber magnatum[2]	*Corylus avellana, Quercus robur, Tilia cordata, Populus tremula, Salix alba, Salix caprea*	*Populus alba* (451), *Tilia* × *europaea* (245), *Tilia platyphyllos* (190), *Populus nigra* (16), *Quercus petrea* (13), *Ostrya carpinifolia* (3), *Quercus cerris* (3), *Quercus pubescens* (1)
T. melanosporum[2]	*Carpinus betulus, Corylus avellana, Quercus robur, Tilia cordata*	*Tilia* x *europaea* (245), *Tilia platyphyllos* (190), *Quercus petrea* (13), *Corylus heterophylla* (7), *Ostrya carpinifolia* (3), *Quercus cerris* (3), *Quercus pubescens* (1)
T. aestivum[2]	*Betula pendula,* ***Carpinus betulus*, Corylus avellana*, Quercus robur*,*** *Tilia cordata, Picea abies, Pinus sylvestris*	***Fagus sylvatica**** (263), *Tilia* × *europaea* (245), *Tilia platyphyllos* (190), *Abies alba* (150), ***Pinus nigra*** * (74), *Corylus colurna* (25), *Castanea sativa* (13), *Quercus petrea* (13), *Ostrya carpinifolia* (3), *Quercus cerris* (3), *Quercus pubescens* (1)
T. borchii[2]	*Corylus avellana, Quercus robur, Tilia cordata, Picea abies, Pinus sylvestris*	*Larix* sp. (> 700), *Fagus sylvatica* (263), *Tilia* × *europaea* (245), *Tilia platyphyllos* (190), *Pinus strobus* (166), *Pinus nigra* (74), *Tilia americana* (39), *Populus nigra* (16), *Quercus petrea* (13), *Ostrya carpinifolia* (3), *Quercus cerris* (3), *Quercus pubescens* (1)
T. macrosporum[2]	*Betula pendula, Corylus avellana, Quercus robur, Salix alba, Salix caprea*	*Populus alba* (451), *Tilia* × *europaea* (245), *Tilia platyphyllos* (190), *Populus nigra* (16), *Quercus petrea* (13), *Ostrya carpinifolia* (3), *Quercus cerris* (3), *Quercus pubescens* (1)
T. mesentericum[2]	*Corylus avellana, Tilia cordata*	*Fagus sylvatica* (263), *Tilia* × *europaea* (245), *Tilia platyphyllos* (190), *Pinus nigra* (74), *Corylus colurna* (25), *Castanea sativa* (13), *Quercus cerris* (3), *Ostrya carpinifolia* (3), *Quercus pubescens* (1)
T. brumale	*Quercus robur, Corylus avellana, Tilia cordata*	*Fagus sylvatica* (263), *Tilia americana* (39), *Quercus petrea* (13), *Ostrya carpinifolia* (3), *Quercus cerris* (3), *Quercus pubescens* (1)
Choiromyces venosus[3]	*Betula pendula, Corylus avellana, Quercus robur, Tilia cordata*	*Fagus sylvatica* (263)

[1] Tree species distribution in Latvia given according to Atlas of Latvian Woody Plants (Laiviņš et al., 2008), number in parentheses indicates number of localities in Latvia for introduced species
[2] Host plants for truffles species is given according to Hall et al., 2007
[3] Host plants according to Wedén, 2007
* bold letters - species suitable for commercial *Tuber aestivum* cultivation

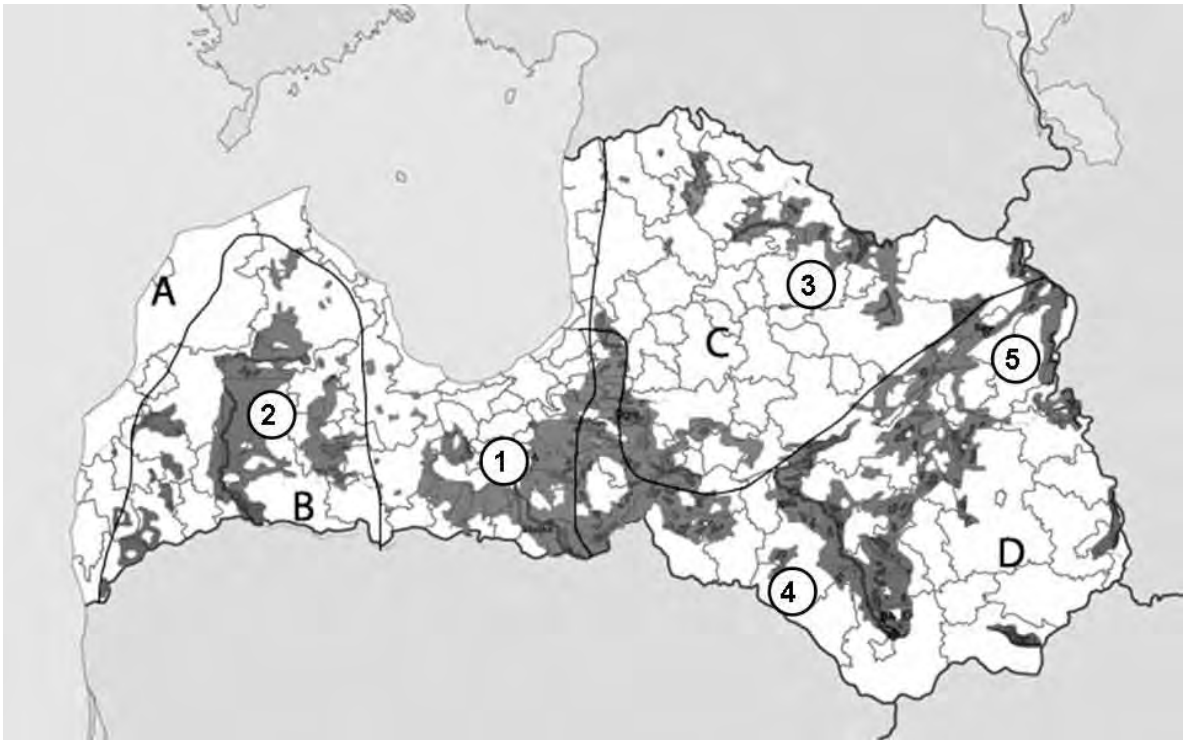

Figure 1. Regions of Latvia potentially suitable for truffle cultivation on the criteria of climate and soil parent material: dark-coloured areas 1, 2 – the most suitable regions for truffle cultivation; dark-coloured areas 3, 4, 5 – regions with only some factors for truffle cultivation satisfied; A, B, C, D – climate regions of Latvia.

Region 1 – southern part of Central Latvia (Figure 1). The western part of this region is the only in Latvia where calcareous soils comprise about 50% of the total agricultural land. In the eastern part of this region, calcareous soils are found close to Daugava river valley. Climate (Figure 1, region A) is relatively dry and warm and the period without freezing is the longest in Latvia (140-150 days). This climatic region is the only one in Latvia where plant species characteristic to maritime climate conditions grow naturally (*Hedera helix, Taxus baccata,* etc.). However, the snow cover in this region could be unstable, which can negatively affect host plant roots in the upper soil layer. Another concern is that the western part of this region has very high percent of clayey soils (up to 31% of agricultural land) (Boruks, 2004) which should be avoided choosing the site for truffle cultivation.

In the region 2 (Rietumkursas, Austrumkursas Uplands, western part of Latvia), distribution of calcareous soils is lower, but they can be found, especially on the upland hills; calcareous deposits are close to the surface in the river valleys (Venta, Abava). Climate (region B) is similar to the previous; except that winters are colder; hence choosing the southern exposition and place protected from northern winds could improve growth conditions.

Region 3 is situated in the Northern part of Latvia, and distribution of calcareous soils is restricted; they are found locally on the geological formations – eskers, kames and dauguls, sometimes also on moraine plains. The climate of this region (Climatic region C) is the wettest and coldest region of Latvia. The vegetation period is short and temperature could drop significantly. Despite suitable soils, this region is therefore not recommended for truffle cultivation.

Climate in regions 4 and 5 is the most continental in Latvia – summers are hot, but winters relatively cold. Both temperature maximum (+36.4 °C) and minimum (-43.2 °C) are registered in this climate region (climate region D). Soils suitable for truffle cultivation could be found in Latgale Upland and Daugava river valey. In region 5 soils are mostly very wet, and there are not many sites suitable for Burgundy truffle cultivation.

Combining the two factors – suitable soils and climate conditions – it could be concluded that in Latvia the most suitable regions for truffle cultivation are in the western part (Rietumkursas, Austrumkursas Uplands) and in the central part (Zemgale Plain). Less suitable area from a climate characteristics point of view and with only locally available suitable soils, is in the in northern and eastern parts (Tālava lowland, Latgale Upland, Daugava river valley). Nevertheless, when choosing the right site for the establishment of a

truffle orchard, it is necessary not only to investigate the soil of particular site, but also take into account other important factors, such as exposition (southern exposition will give higher soil temperature, but also higher water evaporation rate), windiness of the place, distance from adjacent forest trees which may be a possible source of contamination with spores of other mycorrhizal mushrooms, and availability of water for irrigation. Land use history may also be of importance. For example, it is not recommended to use land recently transformed from forest land because of the contamination risk of other mycorrhizal fungi. After the planting of good quality mycorrhized seedlings, the proper management of truffle orchard is essential to ensure a reasonably quick start of truffle production and good harvests. Truffle cultivation is time and labour consuming process, and management will include soil cultivation, weeding, irrigation, pruning and protection from pests and wild animals.

Conclusions

1. Taking into account ecological requirements, fruiting season of commercially cultivated truffle species and comparing them to climatic characteristics of Latvia, the most suitable species for the cultivation at present is the Burgundy truffle *T. aestivum* syn. *T. uncinatum*.
2. For the cultivation of Burgundy truffle in Latvia we recommend to use *Quercus robur, Corylus avellana, Tilia cordata* and *Fagus sylvatica* as host trees.
3. From the superimposing of the climatic region and soil region maps of Latvia analysis we conclude that the areas with the highest potential for truffle cultivation are the arear around Bauska, Dobele, to the south of Jelgava, as well as smaller areas in vicinity of Saldus, Kuldīga, Ogre, Rīga, and less suitable areas - in vicnity of Jēkabpils and Madona.

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FACTORS AFFECTING GOAT MILK YIELD AND ITS COMPOSITION IN LATVIA

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Abstract
The aim of the present research was to evaluate the affect of genetic and environmental factors on the variability of the goat milk yield, fat and protein content in goat milk in Latvia. Data of 6067 lactation records from 2400 goats of six different breeds were analysed in the period of 2001 to 2010. The highest milk yield (662.7±14.58 kg) was obtained in 2002, the highest fat content (40.7±0.05 g kg^{-1}) – in 2004, but highest protein content (32.7±0.19 g kg^{-1}) – in 2008 ($p<0.05$). Basically there are two goat breeds in Latvia: Latvian goats and Saanen goats. In the research, 3261 Latvian and 2032 Saanen goats in closed lactations were analysed. It was found that Saanen goats gave the highest milk yield (579.3±5.01 kg), but Alpine goats – the highest fat and protein content (respectively 41.7±0.63 and 32.3±0.30 g kg^{-1}; $p<0.05$). It was observed that most of all the goats kidded in winter (2379) and spring (3378). The highest milk yield (583.8±7.39 kg) was determined for goats kidded in winter season, but the highest fat content (41.8±0.06) and protein content (32.3±0.03) – for goats kidded in summer season. The average milk yield in the first lactation (1636) was significantly lower than in the third lactation (578.0±8.34; $p<0.05$) when the goats produced the highest milk yield in the research.
Key words: goats, milk productivity traits, breed, environmental factors.

Introduction

The productivity of goat milk is characterized by the quantity of produced milk and its quality in a fixed period of time – by day, by month, by lactation, by year, or by the whole goat lifetime.

The quantity of goat milk and its content vary depending on animal breed traits, genotype, age, the stage of lactation, the speed of milking, the functional condition of hormonal system, illnesses, gestation, season and many other factors (Mioč et al., 2008).

The productivity of the goat milk in lactation mainly depends on a goat breed and conditions of feeding. The milk yield can range from 400 up to 1000 kg, for separate goats of German Noble breed the milk yield can reach up to 1900 kg (Sambraus, 2001). The goat milk contains approximately 87% of water and 13% of dry matter.

The most important of milk chemical content is the proportion of fat and protein which may vary for different goat breeds – from 3.00 to 4.65% and from 3.00 to 3.50% monthly (Gall, 2001).

According to FAOSTAT (2008) data there are 862.9 million of goats in the world and 18.0 million of them are in European countries. The dairy goats constitute 80% of the total number of goats in the world. The largest number of goats is in Greece (4.1 million), in Spain (1.4 million), and in France (0.8 million). In total, there have been found about 110 different goat breeds in the world and just about 10 breeds are used for milk production. The most popular dairy goat breeds are Saanen, Alpinen, Thuringian, Toggenburgs, and Anglo Nubian goats (FAOSTAT, 2008).

The breeding of milk goats is one of livestock breeding branches in Latvia. Sixty-five percent of the goats in Latvia are Latvian goats (LVK), 15% - Saanen goats (ZK), but the rest of 20% include Alpine (AK), German Noble (VBD), and Thuringian (TIR) goats imported from Germany in the period of 2004 to 2006. The milk recording was done for 1158 dairy goats in Latvia in 2010: on average, milk yield was 529 kg, fat content – 3.93%, protein content – 3.21%, and somatic cell count (SCC) – 1056 thousand ml^{-1} (The State Agency (S/A) Agricultural Data Centre).

The aim of the present research was to evaluate the affect of genetic and environmental factors on the variability of the goat milk yield, as well as on fat and protein content in goat milk in Latvia.

Materials and Methods

During the research we used goat herds where the milk recording had been done. The milk yield (kg) and the fat and protein content (g kg^{-1}) of the most popular goat breeds in Latvia were analysed: AK, LVK, TIR, VBD, ZK, and different crossbreeds (XX).

The information of the State Agency (S/A) Agricultural Data Centre of about 2400 goats in 6067 closed standard lactations (240 – 305 milking days) kidded in the period of 2001 to 2010 was used for the analysis of goat milk productivity traits.

To measure the milk yield in farms, electronic scales or the measuring instruments of half-automatic milking equipment of milk measurement were used.

Up to 2004 the milk samples were analysed in the milk control laboratory in Kurzeme Artificial Insemination Station, and starting from 2005 – in the milk control laboratory in Sigulda Artificial Insemination Station. The content of fat and protein was identified according to the method of ISO 9622:1999 with device Milko-Skan 133 B in both laboratories.

In 10 years the milk recording was done in 63 herds of different size. For the goats in Latvia, the seasonal kidding is observed therefore the factor

'kidding season' was as follows: winter (December, January, February), spring (March, April, May), summer (June, July, August), and autumn (September, October, November).

As duration of goat milking varies from the first to the tenth lactation, we made five gradation classes for the fixed factor 'lactation'. It included the animals from the fifth and later lactations.

The statistical data were processed using SPSS program package and Microsoft Excel for Windows. Data in tables and figures are presented as least square mean ± standard error of means (SEM). The coefficient of variation (CV) was used to describe the variability. The linear model of variable factors (GLM – General linear model) was used to identify factors which significantly affected the changes in traits of the goat milk yield productivity.

The model which includes fixed factors as well as random and covariate factors was as follows:

$$y_{ijklm} = \mu + \check{S}_i + L_j + ATG_k + ATS_l + G_m + (ATG_k x\, ATS_l x\, G_m) + DZn + AV_o + e_{ijklmnop},$$

where

$y_{ijklmnop}$ - i-the trait of the animal milk productivity;
μ - the average value of the population;
$\check{S}_i$ - the fixed factor 'breed' (i=1- 6);
L_j - the fixed factor 'lactation' (j=1- 5);
ATG_k - the fixed factor 'milking year' (k=1-10);
ATS_l - the fixed factor 'milking season' (l=1- 4);
G_m - the fixed factor 'herd' (m=1- 63);
DZ_n - the random animal effect (n=2400);
AV_o - kidding age in months, as covariate factor;
$e_{ijklmnop}$ - random residual.

The validity of factors included in the model was identified according to the significance level $\alpha = 0.05$; 0.01; 0.001.

Results and Discussion

The recording of milk goats was started in 2001. It was organised according to the joint rules of the International Committee for Animal Recording (ICAR). These rules prescribe the individual milk yield registration and the analysis of the milk content. The average of the goat milk productivity is represented in Table 1.

It was found that the average milk yield in standard lactation was 547.6 kg, the average milk fat content – 39.3 g kg^{-1}, and the average protein content – 31.8 g kg^{-1}. The greatest variability of milk productivity traits was determined for Latvian dairy goats. It was indicated by high values of the variation coefficient which for the milk yield was 31.3%, for the fat content – 16.8%, and for the protein content - 10.7%.

The great variability of the milk productivity traits shows that there were animals with both high and low productivity in the herds of milk goat breeds in Latvia in the analysed period of time. It means the selection should be done.

Evaluating the affect of the influence of genetic and environmental factors on the goat milk productivity, we concluded that the milk yield variability is affected by all analysed factors ($p<0.001$; Table 2). The milk fat and protein content variability was affected by the goat breed, lactation, goat kidding year and season as well as by the herd the animal lived. The interaction effect of the three fixed factors (a year of kidding, a season of kidding and a herd) was significant too ($p<0.05$, $p<0.01$, and $p<0.001$ respectively).

The quality of the linear model is described by the determination coefficient (R^2). The factors included in the model characterised the changes in the goat milk productivity traits precisely enough: the highest R^2 was for the protein content (64.0%), but the lowest – for the fat content (58.3%). In linear models offered by other authors, when analysing the affects of similar goat milk productivity factors, the determination coefficient values were between 55% and 68% (Crepaldi et al., 1999).

During the analysis of the fixed factors it was found that significantly highest milk yield in standard lactation (662.7±14.58) in the period of 2001 to 2010 was obtained for goats kidded in 2002. The lowest milk yield was obtained in 2005, 2007 and 2008 when it did not reach 500 kg and differed significantly from the milk yields in 2001 and 2002 ($p<0.05$; Figure 1).

Table1

Descriptive statistics of the goat milk productivity traits (n=6067)

Trait	$\bar{x} \pm s_{\bar{x}}$	CV, %	Minimum	Maximum
Milk yield, kg	547.6 ± 2.22	31.7	187.0	1290
Fat content, g kg^{-1}	39.3 ± 0.81	16.8	23.7	71.7
Protein content, g kg^{-1}	31.8 ± 0.42	10.7	21.4	51.9

Table 2

The influence of researched factors on goat milk productivity traits

Factors	Milk yield, kg	Fat content, g kg^{-1}	Protein content, g kg^{-1}
	p-value		
Breed	***	***	***
Lactation number	***	**	***
Year of kidding	***	**	***
Season of kidding	***	*	***
Herd	***	***	***
Herd ×year ×season	***	***	***
Age of kidding	***	n.s.	n.s.
R^2	0.633	0.583	0.640

*p<0.05; ** p<0.01; *** p<0.00; n. s. p>0.05.

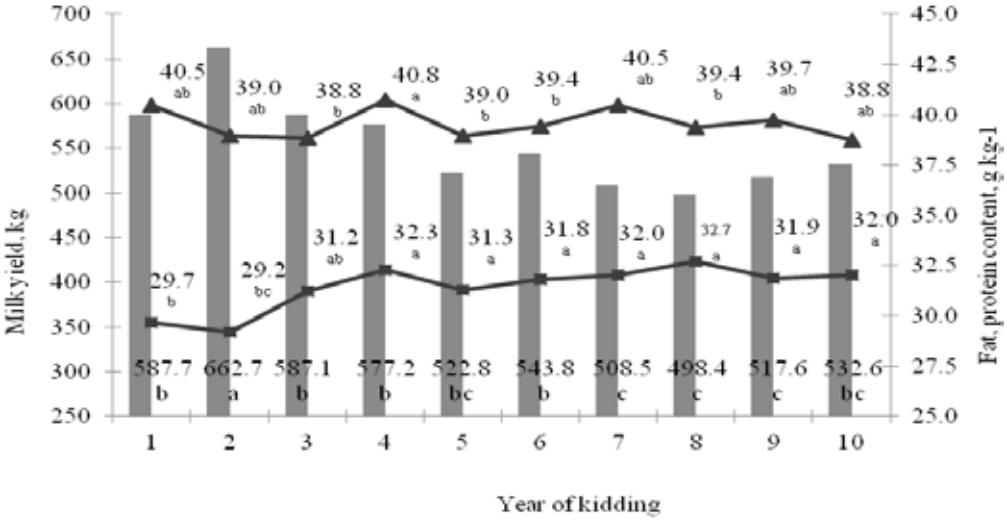

Figure 1. Least squares means of milk yield, and the fat and protein content in the period of 2001 to 2010.
▬ – Milk yield, kg; ▲ – Fat content, g kg^{-1}; ■ – Protein content, g kg^{-1}.
$^{a;b;c}$ – traits with different superscripts significantly differ among the research years (p<0.05).

At the beginning (in 2001 and 2002), goat milk recording was done only for a small number of goats a year (respectively 133 and 258) and only for the goats whose owners were interested in a systematic selection, therefore the best animals were chosen. Gradually the control of milk amount and content was done for a greater and greater number of goats, and finally 1156 goats were included in our research in 2009. The significant differences in the milk yield among the research years can also be explained by unfriendly environmental conditions in 2005 and 2007. These years were characterised by lasting periods of dryness and increased air temperature in the grazing period. Several scientists (Lough et al., 1990; Huth, 1995) who have studied the affect of external environment (regarding temperatures and relative humidity) on cow milk productivity have found that the animals react individually to climatic condition changes; however the behaviour of animals changes with the increase in environmental temperature.

Depending on the heat stress intensiveness and the duration of heat, the dry matter consumption decreased from 5% to 25%, which induces energy deficit.

The animal has troubled rumination when the dry matter consumption decreases therefore the separation of saliva and pH in the rumen decreases. The acidic ambience of the rumen badly affects the formation of evaporable fatty acid, which induces decrease in the milk yield and fat in cows (Osītis, 2005). Goats are ruminants too therefore their reaction to environmental changes and the need for well-balanced feeding could be similar to cows (Sprūžs and Šelegovska, 2003; Korn et al., 2007). The milk productivity decreases significantly also on rainy days because the animal's feed consumption is reduced. It has been found that decrease in temperature below +10 °C reduces the goat milk yield reduces per day (Brito, 2011).

Our research suggests that reduction in goat milk yield in 2008 could be affected by the increase in the number of the first lactation goats.

Table 3

Least squares means (± SEM) of the goat milk productivity traits in different kidding seasons

Season	n	Milk yield, kg	Fat content, (g kg^{-1})	Protein content, (g kg^{-1})
Winter	2379	583.5±7.39^{a}	39.0±0.31^{b}	31.1±0.15^{b}
Spring	3378	526.9±7.20^{b}	39.1±0.30^{b}	31.4±0.15^{c}
Summer	270	441.6±13.50^{c}	41.8±0.63^{a}	32.3±0.25^{a}
Autumn	40	583.8±22.44^{a}	38.5±0.92^{b}	32.0±0.45^{c}

^{a,b,c}- milk productivity traits with different letters differ significantly between different seasons ($p<0.05$).

The goat milk content significantly differed among the research years. The highest milk fat content was (40.8±0.05 g kg^{-1}) for goats kidded in 2004. The milk protein content was between 32.7 g kg^{-1} in 2008 and 29.2 g kg^{-1} in 2002.

Goats, in common with sheep, are polycyclic animals with well-marked rutting time which starts at the end of July and ends at the beginning of August. The productivity of goat kidded at the beginning of summer and autumn could be affected by two important factors – the goat rutting time, and the changes between the grazing period and the period goats spend inside the stables.

The goats are in heat from 2 to 3 days during the rutting time. The rut repeats after each 19 – 21 days, and if the goat does not become pregnant it repeats regularly till January. Besides, the grazing time ends in autumn and the conditions of feeding and keeping are changed, which makes stress for the goats and in such a way affecting the milk productivity.

According to several authors' researches (Crepaldi et al., 1999; Zoa-Mboe et al., 1997), the goat kidding month significantly affects the variability of the milk productivity traits. The group of scientists in Croatia during their research of Alpine and Saanen goats have found that the highest milk productivity was reached by the goats kidded in winter – 627.75±4.06 kg (Mioč et al., 2008). The analysis of the goat kidding seasonality in France has shown that 68.3% of goats kidded in winter and spring (Institut de l'Elevage, 2010).

The results of the present research are summarized in Table 3.

The significantly highest milk yield in standard lactation was determined for goats kidded in winter and spring (583.8±22.44 and 583.5±7.39 kg) from October to March; the lowest milk yield – for the goats kidded in summer (441.6±13.50; $p<0.05$).

The significantly highest fat and protein content was determined for the goats kidded in summer (41.8±0.63 and 32.3±0.25 g kg^{-1}; $p<0.05$). During the research, a negative correlation between the milk yield and the milk fat and milk protein content was observed.

The goat milk yield gradually increases with each succeeding lactation. The maximum can be reached in the third to the fifth lactation if the feeding and keeping conditions are set correctly, but in later lactations it starts to decrease. In the first and the second yield the milk yield reaches 65% – 85% of the grown-up goat milk yield. Starting with the third lactation the fluctuations of the milk yield affected by age are small and the goats could be considered as grownups (Gall, 2001).

The significantly highest average milk yields in the standard lactation were determined for the goats of the third (578.0±8.34 kg) and the fourth (572.2±8.34 kg) lactations. Croatian scientists have observed similar results when investigating productivity traits of Alpine and Saanen goats (Mioč et al., 2008). They found that the lowest milk yield (477.6±7.41 kg) was for the first lactation goats, and it did not reach even 500 kg ($p<0.05$).

In our research, the goat milk content significantly differed among the lactations. The highest milk fat content (from 39.6±0.34 to 39.9±0.39) was determined for the goats in the fourth and later lactations. The milk protein content was between 31.3±0.16 g kg^{-1} in the fourth lactation and 32.1±0.15 g kg^{-1} in the first

Table 4

Least squares means (± SEM) of the goat milk productivity traits in different lactations

Lactation	n	Milk yield, kg	Fat content, g kg^{-1}	Protein content, g kg^{-1}
1	1636	477.6±7.41^{c}	39.4±0.30	32.1±0.15^{a}
2	1521	546.3±7.25^{b}	39.2±0.30^{b}	31.6±0.14^{b}
3	1142	578.0±8.34^{a}	39.2±0.31^{b}	31.5±0.15^{b}
4	827	572.2±8.34^{a}	39.6±0.34	31.3±0.16^{b}
5 and >	941	549.8±9.48^{b}	39.9±0.39^{a}	31.7±0.19^{a}

^{a,b,c}- milk productivity traits with different letters differ significantly between different lactations ($p<0.05$).

Table 5

Least squares means (± SEM) of the milk productivity traits of the goat breeds in Latvia

Breed	n	Milk yield, kg	Fat content, g kg^{-1}	Protein content, g kg^{-1}
AK	384	511.4±15.50^{c}	41.7±0.63^{a}	32.3±0.30^{a}
LVK	3261	560.8±3.86^{b}	38.9±0.16^{b}	31.8±0.08^{a}
TIR	81	543.8±14.88^{b}	36.9±0.61^{c}	30.1±0.29^{b}
VBD	218	533.8±15.21^{b}	41.3±0.62^{a}	32.0±0.30^{a}
XX	91	539.6±15.09^{b}	39.5±0.61^{b}	32.1±0.30^{a}
ZK	2032	579.3±5.01^{a}	39.0±0.21^{b}	31.7±0.10^{a}

^{a,b,c}- milk productivity traits with different letters differ significantly between different breeds ($p<0.05$).

lactation. German scientists have found (Bömke, 2004) that WBD and AK goats reach the highest milk yield in the third lactation. According to them the increase in protein content was determined from the fourth to the thirteenth lactation, but the highest milk fat content was determined in the first lactation. The milk recording results in France in 2010 show that most of all milk is produced during the second and third lactations (from 903 to 906 kg); however also reduced milk fat and protein content is determined in the same lactations (Institut de l'Elevage, 2010).

Analysing the affect of the researched factors on the milk productivity traits variability, it was found that in ten years Saanen goats gave the significantly highest milk yield (579.3±5.01 kg), while Latvian goats reached the second highest milk yield (560.8±3.86 kg) (Table 5)

The highest milk fat and protein content was for AK goats (41.7±0.63 and 32.3±0.30 g kg^{-1}). The milk recording results in Germany show that the highest milk yield in 2010 was reached by German Noble (the milk yield was 717 kg and 33.4 g kg^{-1}), but the highest fat content has been determined for Thuringian goats (36.3 g kg^{-1}) (Milchleistungsprüfung bei Ziegen, 2010).

Milk recording results in France show that the average milk yield of ZK goats was 861 kg, the milk fat content – 35.8 g kg$^{-1,}$ and the protein content – 31.6 g kg^{-1}, but the AK milk yield was 833 kg, the milk fat content – 37.8 g kg^{-1}, and the protein content – 32.8 g kg^{-1} (Institut de l'Elevage, 2010).

Conclusions

1. The goat milk yield, and the fat and protein content varied depending on the year and season of kidding, herd, lactation, breed and interaction between year, season and herd ($p<0.05$; $p<0.01$; $p<0.001$). The determination coefficients were estimated from 0.583 for fat content, to 0.640 for protein content.
2. The significantly highest milk yield (662.7 kg) was determined in 2002, the highest milk fat content (40.7 g kg^{-1}) – in 2004, but the highest protein content (32.7 g kg^{-1}) was determined for the goats kidded in 2008 ($p<0.05$).
3. The goats kidded in autumn and winter seasons gave the significantly highest milk yield (respectively 583.8 and 583.5 kg), but the goats kidded in summer season had the highest milk fat and protein content (41.8 and 32.3 g kg^{-1}; $p<0.05$).
4. The highest milk yield was determined for the third and fourth lactation goats (578.0 and 572.2 kg), but the highest milk fat content (39.9 g kg^{-1}) – for the goats in the fifth and later lactations. The significantly highest protein content (32.1 g kg^{-1}) was determined in the first lactation ($p<0.05$).
5. The Saanen goats reached significantly highest milk yield (579.3 kg) but the highest milk fat and protein content was determined for the Alpine goats (the fat content – 41.7, and the protein content – 32.3 g kg^{-1}; $p<0.05$).

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MILK UREA CONTENT AS INDICATOR FEED PROTEIN UTILIZATION AND ENVIRONMENTAL POLLUTION IN FARMS

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Abstract
Advances in milk production and the expansion of dairy herds have increased the need for improved manure management and whole farm nutrient balance. It is well known in dairy management that the balanced feeding and holding technology is an important level by which milk production and milk composition can be modified. The objective of this study was to evaluate urea content and urea yield in different farms with different holding technologies. Four farms represent three cow breeds (Holstein Black and White, Latvian Brown, and cross breed XP). Individual cow milk samples (n=2740) were collected monthly from September 2009 to November 2010. Milk samples were analyzed for total protein, casein, fat, lactose, and urea content with instrumental infrared spectroscopy method. The average milk yield per cows in farms was significantly different (from 27.9 to 17.0 kg per control day). The urea content in cow milk was between 20.3 to 46.6 mg dL^{-1}. The average urea content in farms A, B and D was up to standard (from 15.0 to 30.0 mg dL^{-1}). In the farm C, average urea content ranged between 17.4 and 79.9 mg dL^{-1}, which indicated problems in feeding or management in the farm. It was established that milk urea content and yield significantly ($p<0.05$) varied in farms with different dairy cow holding and feeding technologies. Urea content can be used to evaluate feed protein utilization in farms and predict environmental pollution.
Key words: dairy cow, milk yield, urea content and urea yield.

Introduction

Urea is a small organic molecule composed of carbon, nitrogen, oxygen, and hydrogen. Urea is a common constituent of blood and other body fluids. Urea is formed from ammonia in the kidney and liver. Ammonia is produced by the breakdown of protein during tissue metabolism. Ammonia is very toxic. The conversion of ammonia to urea, primarily in the liver, prevents ammonia toxicity. Urea is then excreted from the body in urine.

Urea is therefore a normal constituent of milk and comprises part of the nonprotein nitrogen fraction. Although opinions do vary to some extent, milk urea levels between 20 and 30 mg dL^{-1} are generally considered as normal for cow's milk. Urea accounts for roughly 50% of the non-protein nitrogen fraction in herd bulk milk of dairy cows, although this may vary from 35 to 65%. For milk from individual cows, this variation may be even larger (Bijgaart, 2003). The urea content may be used to monitor nutritional status of lactating dairy cows and improve dairy herd nutrition.

Urea in milk has proven to be an easily measurable indicator for protein metabolism efficiency in dairy cattle. The obtained figures can help to identify and correct imbalances in the protein/energy ratio in the diet, sub-optimal feed nitrogen utilization, the potential for reducing ammonia emissions from dairy farms, and fertility problems.

The variation in milk urea concentrations between herds and between cows indicates a wide variation in protein, energy and water intake within dairy cows and herds. If the milk urea content is outside of normal concentration it would suggest problems with the feeding program. Urea concentration in milk may provide an opportunity to look at problems with the feeding and system within farm.

In several countries, essential legislation is for to limit the mineral surplus in animal production. That development forces the farmer to evaluate thoroughly the mineral flows on their farms and in the animals. The main tools for them to avoid mineral, and in cattle especially nitrogen, losses are: reduce the amount of artificial fertilizer, decrease the ammonia emission from the stable, and fine - tune the balance between the protein and energy in take by the cows (BANR, 2001).

For monitoring the last aspect, representative and easily measurable parameters are of great practical value for the farmer. Urea excretion has the potential to serve as a biological tool to monitor nitrogen losses in dairy cows. From 50 to 70% of the nitrogen which is not retained by the cow or used for milk synthesis is excreted in the urine, and the remainder is lost via faeces. The correlation between total urea extraction in urine with the urea content in blood or milk has been 0.88 and 0.77, respectively (Hof et al., 1997).

In order to protect water quality across Europe by preventing nitrates from agricultural sources polluting ground and surface waters, the Nitrates Directive was implemented in the European Union in 1991 Council Directive 91/676/EEC. This directive has put firm ceilings to fertilizer and manure application and rate of N emissions to the environment. Specialization, fertilizer nitrogen (N) applications to grasslands, purchase of concentrate feed and improvement of the management and genetic potentials of the herd have played dominant roles in the intensification of

ruminant production that is increasing the output of milk per farm. High inputs of N fertilizers and protein-rich feeds contribute to allow high production levels, but most of the N ingested is not retained in milk but excreted again in urine and faeces (Dijkstra et al., 2011).

The objective of this study was to evaluate feed protein utilization in farms and predict environmental pollution used urea content and urea yield in different farms with different holding technologies.

Materials and Methods

In the study, individual cow milk samples (n=2740) were collected monthly from four dairy farms (Farm A, B, C, and D) from September 2009 to November 2010. Dairy herds represent three breeds: Holstein Black and White (HB), Latvian Brown (LB), and cross breed XP (cross breed from HB and LB).

Dairy farms were with different number of animals in herds, and with different milking and holding technologies. Farms A and C had a small (n=113 and n=119 accordingly) number of animals and the traditional holding technology in the pasture-based seasonal dairying system. In these farms cows were managed in one feeding group. Whereas farms B and D were big farms (n=1829 and n=679 accordingly) with a balanced feeding and total mixed ration in all years without pasture period. Management in these farms was organized in feeding groups according to lactation stage. Milking frequency was two times per day. The herds were under official performance and pedigree recording.

The monthly control milk samples were analyzed for urea content. Parameter was analyzed in accredited milk quality laboratory SIA 'Piensaimnieku Laboratorija' with accredited instrumental infrared spectroscopy method.

Data regarding breed of cows and date of milk analysis were available from monthly records of the herds from state agency "Agricultural Data Centre" program.

Control day was grouped into four seasons: winter (W) – (December, January, February, n= 601), spring (Sp) – (March, April, May, n=745), summer (S) – (June, July, August, n=693), and autumn (A) – (September, October, November, n=701). Milk urea content unit (mg dL^{-1}) was transformed to % (FOSS, 2005), and afterwards the urea yield (g) in control day was calculated according to International Committee For Animal Recording (ICAR) guidelines (ICAR, 2011).

The statistical analyses were performed using SPSS program package and Microsoft Excel for Windows.

The obtained data were analyzed using descriptive statistics and Pearson correlation analysis. The significance of the differences between the samples was assessed using ANOVA.

Results and Discussion

The study results were analyzed separately for each farm to evaluate cow milk yield, urea content, and urea yield in milk in the different farms (Table 1).

Average milk yield per cow (from 27.9 to 17.0 kg per control day) in farms significantly differed. The significantly lowest milk yield was in farm C. The highest milk yield was in farm D with several breeds' cows, from which HM breed cows predominated and management in this farm was organized in feeding groups according to lactation stage.

The urea content and urea yield per cow in control day in farms varied (20.3 to 46.6 mg dL^{-1}, and 5.1 to 8.2 g) and was statistical significantly difference. The average urea content and urea yield in farm C

Table 1

Average milk yield, urea content, and urea yield in milk per cow in control day during the research

Farms	Traits	$\bar{x} \pm s_{\bar{x}}$	Minimum	Maximum
A	Milk yield, kg	25.2±6.05[a]	9.0	36.8
	Urea content, mg dL^{-1}	20.3±6.76[a]	2.4	37.1
	Urea yield, g	5.1±2.10[a]	0.7	10.0
B	Milk yield, kg	23.7±6.84[b]	5.3	53.7
	Urea content, mg dL^{-1}	27.2±8.42[b]	5.2	56.7
	Urea yield, g	6.4±2.70[b]	1.1	20.4
C	Milk yield, kg	17.0±5.72[c]	6.2	28.8
	Urea content, mg dL^{-1}	46.6±15.78[c]	17.4	79.9
	Urea yield, g	8.2±4.63[c]	1.7	21.9
D	Milk yield, kg	27.9±9.49[d]	3.8	61.1
	Urea content, mg dL^{-1}	26.8±5.48[b]	12.0	44.5
	Urea yield, g	7.4±2.76[d]	0.6	19.0

[a; b; c; d] – traits with unequal letter differed significantly between the farms ($p<0.05$).

were significantly higher (46.6 mg dL^{-1} and 8.2 g accordingly) than in other farms, which indicates problems in cow feeding balance and management. Also Lithuanian researchers (Savickis et al., 2010) have established influence of a farm on the contents of to urea. Farm C had LB breed cows, and management in this farm was organized in one feeding group.

The next in study were established that from seasons and farms had influence urea content in milk (Table 2).

The average urea content and urea yield were higher and significantly different in farm C in summer month. The lowest urea content and yield were in farm A in winter month. A significant difference was established between all farms and between all seasons. In the farm D, urea yield was highest in winter and lowest in autumn. In farms A and C, urea yield was significant high and did not differ between spring and summer. But in farm D, the urea yield decreased and did not differ among the spring, summer and autumn months. The results of this study confirm results of previous researchers (Meijier et al., 1996; Savickis et al., 2010) that milk urea content differ between the periods of sampling and individual cows.

To evaluate relation between cow milk yield and urea content and urea yield, the correlation was estimated in all farms (Fig.1).

Overall, in different farms the correlation between milk yield and milk urea yield was significantly high or very high. A closely positive significant correlation (0.830) was in farm D. Correlation between milk yield and milk urea content in farms B and D was significantly negative low (-0.075 and -0.125 accordingly), but in farms A and C a low positive correlation was observed. In farm C was significant low positive correlation (0.244).

Control day milk yield influenced urea content and urea yield in milk and was different between the farms (Fig. 2 and Fig. 3).

Table 2

Average urea content and yield in milk per cow in control day at different seasons

Farms	Traits	Seasons			
		W	Sp	S	A
A	Urea content, mg dL^{-1}	$13.3\pm1.28^{a,A}$	$19.2\pm1.01^{b,A}$	$22.6\pm1.03^{c,A}$	$23.9\pm1.04^{c,A}$
	Urea yield, g	$3.2\pm0.42^{a,A}$	$5.4\pm0.33^{b,A}$	$6.0\pm0.34^{b,A}$	$5.2\pm0.36^{b,A}$
B	Urea content, mg dL^{-1}	$19.6\pm0.35^{a,B}$	$30.1\pm0.31^{b,B}$	$31.8\pm0.36^{c,B}$	$26.2\pm0.34^{d,A,C}$
	Urea yield, g	$5.1\pm0.12^{a,B}$	$8.1\pm0.11^{b,B}$	$7.0\pm0.11^{c,B}$	$5.0\pm0.11^{a,A}$
C	Urea content, mg dL^{-1}	$38.1\pm3.11^{a,C}$	$49.0\pm2.76^{b,C}$	$55.3\pm2.58^{b,C}$	$42.3\pm2.40^{b,a,B}$
	Urea yield, g	$5.6\pm0.89^{a,B}$	$9.6\pm0.79^{b,C}$	$10.7\pm0.74^{b,C}$	$6.4\pm0.69^{a,A,B}$
D	Urea content, mg dL^{-1}	$25.7\pm0.4^{a,D}$	$25.0\pm0.39^{a,D}$	$29.3\pm0.42^{b,D}$	$27.2\pm0.38^{c,C}$
	Urea yield, g	$8.4\pm0.22^{a,C}$	$7.2\pm0.20^{b,D}$	$7.4\pm0.22^{b,B}$	$6.8\pm0.20^{b,c,B}$

$^{a; b; c; d}$ milk urea content and urea yield with unequal letter differed significantly between seasons ($p<0.05$);
$^{A; B; C; D}$ milk urea content and urea yield with unequal letter differed significantly between farms ($p<0.05$).

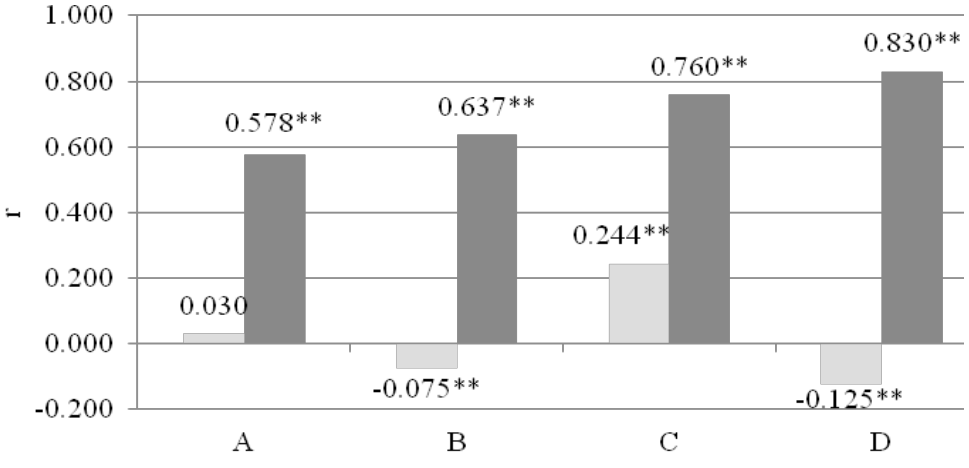

Figure 1. Correlation between cow milk urea content, yield and milk yield in farms (**$p<0.01$):

Urea content in farms B and D was similar with no significant differences between milk yield levels, whereas in farm C it was significantly higher and increased with increase in milk yield. Farm A had the lowest urea content in milk in all milk yield levels. The results of our study confirm previous researchers (Oltner et al., 1985) that milk urea content increase than increase milk yield.

Milk urea yield differed between farms and between milk yield levels (Fig.3). Our study demonstrated that milk urea yield increased with increase in milk yield. In farms B and D urea yield were similar which can suggests a good balanced feeding management according to the lactation stage in farms. Whereas in farm C, average urea yield was higher than in other farms, indicating problems with the feeding balance and management. Also German researchers (Spiekers and Obermaier, 2012) have established influence of the milk yield level on urea content in cow milk.

Many researchers (Jonker et al., 2001; Dijkstra et al., 2011; Gruber and Poetsch, 2012) indicate usability of milk urea content in practice to control protein utilization in farm.

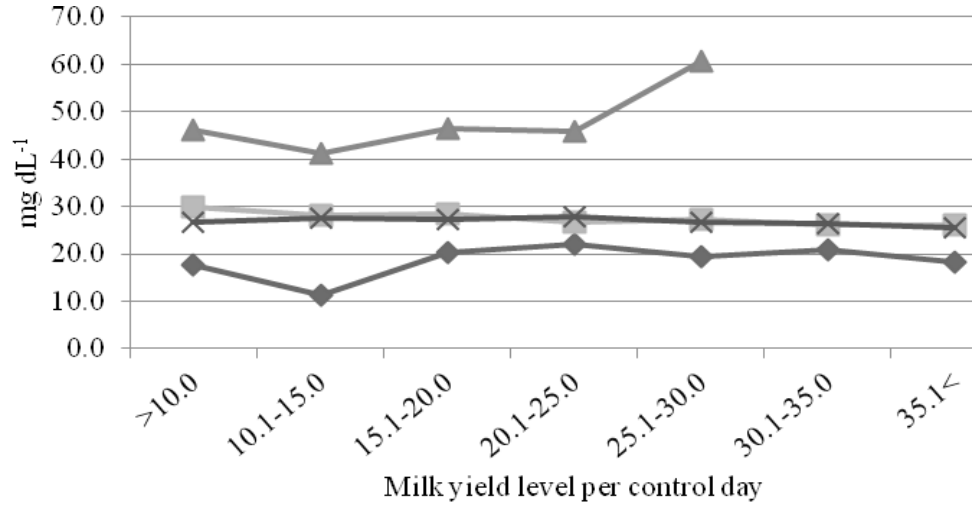

Figure 2. Urea content depending on the level of control day milk yield per cow:

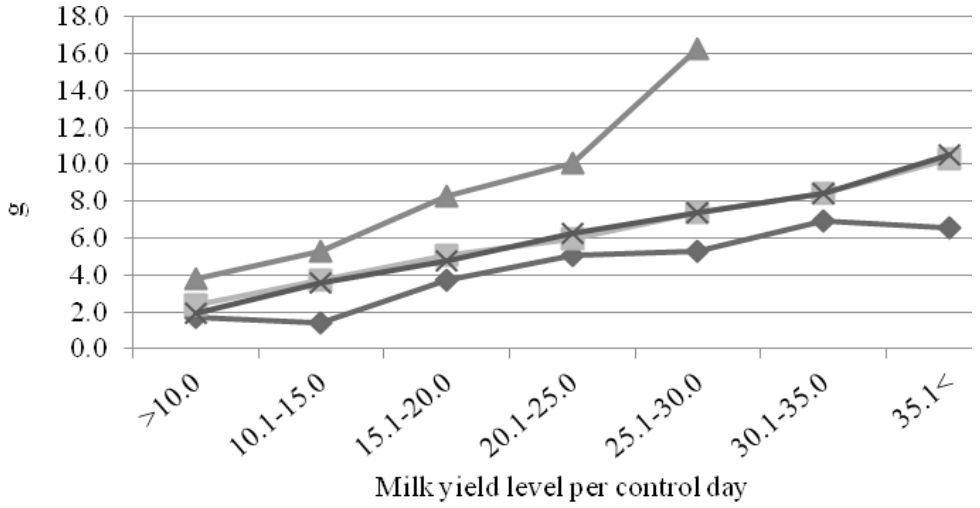

Figure 3. Urea yield depending on the level of control day milk yield per cow:

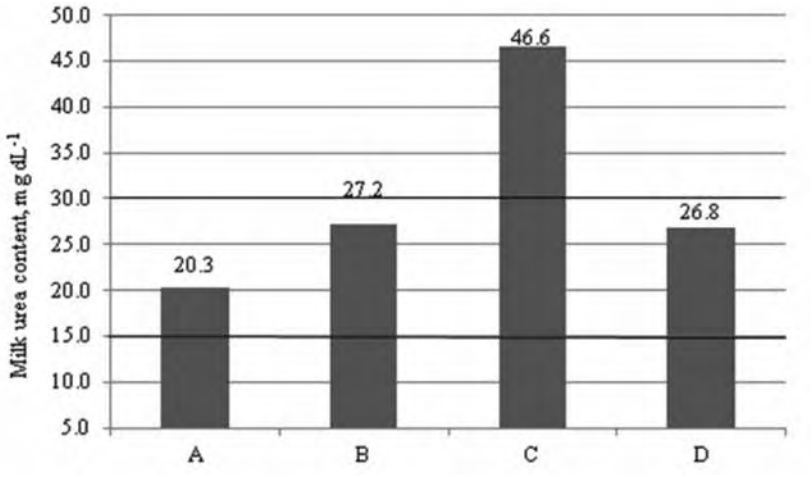

Figure 4. Milk urea content per control day in farms.

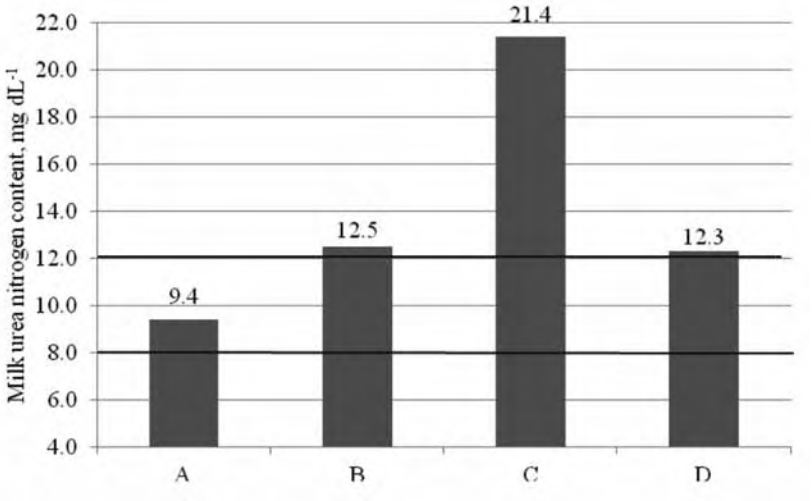

Figure 5. Milk urea nitrogen content per control day in farms.

Researchers from Europe (Bijgaart, 2003) confirm that normal milk urea content in milk is from 15.0 to 30.0 mg dL^{-1}. In our study, milk urea content differed among the farms. Farm C were urea content in milk significantly highest than recommended. The measurements of milk urea content could be used to assess the adequacy of protein feeding in dairy cows and the efficiency of N utilization for milk production (Jonker et al., 1998; 2002; Nousiainen et al., 2004). The value of 20.8 mg 100 mL^{-1} milk urea content has turned out to correspond to an optimal crude protein content of the ration, i.e., a ruminal N balance of zero. The statistical evaluation of the official milk recording and breeding organisation in Austria indicates that the average milk urea content is around 20 – 22 mg 100 mL^{-1} in the relevant milk yield classes (3000 – 7000 kg milk) (Gruber and Poetsch, 2012).

Researchers from United States use milk urea nitrogen (MUN) content for evaluation of the utilization of feed protein (Depeters et al., 1992). Milk urea nitrogen content makes 46% from the milk urea content (Spiekers and Obermaier, 2012). We estimate milk urea nitrogen content for our study results following the principles (Fig. 5).

In the United States, normal MUN content in milk is from 8.0 to 12.0 mg dL^{-1}. In our study, farm A had the recommended MUN content, which suggests a good feed protein utilization in the farm. Farms B and D had a similar MUN content, which was higher than the normal content, whereas farm C had a two times higher MUN content than the norm, which indicates the problem with farm feeding or management technology. The MUN content used for evaluation of nitrogen utilization is more sensitive than urea content.

The results of our study approval monitoring nitrogen utilization in the farms urea content are useful to indicate problem with feeding management and potential nitrogen loses.

Conclusions

1. It was established that milk urea content and yield significantly ($p<0.05$) varied in farms with different

dairy cow holding and feeding technologies and in different seasons.
2. In farms A, B, and D, milk urea content was not higher than the allowable level (from 15.0 to 30.0 mg dL^{-1}) which suggests balanced feeding or good management in the farm.
3. The evaluation between milk yield with milk urea content and yield were significant from low negative (r= -0.125) to high positive (r=0.830) was established.
4. The milk urea content significantly ($p<0.05$) varied for cows with different milk yield per day. The cow with the highest milk yield had the highest milk urea content.

Acknowledgements

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REHYDRATION KINETICS OF DRIED LATVIAN CRANBERRIES AFFECTED BY DRYING CONDITIONS

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Abstract
The aim of the current research was to study the effect of drying conditions on the rehydration kinetics of Latvia wild grown and on cultivated cranberries. The research was accomplished on fresh wild cranberries and cultivated cranberry cultivars 'Ben Lear' and 'Pilgrim' harvested in Kurzeme region of Latvia in the first part of October 2010 and immediately used in the drying experiment. Three methods were used for pre-treatment of berries: perforation, halving and steam-blanching. Before drying in a convective drier the berries were pre-treated using all three methods and berries were dried in a microwave vacuum drier using two pre-treatment methods – steam-blanching and halving. Parts of berries were dried in the microwave vacuum drier without pre-treatment (whole berries). For drying experiments, convective and microwave vacuum drier were used. Cranberry samples were rehydrated in water at +20±1 °C and +40±1 °C. The moisture content of the cranberry samples after rehydration was estimated as oven-dry method. The rehydration properties of cranberries increased with the increase in temperature, up to +40±1 °C, the increase being more significant at the initial stages of the process. Microwave drying possibly produces a sample with increased porosity, which in turn leads to improved rehydration characteristics and a softer product and may reduce processing time. Pre-treatment of berries did not significantly influence the increasing intensity of moisture content during rehydration, but the drying methods within rehydration at the temperature of +40±1 °C significantly influenced the increasing intensity of moisture content.
Key words: rehydration, cranberries, convective drying, microwave vacuum drying.

Introduction

Dehydrated products can be used in many processed or ready-to-eat foods in place of fresh foods and have several advantages such as convenience in transportation, storage, preparation and use (Lewicki et al., 1998). Dehydrated products need to be rehydrated before consumption or further processing (Oliveira and Ilincanu, 1999). During rehydration, absorption of water into the tissue results in an increase in the mass (Krokida and Marinos-Kouris, 2003). Rehydration is influenced by several factors, grouped as intrinsic factors (product chemical composition, pre-drying treatment, product formulation, drying techniques and conditions, etc.) and extrinsic factors such as composition of immersion media, temperature, and hydrodynamic conditions (Oliveira and Ilincanu, 1999). Some of these factors induce changes in the structure and composition of the plant tissue, which results in the impairment of the reconstitution properties (Taiwo et al., 2002).

Rehydration of dried plant tissues is composed of three simultaneous processes: the imbibition of water into dried material, the swelling and the leaching of soluble (McMinn and Magee, 1997).

Rehydration is a complex process aimed at the restoration of raw material properties when dried material is in contact with a liquid phase. Pre-drying treatments, subsequent drying and re-hydration induce many changes in structure and composition of plant tissue (Lewicki et al., 1997), which result in impaired reconstruction properties. Hence, rehydration can be considered as a measure of the injuries to the material caused by drying and treatments drying dehydration (Lewicki, 1998).

The term *quality* implies the degree of excellence of a product; it is a human perception encompassing many properties or characteristics (Abbot, 1999).

Cranberries belong to a group of evergreen dwarf shrubs or trailing vines in the genus *Vaccinium* subgenus *Oxycoccus*. Traditionally they grow in acidic bogs throughout the cooler parts of the world; when cultivated are grown on low trailing vines in great sandy bogs. Berries are rich in a vitamin C, organic acids, mineral substances, aroma, and phenol compounds. Many cultivars and native species of berries exist with substantially higher antioxidant levels than others (Moyer et al., 2002; Popleva, 2000).

Cranberries and their products have been historically associated with many positive benefits for human health. For many decades, cranberry juice has been widely used as a folk remedy to treat urinary tract infections. Cranberry juice extracts have also been suggested to exhibit anticancer effects and to inhibit the oxidation of low-density lipoprotein *in vitro*, potentially preventing the development of heart diseases (Vattem et al., 2005).

Drying is one of the oldest methods of food preservation and it is a difficult food processing operation mainly because of undesirable changes in quality and the removed water from a food product using conventional air drying may cause serious damage to the dried product (Wang and Xi, 2005). It is the most common and most energy-consuming food preservation process. With literally hundreds of

variants actually used in drying of particulate solids, pastes, continuous sheets, slurries or solutions, it provides the most diversity among food engineering unit operations. Air-drying, in particular, is an ancient process used to preserve foods in which the solid to be dried is exposed to a continuously flowing hot stream of air where moisture evaporates. The phenomena underlying this process is a complex problem involving simultaneous mass and energy transport in a hygroscopic, shrinking system. Air-drying offers dehydrated products that can have an extended shelf life of a year but, unfortunately, the quality of a conventionally dried product is usually drastically reduced from that of the original foodstuff (Ratti, 2001; Feng and Tang, 1998).

Microwave drying is rapid, more uniform and energy efficient compared to a conventional hot air drying. In this case, the removal of moisture is accelerated and, further-more, heat transfer to the solid is slowed down significantly due to the absence of convection. Also because of the concentrated energy of a microwave system, only 20–35% of the floor space is required, as compared to conventional heating and drying equipment. However, microwave drying is known to result in a poor quality product if not properly applied (Drouzas and Shubert, 1996; Yongsawatdigul and Gunasekaran, 1996).

Water accounts for the bulk of the dielectric component of most food systems especially for high moisture fruits and vegetables. Hence, these products are very responsive to microwave applications and will absorb the microwave energy quickly and efficiently as long as there is residual moisture. Microwave application for drying therefore offers a distinct advantage. Proteins, lipids and other components can also absorb microwave energy, but are relatively less responsive. A second advantage of microwave application for drying of vegetables is the internal heat generation (Wang and Xi, 2005).

The aim of the current research was to study the effect of drying conditions on the rehydration kinetics of wild and cultivated cranberries in Latvia.

Materials and Methods

The research was accomplished on fresh, wild (*Vaccinium oxycoccus* L.) and cultivated (*Vaccinium macrocarpon* Ait.) cranberries harvested in Kurzeme region in the first part of October 2010 and immediately used in the current drying experiment. Cranberry cultivars were: 'Ben Lear' and 'Pilgrim'. Cranberries were used in the experiment immediately after drying.

Three methods were used for pre-treatment of berries: perforation, halving, and steam-blanching. Before drying in a convective drier, berries were pre-treated using all three methods, and berries were dried in a microwave vacuum drier using two pre-treatment methods – steam-blanching and halving. Part of berries were dried in the microwave vacuum drier without pre-treatment (whole berries).

Perforation of berries (3.000±0.001 kg) was realised manually with a needle (1 mm in diameter) - about 20 pricks on each berry surface; halving (3.000±0.001 kg) was realised manually with knife; steam-blanching (3.000±0.001 kg) was realised using "TEFAL VC4003 VITAMIN+" (Tefal, China) vessel at the temperature of +94±1 °C.

Drying conditions was elected accordingly to the results of previous experiments for maximal biological compounds preservation in cranberries during processing in elevated temperatures (Dorofejeva et al., 2010).

For air drying experiments, the convective drier "Memmert" Model 100-800 (Memmert GmbH Co. KG, Germany) was used; drying parameters were as follows: temperature - 50±1 °C, and air flow velocity - 1.2±0.1 m s^{-1}. Berries were placed on a perforated sieve (diameter − 0.185 m), with the diameter of the holes – 0.002 m.

For drying experiments the vacuum microwave drier „Musson-1" (OOO Ingredient, Russia) was used (at 2450 MHz frequency and 12.5 cm wavelength) (Vacuum microwave drier MUSSON-1, 2007). The power of each of the four magnetrons was 640 W. The necessary amount of microwave energy (magnetron minutes) was calculated. The following drying conditions for processing of cranberries in the microwave vacuum drier were selected: the first drying stage at 4 magnetrons – energy of 2100 kJ, the second stage at 3 magnetrons – energy of 2520 kJ, the third stage at 2 magnetrons – 1260 kJ, and the fourth stage at 1 magnetron – 756 kJ. Temperature in the microwave vacuum drier was 36±2 °C (moisture content - 9±1%).

Cranberry samples were rehydrated in water at +20±1 °C and +40±1 °C (temperature not higher than +40±1 °C was chosen mainly for maintenance of berries biological value). Samples were rehydrated by immersion: 3g of each sample in 100±1 ml of distilled water. The samples were weighed after 60 minutes of rehydration till unchanged weight. Sample weight changes were controlled throughout the hydration process. Before weighing, samples were gently blotted in order to remove the superficial water (Singh et al., 2008).

The moisture content of the cranberry samples after rehydration was estimated as oven-dry method at +105±1 °C (Temmingoff and Houba, 2004). All the experiments were performed in triplicate and average values were reported.

Data were expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 was used to determine the significant differences.

Two-way ANOVA and three-way ANOVA analysis by IBM SPSS 20.0 were used to investigate the influence of factors and interactions among them.

Results and Discussion

Dehydration operations are important steps in the chemical and food processing industries. The basic objective in drying food products is the removal of water in the solids up to a certain level, at which microbial spoilage is minimized. The wide variety of dehydrated foods, which today are available to the consumer (snacks, dry mixes and soups, dried fruits, etc.), and the interesting concern for meeting quality specifications and conserving energy emphasize the need for a thorough understanding of the operation and the problems related to the design and operation of dehydration and rehydration plants (Vagenas and Marinos-Kouris, 1991).

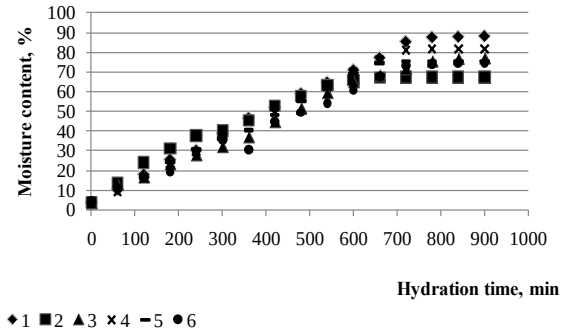
The effect of temperature on the water absorption of severally pre-treated and dried cranberry cultivars "Ben Lear", "Pilgrim" and wild berries are shown in Figures 1, 2 and 3.

In the present research it was detected that temperature had an influence on rehydration behaviour with initial rehydration rates increasing with temperature of up to +40±1 °C (Figs. 1, 2 and 3). In experiments it was found that the cultivar of berries, pre-treatment, drying conditions and hydration solution (water) temperature influence the rehydration capacity. As a result, hydration water quantity was different too.

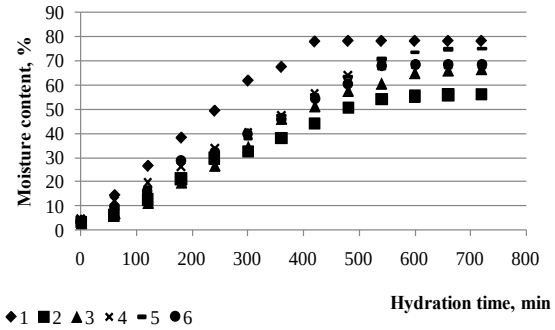
In other researches it has been detected that moisture content of fresh wild berries initially was 85.91±2.40%, of cranberry cultivar "Pilgrim" – 87.36±2.12%, and of cranberry cultivar "Ben Lear" – 87.36±2.91% (Dorofejeva et al., 2011).

For cranberry cultivar "Ben Lear", after (perforated and dried in convective drier) rehydrating the berries in water at the temperature by +20±1 °C the initial moisture content (i.e. 88.56±1.20 %) was reached after 14 h (Fig. 1). However, if severally ways pre-treated berries were dried in the microwave vacuum drier – maximal moisture content (75.00±1.14%), was reached after 13 h. For berries rehydrated in water at +40±1 °C, the moisture content was lower (70.00±1.36% in average) and rehydration time was shorter (9 h) (Fig. 1). The obtained results could be explained with drying methods particularity. Within berries processing in microwave vacuum drier, structure of berries was changed less, than if cranberries were dried in oven-dry drier. As a result the cells of berries absorb water faster and swell more rapidly.

Similar results were acquired in experiments with cranberry cultivar "Pilgrim". However, for cranberry cultivar "Pilgrim", the maximal moisture content (75.00±0.32%) during rehydration was reached at the temperature by +20±1 °C (Fig. 2), which was by ~12% less compared the moisture content by fresh berries. Rehydration time at the temperature by +20±1 °C was 13−14 h. For berries rehydrated at +40±1 °C, the maximally reached moisture content was 85.14±2.18% (Fig. 2), which was very similar to parameters of fresh berries. The rehydration time was 13 h.

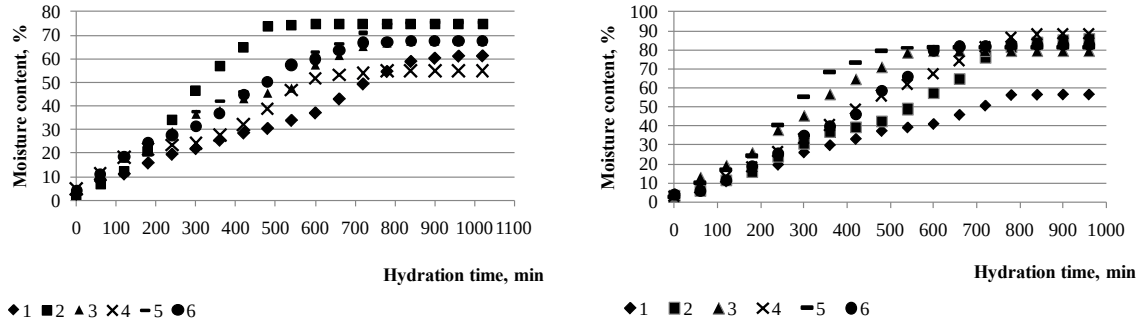


Rehydration temperature +20±1 °C



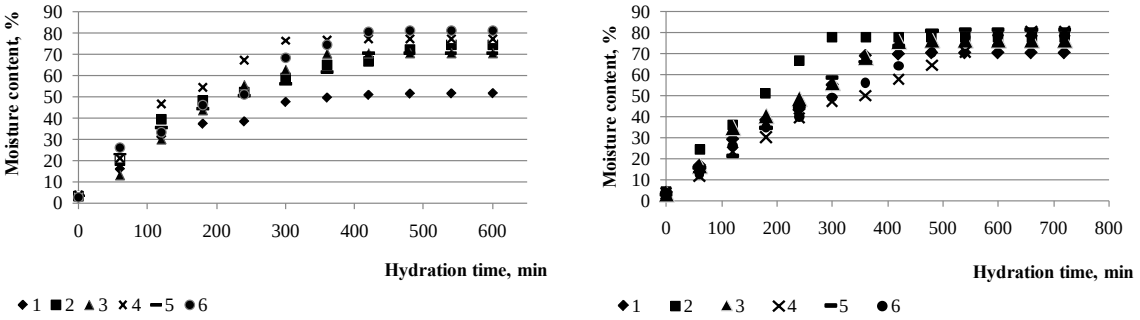
Rehydration temperature +40±1 °C

Figure 1. Rehydration water quantity of cranberry cultivar "Ben Lear".
1 *– perforated berries dried in convection drier;* ***2*** *– halved berries dried in convection drier;* ***3*** *– steam-blanched berries dried in convection drier;* ***4*** *– whole berries dried in microwave-vacuum drier;* ***5*** *– halved berries dried in microwave-vacuum drier;* ***6*** *– steam-blanched berries dried in microwave-vacuum drier.*



Hydration temperature +20±1 °C Hydration temperature +40±1 °C

Figure 2. Rehydration water quantity of cranberry cultivar "Pilgrim".
1 *– perforated berries dried in convection drier;* ***2*** *– halved berries dried in convection drier;* ***3*** *− steam-blanched berries dried in convection drier;* ***4*** *– whole berries, dried in microwave-vacuum drier;* ***5*** *− halved berries dried in microwave-vacuum drier;* ***6*** *− steam-blanched berries dried in microwave-vacuum drier.*



Hydration temperature +20±1 °C Hydration temperature +40±1 °C

Figure 3. Rehydration water quantity of wild cranberries.
1 *– perforated berries dried in convection drier;* ***2*** *– halved berries dried in convection drier;* ***3*** *− steam-blanched berries dried in convection drier;* ***4*** *– whole berries dried in microwave-vacuum drier;* ***5*** *− halved berries dried in microwave-vacuum drier;* ***6*** *− steam-blanched berries dried in microwave-vacuum drier.*

Opposite results were obtained in rehydration experiments with wild cranberries. Were moisture content in berries ranged from 52% to 81% (Fig. 3). However, the highest moisture content was found in berries rehydrated at +40±1 °C. Rehydration time at the temperature by +20±1 °C and +40±1 °C was similar – 8−9 h.

In our experiments the acquired results were very various. Therefore, for the detection of significance of differences mathematical data processing was used. As a result, Greenhouse-Geisser test was suited. It was established that, for severally processed berries rehydration in water at temperature +20±1 °C, that changes in moisture content within the tested time period were significant with p=0.0001; however, pre-treatment of berries and drying methods did not significantly influence the changes in moisture content (p=0.6830) (Table 1) during rehydration.

Very similar results were established in mathematical data processing of results acquired during berries rehydration at the +40±1 °C temperature (Table 2). Significant moisture content changes were found within rehydration time. It was proved that pre-treatment by berries does not significantly (p=0.311) influence the intensity of moisture content increases within hydration at the temperature by +40±1 °C (Table 2). However, drying methods significantly (p=0.001) influenced the intensity of moisture content increases within rehydration, mainly could

Table 1

Tests of within-subjects effects (hydration at temperature +20±1 °C)

Source	Type III sum of squares	Degree of freedom	Mean square	Variance ratio, F	Significance, p value
Rehydration time	465511.918	1.742	267186.531	525.119	0.000
Rehydration time and pre-treatment method	2021.390	3.485	580.101	1.140	0.341
Rehydration time and drying	301.478	1.742	173.037	0.340	0.683
Rehydration time, pre-treatment and drying method	1980.906	3.485	568.483	1.117	0.351
Error	42551.424	83.629	508.811	-	-

Table 2

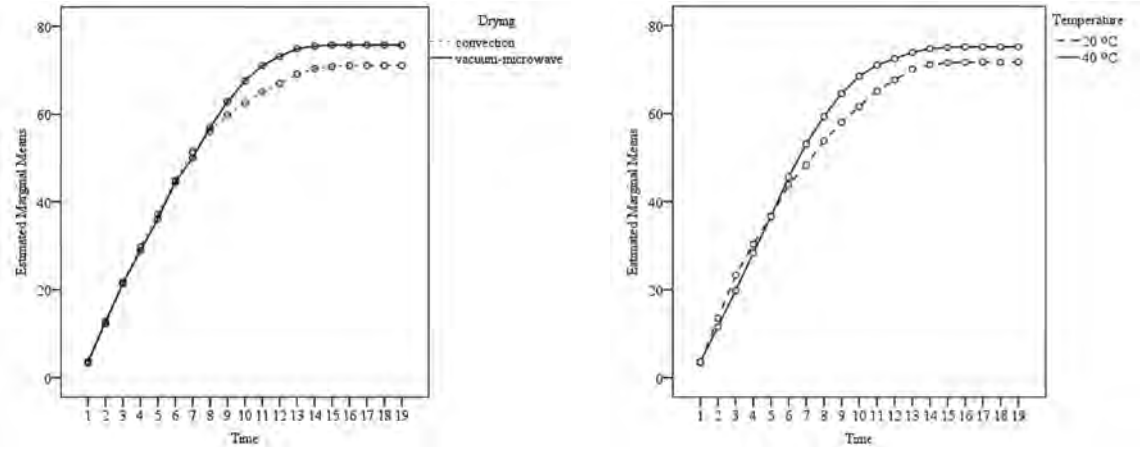
Tests of within-subjects effects (hydration at temperature +40±1 °C)

Source	Type III sum of squares	Degree of freedom	Mean square	Variance ratio, F	Significance, p value
Rehydration time	569335.708	1.731	328871.637	936.114	0.000
Rehydration time and pre-treatment method	1476.381	3.462	426.409	1.214	0.311
Rehydration time and drying	5299.336	1.731	3061.113	8.713	0.001
Rehydration time, pre-treatment and drying method	3862.506	3.462	1115.571	3.175	0.023
Error	29193.150	83.097	351.316	-	-

be explained with the influence of drying temperature and time on the structure of berries. Interconnection in berries pre-treatment and drying methods significantly (p=0.023) influenced the changes in moisture content during rehydration, with maximal probability P=97.70%.

Pre-treatment method, berry cultivar and rehydration temperature had no significant influence on rehydration capacity by berries if berries were dried in convection drier. However, if berries were dried in microwave-vacuum drier the rehydration temperature had the major influence on rehydration capacity.

If time factor is not taken into consideration in mathematical data analyse, interconnection (berry pre-treatment and drying methods, rehydration temperature) of analysed factors has not significant influence on rehydration capacity with probability P=95.00%.



(A) (B)

Figure 4. Influence by drying conditions (A) and rehydration temperature (B) on berry hydration capacity.

It was ascertained that pre-treatment methods by berries do not significantly (P=83.90%) influence the rehydration capacity. Whereas, drying methods (P=99.10%) and rehydration temperature (P=99.50%), on the contrary have significant influence on the rehydration process by berries (Fig. 4).

Conclusions

1. The rehydration properties of cranberries increased with the increase in temperature, up to +40±1 °C, the increase being more significant at the initial stages of the process.
2. Berries pre-treatment did not significantly influence the intensity of moisture content increase during rehydration. Whereas, drying methods significantly influenced the intensity of moisture content increase during rehydration at the temperature of +40±1 °C.
3. Pre-treatment method, berry cultivar and rehydration temperature had no substantial influence on cranberry rehydration capacity if berries were dried in convection drier. However, if berries were dried in microwave-vacuum drier, rehydration temperature had the main influence on the rehydration process.

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PRELIMINARY RESULTS OF 1-METHYLCYCLOPROPENE INFLUENCE ON APPLE QUALITY DURING STORAGE

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Abstract
Apples are the most popular and common fruits in Latvia. Storage technology is crucial to preserve fruit quality as long as possible. Choosing the appropriate gas content of the storage environment can prolong storage life two to three times for apples. The aim of the research was to compare six different type of apple grown in Latvia, which was stored in diverse conditions. All experiments were performed at Latvia State Institute of Fruit-Growing through 2011 – 2012. For fruits before storage, 1-methylcyclopropene (1-MCP), which blocks the emission of ethylene, was used. Apples were stored in a cooler and in ULO type plastic bags in a modified environment with two different gas contents. The temperature of the environment was +2 ±1 °C with 90% relative humidity. Changes in physico-chemical (soluble solids, total acids and flesh density) indexes were examined before and during the storage. The results showed 1-MCP has a positive effect on quality preservation of fruit. Fruits stored in ULO type plastic bags (gas content: 1.5% O_2 and 2.5% CO_2) had the best results in preservation of physico-chemical indexes. Examination of results revealed that physico-chemical indexes changed the most in samples stored in the cooler.
Key words: acid, density, soluble solids, 1-MCP, ULO.

Introduction

Fresh fruits are the basis of a healthy diet because they supply human body with the necessary biologically active elements. Apples contain organic acids like apple and citric acids (0.2 - 0.7%) which is why they have sour taste (Dofer, 2006). The amount and content of vitamins are closely related to storage time and conditions. Storage in non-modified environment with lowered temperature for six months results in the decrease of the amount of C vitamins by ~50%, whereas it does not significantly affect vitamins of group B, microelements and fibres (Wilkinson, Perring, 2006). Chemical composition and quality changes depend on apple cultivar, growing conditions, and harvesting time. If fruits are not ripe enough before the storage they dehydrate more intensively, but if harvested too late apples will rot during or just after the storage. Shelf life period would be very short for such apples (Sfakiotakis et al., 2008).

For the last 50 years fresh fruits are stored in modified gas environment all over the world. Modified gas environment allows storing them for up to one year (Akbudak et al., 2002). According to Food and Agriculture Organization of the United Nations, 79% of apples grown in Europe are stored in modified atmosphere. However, it has several drawbacks, for example flavour does not develop and the colour of apples remains exactly the same as at the beginning of the storage. It is advised to sell the fruits within a month after the storage. If the gas content of the modified environment is chosen incorrectly, the unpleasant hint of taste and flavour, and physiological disorders, such as skin burn, flesh breakdown and conducting tissue browning can develop (Marin et al., 2009).

Ten years ago, scientists from North Carolina University, USA, discovered a substance – methylcyclopropene (1-MCP) - which reacting with water emits a gas that blocks the emission of ethylene. Ethylene is a substance responsible for apple aging process, but 1-MCP blocks its effect so quality indexes of the fruits can be preserved (Beaudry, Watkins, 2003). It has been five years from now since 1-MCP is used on an industrial scale. According to European Food Safety Authority EFSA, MCP was used for storage of vegetables and cut flowers in 26 countries in 2010. During five years of research the effectiveness of 1-MCP has been proved. In order to use the 1-MCP it is essential to determine the precise level of ripeness of apples, processing must be performed in airproof environment and total fruit mass and cabinet capacity ratio must be considered (Watkins et al., 2008). Until now, no research has been conducted on the effect of 1-MCP on apple quality during the storage in Latvia. The aim of the presented study was to investigate preliminary results of apple storage in different conditions.

Materials and Methods

Experiment scheme

The research was conducted in the Unit of Fruit and Berry Experimental Processing of Latvia State Institute of Fruit-Growing. Six apple cultivar fruits of the 2011 yield were used: 'Auksis', 'Orlik', 'Antej', 'Zarja Alatau', 'Belorusskoje Malinovoje', and 'Sinap Orlovskij'. Before storage, fruits were treated with 1-MCP that was diluted with water at 1:30 ratio in a conical flask. After dilution, the flask was immediately closed as well as the processing

Table 1

Modified atmospheres for apple storage

Atmosphere	O_2 (%)	CO_2 (%)	N_2 (%)
Modified atmosphere (ULO 1)	1.00	2.00	97.00
Modified atmosphere (ULO 2)	1.50	2.50	96.00
Non-modified atmosphere (cooler)	20.96	0.03	78.06

cabinet was hermetically closed. The treatment with 1-MCP took 12 hours at +18 °C temperature. After processing, apples were stored in type ULO plastic bags at +2 ±1 °C with relative humidity of 90% in two differently modified atmospheres (Table 1). Control samples (not processed) were stored in a cooler with the same temperature and humidity. The duration of the research was 9 months. In the paper intermediate results after 3 months were evaluate.

Before and after the storage the following physico-chemical indexes of apples were determined using standardized methods:

- content of soluble solids according to LVS EN 12143:2001 standard;
- content of titrable acids according to LVS EN 12147:2001 standard;
- density was measured with penetrometer according to LVS EN 1131:2001 standard.

Mathematical data processing

SPSS software version 11.0. was used for data processing using the methods of descriptive statistics and analysis of variance (ANOVA). Statistical difference would be considered significant if $p<0.05$.

Results and Discussion

Changes in the amount and content of acids in apples depend on ripeness. Results of the research (Table 2) show that all cultivars had the highest amount of acids before the storage. Similar results have been obtained with the cultivar 'Empire' (Fawbush et al., 2009). In our research, conducted in Dobele, cultivar 'Auksis' showed the largest decrease in acid amount regardless of the storage technology. These apples had the lowest acid content when stored in the cooler, but when stored in 1-MCP+ULO 2 the acid content was the highest. Modified environment slows down the metabolism of fruits (Blažek et al., 2003) which explains why apples stored in modified atmosphere had the highest total amount of acids.

Also for the cultivar 'Orlik', the amount of acids significantly decreased in samples stored in the cooler ($p<0.05$). This changes can be explained similarly as with the cultivar 'Auksis': modified environment cabinet and processing with 1-MCP inhibitor slows down the metabolism of the fruit (Villatoro et al., 2008). A relatively high level of acids before the storage was observed in cultivar 'Antej'. During storage it significantly decreased ($p<0.05$) with all storage types. The amount of acids in cultivar 'Zarja Alatau' regardless of storage type changed notably ($p<0.05$). Apples of the cultivar 'Belorusskoje Malinovoje' that were stored in the cooler had the largest decrease in acid amount (almost 50%), but for the ones stored in ULO environment it did not change markedly. The highest total amount of acids before storage was detected in cultivar 'Sinap Orlovskij', but later it significantly decreased regardless of the storage, which means that ripening process continued during the storage. The amount of acids was preserved the best in the environment 1-MCP+ULO 2 for all cultivars.

Table 2

Change in total amount of acids depending on storage type (%)

Storage type	Cultivar					
	'Auksis'	'Orlik'	'Antej'	'Zarja Alatau'	'Belorusskoje Malinovoje'	'Sinap Orlovskij'
Before storage	0.65±0.01	0.41±0.01	0.70±0.00	0.59±0.02	0.67±0.01	0.82±0.02
Cooler	0.40±0.02*	0.37±0.00*	0.57±0.01*	0.49±0.01*	0.34±0.01*	0.63±0.00*
1-MCP+cooler	0.43±0.02*	0.38±0.01	0.59±0.01*	0.49±0.01*	0.51±0.01*	0.65±0.01*
1-MCP+ULO 1	0.44±0.01*	0.39±0.01	0.59±0.01*	0.49±0.01*	0.62±0.02	0.67±0.01*
1-MCP+ULO 2	0.50±0.00*	0.41±0.01	0.65±0.01	0.51±0.02	0.60±0.02	0.66±0.02*

Data expressed as a mean ± standard deviation; n=3; * Significant changes observed when $p<0.05$

Table 3

Change in the content of solids depending on storage (°Brix)

Storage type	Cultivar					
	'Auksis'	'Orlik'	'Antej'	'Zarja Alatau'	'Belorusskoje Malinovoje'	'Sinap Orlovskij'
Before storage	14.50±0.01	11.52±0.01	11.29±0.02	11.87±0.01	10.25±0.02	11.84±0.02
Cooler	13.22±0.03*	11.50±0.02	10.54±0.01	11.86±0.05*	10.31±0.01	11.81±0.06*
1-MCP +cooler	13.56±0.10*	11.57±0.04	12.34±0.04*	11.94±0.03*	10.42±0.07	11.99±0.06*
1-MCP +ULO 1	13.39±0.03*	11.62±0.02*	11.55±0.01*	11.94±0.03*	10.76±0.01*	12.10±0.02
1-MCP +ULO 2	13.30±0.10*	11.62±0.02*	12.34±0.04	12.65±0.01*	11.55±0.51*	12.79±0.02*

Data expressed as a mean ± standard deviation; n=20; * Significant changes observed when $p<0.05$

The amount of soluble solids in apples depends on the cultivar and ripeness of the fruit. It increases during the storage when apples are still ripening but after they are fully ripe it decreases. Amount of soluble solids also depends on the weather during the season (Wiley and Porteous, 2005).

The highest level of soluble solids amount before the storage was observed in cultivar 'Auksis' (Table 3) but it notably decreased during the storage, which means that apples were harvested too late. The amount of soluble solids in cultivar 'Antej' stored in ULO environment slightly increased as apples continued to ripen. Changes in the solids of 'Orlik' after processing with 1-MCP and when in ULO were insignificant. Similarly to the cultivar 'Auksis' all the other winter apples showed the increase in the amount of solids as they continued to ripen during the storage.

Significant changes were observed in cultivars 'Auksis', 'Orlik' and 'Belorusskoje Malinovoje' stored in the cooler and processed with 1-MCP before the storage. Cultivars 'Sinap Orlovskij', 'Zarja Alatau' and 'Antej' did not have notable changes in the content of soluble solids.

Apple flesh density depends on the cultivar and ripeness of the fruit during harvesting (Jung, Watkins, 2008). Riper apples have lower density. In our research all cultivars had higher density during the harvesting (Table 4). Storage type has an effect on flesh density (Zanella, 2003). Winter and late-season cultivars were stored together in our research, although it is suggested to store them separately (Watkins, 2003).

Flesh density significantly changed in all cultivars ($p<0.05$). In cultivar 'Antej' it decreased almost three times. Late-season cultivars 'Orlik' and 'Auksis' had lower density before the storage than winter cultivars. Cooling technology was minimally change density for cultivars 'Sinap Orlovskij' and 'Zarja Alatau'. When apples treated with 1-MCP and stored in cooler cultivars 'Orlik', 'Auksis', and 'Belorusskoje Malinovoje' had similar results 'Sinap Orlovskij', 'Antej', and 'Zarja Alatau' maintained the highest density ($p<0.05$). In both 1-MCP ULO environments density changes of different cultivars were similar, but 'Zarja Alatau' demonstrated a notably higher density (5.38 kg cm^{-2}). Reliable conclusions cannot be made now because this is the first research of such kind, but the obtained results will help in developing the method of research.

Table 4

Changes in apple flesh density depending on storage type (kg cm^{-2})

Storage type	Cultivar					
	'Auksis'	'Orlik'	'Antej'	'Zarja Alatau'	'Belorusskoje Malinovoje'	'Sinap Orlovskij'
Before storage	8.26±0.39	8.24±0.28	11.06±0.27	13.39±0.25	12.45±0.83	14.79±0.53
Cooler	3.28±0.16*	3.31±0.13*	3.43±0.16*	4.21±0.12*	2.99±0.12*	4.21±0.18*
1- MCP +cooler	3.56±0.17*	3.46±0.14*	4.41±0.12*	5.23±0.12*	3.47±0.07*	4.29±0.16*
1-MCP +ULO-1	3.70±0.17*	3.90±0.11*	4.56±0.14*	5.38±0.13*	3.94±0.18*	4.39±0.10*
1-MCP +ULO-2	4.01±0.18*	4.01±0.15*	4.66±0.17*	4.71±0.13*	3.70±0.14*	3.53±0.21*

Data expressed as a mean ± standard deviation; n=20; * Significant changes observed when $p<0.05$

Conclusions

Modified environment slows down the aging of apples thus changes in their quality during the storage are minimal, especially in winter cultivars 'Belorusskoje Malinovoje', 'Antej', 'Sinap Orlovskij', and 'Zarja Alatau' treated with 1-MCP before the storage in ULO 2-type plastic bags with the following gas content: O_2 (1.50%) and CO_2 (2.50%). Examination of the results revealed that physico-chemical indexes changed the most in samples stored in the cooler.

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SENSORY PROPERTIES AND CHEMICAL COMPOSITION OF CIDER DEPENDING ON APPLE VARIETY

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Abstract
Apple variety influence chemical composition and sensory properties of products obtained from apples. This paper reports the influence of apple varieties on the sensory properties of the cider evaluated by two differently trained panel groups. Juices of five varieties apples ('Auksis', 'Lietuvas Pepins', 'DI-93-4-14', 'Remo' and 'Kerr') were fermented with *Saccharomyces bayanis* EC-1118 (Lalvin, Canada). The sensory evaluation of samples was carried out with two panel groups – experts (experienced in the field of beverage technology and evaluation) and trained panellists (finished basic course of sensory evaluation). Experts identified flavours of cider, and evaluated intensity of sensory properties, namely, clarity, aroma (apple, fruit, yeast), taste (apple, yeast, sour, astringent, bitter) using line scale. Trained panellists evaluated the samples only using line scale. Four descriptors were significant for characterization of differences in ciders from various variety apples, namely, sour taste, apple taste, apple aroma and clarity. The research suggests that, varieties with more intense apple and fruit aroma, apple taste and additionally with astringent and bitter taste notes are preferable. Taking into account these results, higher evaluations for cider 'DI-93-4-14' were observed, followed by 'Remo' and 'Kerr'.
Key words: variety, cider, sensory properties, fermentation.

Introduction

Apple juice is the raw material of different fermented drinks, like apple wine and cider. The fermentation of apple must is a complex process involving several biochemical reactions. Overall quality of cider is influenced by many factors, namely, apple variety, yeasts strains, fermentation conditions, the production process and aging treatments (Hidalgo et al., 2004; Martınez-Rodrıguez and Polo, 2003; Beech, 1993). Cider flavour is composed by a wide variety of compounds with different aromatic properties. Moreover, the main cider aroma holds a close relationship with the type and concentration of aromatic compounds derived from apples (varietal flavour), other compounds are produced by yeasts and bacteria during alcoholic and malolactic fermentation (fermentative flavour) and compounds that appear during the ageing process (post-fermentative flavor) (Boulton et al., 1995). The esters and alcohols which are the products of fatty acid metabolization are the major groups in the apple juice. Esters associated with 'fruity' attributes, account up to 80–98% (Lopez et al., 1998). Phenolics have been shown to be important in the appearance and taste of cider and have been implicated in the quality of the beverage; and the polyphenolic profile of apples and apple drinks is also influenced by several factors: variety, climate, maturity, storage, processing (Van der Sluis, 2001; Ruiz-Rodriguez, 2008; Lata, 2007). Phenolics are associated with bitterness, astringency, and colour stability, and some of them have been used for detecting adulterations in apple products and could be inhibitors for microbiological growth-avoiding process spoilages (Madrera et al., 2006). But last investigations show that, for example, manipulating pressing conditions of apple juice bitterness and astringency decreased much less than the concentrations of native polyphenols (Renard et al., 2011), whichthat bitterness is the result of a more complex process. Not all ciders are made from 'true' cider apples. Many modern ciders have a high proportion of dessert and culinary apple varieties (Lea, 1995). In this work, special attention is drawn to the use of culinary apples for cider production. The apple variety 'Auksis' is the most popular commercially grown variety in Latvia. The apple varieties 'Lietuvas Pepins' and 'Remo' are used as culinary apple for juice production. The apple variety 'DI-93-4-14' is a new perspective apple variety for Latvian growers suitable for juice and wine production. Scrab apple variety 'Kerr' is grown in small areas on the Latvian farms, it is characterized by a clear, fragrant juice with considerable tannins contents, suitable for cider. Sensory properties are some of the most important factors on consumer liking and preference; thus it is very important to determine factors affecting the product attributes, acceptance and preference especially for foods (Dos et al., 2005; Medeiros de Melo et al., 2009). Sensory descriptive analysis is a primary tool of food scientists, which involves the evaluation of both the qualitative and quantitative sensory characteristics of products (Meilgaard et al., 1999). For evaluation of cider sensory properties, Williams (1975) developed 12 classes of descriptors that characterise the main typical flavours.

The aim of current research was to assess sensory properties of cider depending on apple variety evaluated by two differently trained panel groups.

Materials and Methods

Raw materials

Five apple varieties – 'Auksis' (A), 'Lietuvas Pepins' (LP), 'DI-93-4-14' (DI), 'Remo' (R), and 'Kerr' (K) –grown in the Latvia State Institute of Fruit Growing and harvested in September and October 2010 were used In the research. Juice was obtained by mechanical press Voran Basket Press 60K (Voran Maschinen GmbH, Austria). For stabilization of juice before fermentation, 'Tannisol' (Enartis, Italy) was added. Tannisol capsules consist of potassium metabisulphite (added amount to the juice – 9.5 g 100 L^{-1}), ascorbic acid (0.3 g 100 L^{-1}), and tannin (0.2 g 100 L^{-1}). Sulphites have various permitted uses, their primary function is that of a preservative and an antioxidant to prevent or reduce spoilage (Fazio and Warner, 1990), and they help to stabilize colour of the product and inhibit discolouration, thereby improving the appearance and flavour of many foods during preparation, storage and distribution (Adams, 1997).

Fermentation conditions

Fermentation was performed using commercial yeast *Saccharomyces bayanis* EC-1118 (Lalvin, Lallemand Inc., Canada) that is recommended for all types of wines, including sparkling, and cider. Fermentation was carried out at 16±1 °C for 28 days. The apple juice was fermented in a glass bottles (for each cider type n=5) with a volume of 750 ml. For analysis, the average juice samples were combined from the five bottles in equal proportions.

Sensory analysis

Sensory evaluation of fermented apple juices was carried out with two panels – experts (9 women and 2 men, aged 21–51) and trained panellists (31 women and 5 men, aged 21–58). Experts were specialists in the field of beverage technology and experienced in sensory analysis of beverages. Trained panellists had studied the basics of sensory evaluation methods and were experienced in several sensory panels. This group included students and staff of the Faculty of Food Technology.

Two methods of sensory analysis were used:

1) identification of cider flavour using characteristic descriptors divided in 12 classes (Table 1) developed by Williams (1975) – experts;
2) line scale (ISO 4121:2003) for measuring intensity of sensory properties (clarity, aroma (apple, fruit yeast), taste (apple, yeast, sour, astringent, bitter)) – experts and trained panellists.

Table 1

Characterization of cider flavours (Williams, 1975)

Classes	General characterization	Characterization of subclasses
1	Sour, acidic	Acidic, apple (sharp) acid, vinegar, lactic (soft) acid, citrus sour
2	Aromatic, fragrant, fruity, floral	Alcoholic (fusel), solvent-like (plastics, can-liner, acetone), estery (pear drops, apple-like with aniseed note, light fruity), fruity (citrus fruit, banana, blackcurrant, melon, pear, forest fruit, culinary apple, bittersweet apple), acetaldehyde, floral (rose-like, perfume-like, geranium)
3	Spicy, nutty, grassy	Spicy (resinous, woody, spicy bittersweet), nutty (walnut, almond), grassy
4	Caramelised, toasted	Caramel (molasses, raisins), burnt (toasted, rubbery)
5	Chemical	Phenolic (tarry, carbolic, antiseptic, iodoform), plastic, oily (mineral oil, vegetable oil), indole
6	Soapy, fatty, diacetyl, rancid	Fatty acid (soapy, cheesy, rancid butter), butterscotch, rancid
7	Sulphury	Sulphury, sulphur dioxide, sulphidic (rotten egg, drains, autolysed, burnt rubber, shrimp-like, cooked vegetable, cooked cabbage), yeasty
8	Oxidised, stale, musty	Stale, catty, papery, leathery, sherry-like, mouldy (earthy, musty), biscuit
9	Sweet	Honey, artificial (saccharin), vanilla, syrup
10	Bitter	Bitter
11	Mouthfeel	Alkaline, metallic, astringent (drying), powdery, creamy, carbonation (flat, gassy), warming
12	Fullness	Body (watery, characterless, satiating, thick)

Table 2

Chemical and physical parameters of cider

Variety	LP	A	R	K	DI
Titratable acidity, g 100 g^{-1}	0.88 ± 0.01	0.53 ± 0.01	1.01 ± 0.02	1.03 ± 0.02	1.10 ± 0.01
Total phenol content, mg 100 g^{-1} (Riekstina-Dolge et al., 2011)	67.01 ± 1.19	52.76 ± 0.9	45.23 ± 0.47	92.32 ± 0.92	54.01 ± 0.34
Soluble solids, g L^{-1}	0.77±0.02	0.51±0.03	1.03±0.02	1.28±0.05	1.28±0.04
Ethanol content, % vol.	5.07 ± 0.05	5.11 ± 0.09	5.10 ± 0.10	4.81 ± 0.13	4.99 ± 0.08

Chemical analysis

The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colometric method. Fermented apple juice was diluted with ethanol/acetic acid solution (1:20 v/v). Ethanol/acetic acid solution was prepared using acetic acid water solution (2.5%) and ethanol (98% vol.) in ratio 10:90 (v/v). A total of 0.5 ml of aliquot was mixed with 0.25 ml of Folin-Ciocalteu reagents. After 3 min 1 ml of 20% Na_2CO_3 and 3.25 ml of distilled water were added. Samples were heated for 10 min at 70 °C and kept for 30 min at 18 ±2 °C temperature. The absorbance was measured at 765 nm using the spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (mg L^{-1}). Each determination was performed in triplicate and results are expressed as mean ± SD. Titratable acidity was determined according to standard (LVS EN 12147:2001) procedure. Ethanol and soluble solids were separated using distillation procedure, and analysed gravimetrically – distillate for ethanol content and residues for soluble solids determination.

Statistical analysis

Analysis of variance was performed by ANOVA procedure, and $p<0.05$ was considered as statistically significant. Linear correlation analysis was performed with the software SPSS 17.00 for Windows.

Results and Discussions

Chemical and physical parameters of cider

Sensory perceptions are due to the physicochemical composition of ciders. Quality parameters of the analysed samples are given in Table 2. Titratable acidity of the samples ranged from 0.53 to 1.10 g 100g^{-1}

Table 3

Characterization of cider flavours by experts

Classes	General characterization	Characterization of subclasses	Samples				
			LP	K	A	DI	R
1	Sour, acidic	apple (sharp) acid	+	+	+	+	+
		vinegar	+	+	-	+	+
		lactic (soft) acid	-	-	+	+	-
		citrus sour	+	-	+	+	+
2	Aromatic, fragrant, fruity, floral	alcoholic (wine, fusel)	+	+	-	+	+
		esters (apple-like)	+	+	+	+	+
		citrus fruit	+	+	+	+	+
		pear	-	+	-	-	-
3	Spicy, nutty, grassy	woody	-	+	-	-	-
		nutty	+	-	-	+	-
		spicy	+	-	-	-	-
		grassy	-	+	-	-	-
4	Caramel	caramel	-	+	-	-	+
6	Soapy, fatty, diacetyl, rancid	fatty acid	-	+	-	-	-
10	Bitter	bitter	-	-	+	+	+
11	Mouthfeel	astringent	+	+	+	+	+
12	Fullness	body (characterless)	-	+	+	-	+
		body (satiating)	+	-	-	+	-

+ flavours identified in cider; - flavours not identified in cider

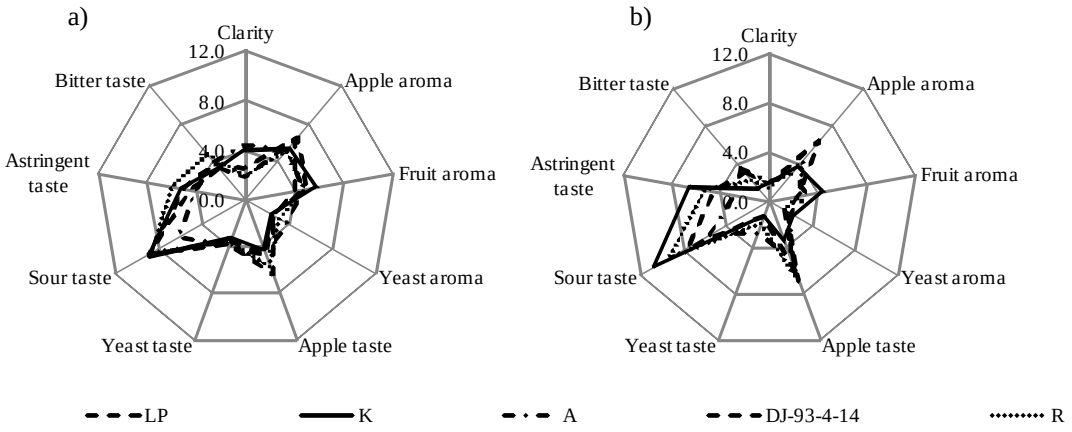

Figure 1. Evaluation of sensory properties of cider by trained panellist's (a) and experts (b).

– the highest content was determined in samples DI, K and R, whereas the lowest – in sample A. The content of total phenols varied depending on apple variety: the highest content was found in sample K (Table 2) but the lowest – in cider R. Variety 'K' belongs to scrab apple varieties and is suitable for cider fermentation (Riekstina-Dolge et al., 2011). Sanoner P. et al. (1999) also reported that polyphenol concentration is higher in cider varieties apples comparing to dessert apples.

Ethanol content of fermented drinks is critical for sensory evaluation. In mixtures without ethanol the fruity smell is strong; however, the intensity of the smell decreases with the increasing concentration of ethanol (Escudero et al., 2007). Also ethanol - induced palate warmth and perceived viscosity may indirectly affect both aroma and flavour perception (Delwiche, 2004). In all analysed samples, alcohol content did not differ significantly (Table 2), and it could not influence evaluation of other sensory properties.

Sensory evaluation of cider flavours

In analysed samples experts identified descriptors of eight classes of cider flavours: 1) sour, acidic; 2) aromatic, fragrant, fruity, floral; 3) spicy, nutty, grassy; 4) caramel; 5) soapy, fatty, diacetyl, rancid; 6) bitter; 7) mouthfeel and 8) fullness (Table 3).

In all samples, sharp acidity was found. Experts identified citrus notes in all samples but pear flavour only in cider K. In cider K also alcohol, wine and fusel notes, were observed.

In the identification of flavours, experts characterised the mouthfeel of fermented juices 'K', 'LP' and 'R' as astringent and dry. Mouthfeel of samples DI, K and LP was described as fullness, but R and A – as characterless.

Evaluation of the intensity of sensory properties

The evaluation of sensory properties by experts and trained panellists was analysed separately, and the results are presented in Fig.1.

According to ANOVA, the effect of apple variety was significant ($p<0.05$) for the following sensory properties: clarity, and apple and sour taste – for trained panellists, and apple aroma, and sour and apple taste – for experts.

Evaluation by trained panellists showed differences in juice clarity; significantly higher results for cider A were identified. It could be explained by pulp structure and maturity of apples. Maturity is an important factor influencing fruit quality (Streif, 1996); and if apples are harvested too early taste could be sour or starchy, and apples harvested too late may be soft and mealy. Starch also causes problems in juice clarification.

Experts and trained panellists evaluation showed that there is not significant differences ($p<0.05$) between samples in yeast taste intensity. Generally intensity of yeast taste for all fermented apple juices samples was not very intensive, and also yeasty flavours was not identified in analysed samples. According to experts and trained panellist assessment there is a significant difference ($p <0.05$) in the ciders sour taste intensity. The lowest intensity of sour taste in fermented juice A, whereas the higher intensity in fermented juices K, R, DI was determined. Correlation between titratable acidity and intensity of sour taste was performed. Trained panellists evaluation of sour taste intensity correlated very close ($r=0.94$), but experts evaluation correlated moderately ($r=0.74$) with titratable acidity. Assessment showed that more intense apple taste is in fermented juices R and DI, and also experts marked significantly higher intensity of apple aroma in those samples. Fruit aroma was not considered as significant properties for differentiation of ciders.

Bitterness and astringency contribute to the good taste of ciders and wines (Lule and Xia, 2005). There were not significant differences ($p>0.05$) in terms of bitterness and astringency between analysed samples. In cider bitterness and astringency are due to the polyphenols especially procyanidins which

Table 4

Correlation matrix among the descriptive terms of experts' estimate

	Clarity	Apple aroma	Fruit aroma	Yeast aroma	Apple taste	Yeast taste	Sour taste	Astringent taste	Bitter taste
Clarity	1	-	-	-	-	-	-	-	-
Apple aroma	0.064	1	-	-	-	-	-	-	-
Fruit aroma	0.068	0.308*	1	-	-	-	-	-	-
Yeast aroma	0.411**	0.092	0.163	1	-	-	-	-	-
Apple taste	-0.007	**0.526****	0.288*	0.233*	1	-	-	-	-
Yeast taste	0.236	0.046	0.272*	**0.642****	0.365*	1	-	-	-
Sour taste	0.025	0.057	0.064	0.099	0.116	-0.096	1	-	-
Astringent taste	0.158	0.092	-0.095	0.194	0.177	0.169	0.385**	1	-
Bitter taste	0.333**	0.041	0.056	0.438**	0.178	**0.510****	-0.069	0.240*	1

** Significant at p< 0.01

* Significant at p<0.05

are polymers of catechins (Noble, 2002). Correlation analyses between total phenol content and intensity of two sensory properties (intensity of astringent and bitter taste) were performed. Experts evaluation of astringent taste intensity correlated moderately (r=0.59), but trained panellists evaluation has not correlation (r=0.06) with total phenol content. Experts and trained panellists evaluation of bitter taste intensity correlated moderately negative r= -0.56 and r= -0.76 with total phenol content. There is often an interaction (Vidal et al., 2004) with other constituents of the beverage: alcohol and polysaccharides reduce astringency while pH can increase it without changing the bitterness (Kallithraka et al., 1997).

Intensity of sensory properties between experts and trained panellists evaluation differed. Mainly trained panellist's marks were higher, especially for apple and fruit aroma.

Correlation between sensory properties

Correlation analysis was performed to determine interactions between different sensory properties. Correlation matrix among the descriptive terms of experts' estimate is presented in Table 4. The expert and trained panellists' evaluation showed moderate correlation between yeast taste and yeast aroma (r=0.543).

In cider, bitterness alteration is characterized for an unpleasant bitter taste associated with the presence of acrolein combined to polyphenols (Sanchez et al., 2010). Heterofermentative *lactobacilli*, and mainly *L. collinoides*, are involved in bitterness production via glycerol dehydratase pathway (Garai-Ibabe et al., 2008). Bitterness results from glycerol degradation in apple-derived products. This degradation is leading to the formation of 3-hydroxypropanal under the action of lactic bacteria present in apple juice. Due to its high instability, 3-hydroxypropanal is spontaneously transformed in acrolein by dehydration (Sauvageot et al., 2000). There is no data about how bitter taste could be influenced by yeast or its metabolites.

Evaluation of sensory properties made by trained panellists showed moderate correlation (p<0.01) only between yeast taste and yeast aroma (r=0.501).

Correlation between the intensity of sensory properties was moderate or weak, and it is not possible to find relationship between the different attributes.

Conslusions

This work is the first study on sensory descriptors of ciders produced from five apple varieties grown in Latvia. Variation in the sensory properties of ciders depending on the used apple variety is attributed to physicochemical composition. Four descriptors, namely, sour taste, apple taste, apple aroma, and clarity were significant for characterization of differences in ciders from various varieties of apples. For development of qualitative cider, varieties with more intense apple and fruit aroma, apple taste and additionally with astringent and bitter taste notes are preferable. Taking into account these results, higher evaluation for cider 'DI-93-4-14' was observed, followed by 'Remo' and 'Kerr'. Experts and trained panellists evaluated intensity of sensory properties

differently, and for new product development it is necessary to analyse both – experts' and potential customer's evaluation.

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THE SUITABILITY OF DIFFERENT ROWANBERRY CULTIVARS FOR PRODUCTION OF FRUIT MARMALADE

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Abstract
The rowanberries (*Sorbus aucuparia* L.) are small orange-red fruits of a rowan tree and belong to the family *Rosaceae*. These berries have been described as an important source of flavonoids, and their antioxidant activity affects reactive oxygen species and lipid peroxidation; therefore they are suitable for production of health-food products. The ripe wild rowanberries have traditionally been used for jellies and jams, but their use as a food ingredient has been less popular because of their bitter taste. Sweeter and less astringent than wild rowanberries are different cultivars of sweet rowanberries and hybrids with other species. The aim of the current research was to determine physical and chemical parameters and sensory properties of rowanberry marmalades. The experiments were carried out in the Faculty of Food Technology of Latvia University of Agriculture. The purees of wild rowanberry and six different rowanberry cultivars were chosen for the production of marmalades. Chemical, physical and sensory indices of the product – moisture, total carotenoids, tannins, colour, hardness and intensity of sensory properties (flavour, colour, bitterness) – were determined as quality indicators. The results showed large variability in the physical and chemical parameters between the marmalades of different rowanberry cultivars and hybrids. The sensory evaluation of marmalades from rowanberry cultivars 'Moravica', 'Mitchurinskaya krasnaya', 'Sorbinka' and hybrid of rowanberry × hawthorn 'Granatnaya' showed that the degree of liking was from "neither like nor dislike" to "like moderately", and marmalades from wild rowanberry, *S. aucuparia* var. *sibirica* and hybrid of rowanberry × pear 'Alaya Krupnaya' – from "dislike moderately" to "dislike slightly".
Key words: Rowanberry marmalade, carotenoids, tannins, colour, hardness, sensory evaluation.

Introduction

The rowanberries (*Sorbus aucuparia* L.) belong to the subfamily *Maloideae* of the family *Rosaceae* and their berries have been promoted as a health-food or can be a source for health-promoting components. The ripe wild rowanberries are picked in the autumn and they are eatable, but very tart in flavour and taste although contain lots of sugar. Rowanberries have been traditionally used to make purees, juices or wine and they make an excellent jelly because of their high amount of pectin, but their use as a food ingredient has been less popular because of their bitter taste (Hukkanen et al., 2006; Poyrazoğlu, 2004; Gough, 2008; Wang, 2007).

Sweeter and less astringent than wild rowanberries are different cultivars of sweet rowanberries and hybrids with other species. The first sweet rowanberry clones were selected in the Sudety mountain area, in the current Czech Republic area in the 19th century. A breeding program for sweet rowanberries was started by Michurin in Russia at the beginning of 20th century, resulting in interesting hybrids of the rowanberry (*Sorbus aucuparia* L.) with the *Aronia, Malus, Mespilus,* or *Pyrus* species. Sweet rowanberries have been bred particularly for northern conditions and have shown excellent winter-hardiness in Russia and Finland (Hukkanen et al., 2006). According to food composition and nutrition tables, sweet rowanberry *S. aucuparia* L. var. *edulis* contains 1600–2420 mg of organic acids per 100 g of edible portion, 98 mg of vitamin C per 100 g, and 2.5 mg of total carotenoids per 100 g (Souci et al., 2008). Comparison of the data obtained to wild rowanberries sugar content indicated that cultivars of the sweet rowanberries were really the sweetest – sugar content in their fruits were considerably 1.2–2.1 times higher (Navys, 2001). The content of reducing sugars (i.e. total amount of glucose and fructose) ranged from 5 to 18 g 100 g^{-1} (Souci et al., 2008, Eder et al., 1991). The content of sorbitol, a sweetening agent that diabetics can tolerate, was high and varied from 3.5 to 12 mg 100 g^{-1} (Eder et al., 1991; Стрельцина et al., 2010).

Sweets constitute the group of food for which human beings have always had some inborn preferences because the majority of sweet fruits or edible parts of plants found in the natural environment are not poisonous. The group of sweets includes honey, candies, jellies and marmalades, candied fruits, sweets made of cacao, and other (Borawska, 2007). Historically, jams and jellies may have originated as an early effort to preserve fruit for consumption in the off-season. Processing of different fruits into juice, marmalade or jam is important for insuring of fruits during all year. Jellies, jams and marmalades are primary distinguished by the form from which their fruit is incorporated. Marmalades are basically jellies with fruit purée and sugar-acid-pectin gel or low-methoxyl pectin-calcium gels. Pectin is traditionally used in a wide range of fruit-based products in which it acts as a thickeners agent (Figuerola, 2007; Grujić et al., 2010; Baker et al., 1996; Willats et al., 2006). Marmalade is a spreadable preparation made from pulp, slurry, juice, aqueous extracts or peels of citrus fruits and sugars. The product has to contain at least 60% by weight of

soluble solids. The addition of fruit pectin and starch syrup is customary. For the production of marmalade, the fresh fruits or intermediary products, such as fruit pulps or slurries, are boiled with the addition of sugar. Other ingredients (gelling agents, starch syrup and acids) are added before the thickening is completed by boiling (Belitz, 2009).

Berries are one of the most suitable fruits for processing into jams and jellies because of their quality, acidity, colour, normally high pectin content, flavour, and aroma. If the fruit does not have enough pectin, commercial pectin has to be used, where a mixture of the required pectin with a similar portion of sugar should be added after the concentration process to avoid heat damage to the pectin molecules. Sugar and other minor ingredients help to develop texture of jams and marmalades because of the formation of a gel between sugars and pectin substances along with fruit acidity. The proportions of fruit and sugar for mixtures should not be less than 45–47 parts by weight of fruit to each 55 parts by weight of sugar (Grujić et al., 2010; Figuerola, 2007).

Acidity of the fruit or its pH value is one of the most important factors in jam process. If the fruit does not have enough acid, a controlled amount of organic acid, such as citric or malic acid, sodium citrate or other is added to reach the required pH to produce gel formation. For gel formation and its stability it is important to insure optimal pH range – 2.5-4.5 (Figuerola, 2007; Javanmard and Endan, 2010; Grujić et al., 2010; Willats et al., 2006).

The purpose of the current research was to determine physical and chemical parameters and sensory properties of fruit marmalades made from sweet rowanberries.

Materials and Methods

Experimental design

The research was carried out at the Faculty of Food Technology, Latvia University of Agriculture, in 2011. The object of the research was fruit marmalade from berries of different rowanberry cultivars grown in Latvia. The rowanberries were picked in the Pure Horticultural Research centre; description of used rowanberry cultivars is given in Table 1. The fruit marmalades were made from rowanberry purees. The rowanberry purees were made from frozen and thawed fruits that were scrubbed through sieve. The mass, mixed with sugar (sucrose – 23.5% from the total amount of product), was heated till 85–90 °C to evaporate part of the water, adding 5.0% pectin (Gen pectin LM-104-AS powder) mixed with part of sugar. The samples of rowanberry marmalade were filled in polypropylene boxes and stored three days at room temperature (22±2 °C) for ripening and thickening. Dimensions of one piece of marmalade on average was 90×70×20 mm, and mass – 100±5 g.

Seven samples of rowanberry marmalades were prepared in this research. Chemical, physical and sensory analyses of the products – moisture content, pH, total carotenoids and tannins, colour L*a*b* values, hardness and intensity of sensory properties (flavour, colour, bitterness) – were determined as quality indicators.

Methods

The moisture content of fruit marmalade was determined with an oven method. The marmalade samples (10 g) were dried at 97 °C overnight (Mattila et al., 2006). For analysis, vacuum drying oven VD53 (Binder) and analytical scales BP-210s (Sartorius)

Table 1

The description of rowanberry cultivars used for research

Rowanberry cultivar	Sort characteristic	Description of fruits
Sorbus aucuparia	Wild rowanberry	Orange or bright red coloured fruits with bitter taste
S. aucuparia var. *sibirica*	Variety of *S. aucuparia*	Orange coloured fruits with bitter taste
'Moravica'	Moravian group variety of *S. aucuparia*	Orange-red coloured fruits with sweet and sour taste
'Sorbinka'	Moravian group variety of *S. aucuparia*	Orange coloured fruits with sweet and sour taste
'Mitchurinskaya krasnaya'	Variety of *S. aucuparia*	Dark red coloured fruits with sweet and sour taste
'Granatnaya'	Hybrid of rowanberry × hawthorn (*Sorbus aucuparia* × *Crataegus sanguinea Pallas)*	Dark red or brown coloured fruits with sweet and sour taste
'Alaya Krupnaya'	Hybrid of rowanberry × pear (*Sorbus aucuparia* × *Pyrus* sp. × *Sorbus aucuparia* var. *moravica*)	Bright red-brown coloured fruits with littlebit bitter taste

were used. Measurements were carried out in three replications.

The pH value was measured by FieldLabpH pH-meter (Schott), using standard method LVS ISO 5542:2010.

The total carotenoids content was analysed by the spectrophotometric method at 440 nm (Ермаков, 1987) extracted with petroleum ether (boiling temperature range – 80–110 °C) and measured with UV-VIS-NIR spectrophotometer UV-3100PC (Shimadzu) in 10 mm cuvettes. A total of 2–3 grams of homogenized marmalade were placed in a conic retort (100 ml) and 96% ethanol (20 ml) was added, and then samples were stirred by a magnetic stirrer for 20 min. Then petroleum ether (25 ml) and water (1 ml) were added, and stirring was continued for one more hour. After 3–4 hours, the top (yellow) layer was used for the detection of total carotenoids. The carotene equivalent (KE) was found, using graduating curve with $K_2Cr_2O_7$. Measurements were carried out in two replications for each sample.

The total tannins content was detected using the traditional method by titration with 0.1 N $KMnO_4$ (Шмыдт, 1960). Measurements were carried out in two replications for each sample.

The colour of rowanberry marmalades was measured in CIE L*a*b* colour system using a *ColorTec-PCM/PSM* (Accuracy Microsensors Inc.). Before measuring, the colorimeter was calibrated using a white reference tile and a light trap (black tile). Ten random areas were measured through the plastic pockets and mean values were reported for each sample. In colour measurement, CIELAB coordinates show the degree of brightness (L), the degree of redness (+a), or greenness (–a), and the degree of yellowness (+b), or blueness (–b), respectively (Coultate, 2002; Chakrborty et al., 2011).

The structure parameter – hardness (cutting force in N) – of the rowanberry marmalades was determined on the Texture Analyser *TA.XT.plus* (Stable Micro Systems Ltd.) and the measuring probe A/BC (butter cutter can be used for soft samples, supplied in association with the Texture Analyser) according to the method described by Muizniece-Brasava et al. (2011). The system was equipped with compression cell of 50 kg and software Texture Exponent 32. Hardness was measured as the maximum penetration force (N) reached during breakage of tissue. Hardness was measured as the maximum penetration force (N) reached during breakage of tissue. The measuring parameters were: pre-test speed – 1 mm s^{-1}; test speed – 1 mm s^{-1}; post-test speed – 10 mm s^{-1}; cutting distance – 10 mm pressing into the sample. The maximum force required for sample compression was calculated as an average of 10 measurements.

Sensory evaluation of the rowanberry marmalades was performed in the Laboratory of Sensory Evaluation at the Faculty of Food Technology of the Latvia University of Agriculture. All rowanberry marmalade samples were evaluated by 25 trained panellists (18 females and 7 males, mean age – 32). Rowanberry marmalade samples were manually divided in 20×20 mm pieces with a knife and presented in three-digit coded containers, and the order of serving was determined by random permutation. The intensity of sensory properties of rowanberry marmalades (colour, flavour and bitterness) was evaluated using line scale, but the degree of liking was evaluated by nine-point hedonic scale (ISO 4121:2003). The obtained data was averaged across panellists. Hedonic scale includes 9 points, which allows evaluating the degree of liking. The points are from “like very much” (9) to “dislike very much” (1) and middle point “neither like nor dislike” (5).

The results represent the mean ± standard deviations. The obtained results were analysed using analysis of variance (ANOVA) and Tukey’s test when significant differences among the rowanberry marmalade samples were found. Statistical differences with *p*-values under 0.05 were considered as significant. Closeness of the relationship between the parameters was determined by analysis of Pearson correlation coefficient.

Table 2

The physical and chemical parameters of rowanberry marmalades

Rowanberry cultivar	pH value	Moisture content, %	Carotenoids, mg 100 g^{-1} DW	Tannins, g 100 g^{-1} DW
‘Granatnaya’	3.33±0.01 ^{c}	36.72±0.07^{c}	2.02±0.14 ^{bc}	0.16±0.02^{c}
‘Moravica’	3.27±0.02^{d}	36.08±0.06^{c}	1.59±0.06 ^{bcd}	0.06±0.02^{d}
Wild rowanberries	3.25±0.02^{d}	38.98±0.16^{b}	2.24±0.19 ^{bc}	0.28±0.04 ^{ab}
S. aucuparia var. *sibirica*	3.46±0.02 ^{a}	39.07±0.14^{b}	3.52±0.68 ^{a}	0.30±0.03 ^{a}
‘Sorbinka’	3.30±0.02 ^{cd}	42.59±0.09^{a}	1.08±0.09^{cd}	0.14±0.01^{cd}
‘Mitchurinskaya krasnaya’	3.40±0.01 ^{b}	43.47±0.12^{a}	2.88±0.04 ^{b}	0.14±0.02^{cd}
‘Alaya krupnaya’	3.15±0.02^{e}	42.98±0.05^{a}	0.43±0.07^{d}	0.21±0.02^{bc}

***** – values, marked with the same superscript letters in a column, are not significantly different (p>0.05).

Results and Discussion

The physical and chemical parameters (pH value, moisture, total tannins and the content of carotenoids) of the samples of rowanberry marmalades are given in Table 2. The moisture content of the mass of raw material varied from 55.42% to 64.79%. It was observed that during preparation of rowanberry marmalades, the moisture decreased on average by 29.5–42.5%.

The results of the statistical analysis indicate that there are significant differences (p=0.000) in the moisture content among the samples of rowanberry marmalade. We can compare our results with moisture content of the rowanberry marmalade and apple-black currant marmalade candies (Berna and Kampuse, 2011; Muizniece-Brasava et al., 2011), where moisture content of marmalade was 26.50–37.92% and 44.78%, respectively. According to these data we can conclude that marmalade can be called as intermediate moisture food similar to jams and jellies, where moisture content of different berries and fruit jams vary between 27.80 and 34.06% (Figuerola, 2007; Ndabikunze et al., 2011).

Based on the fact that the pH is critical to successful gel formation with pectins and low pH increases the percentage of unionized carboxyl groups, the optimum pH for slow-set pectins being about 3.1 and for rapid set pectins 3.4 is recommended (Baker, 1996). Normally fruits used for making jams and jellies have low pH; most have less than pH 4.0 and some have less than pH 3.5. Organic acid (for example, citric acid) stabilizes the relation between pectin and sugar. High acidity, represented by a pH of 3.2 to 3.4, permits an increased number of unionized carboxyl groups in pectin molecules, reducing the electrostatic repulsion between pectin chains (Figuerola, 2007).

The pH values of rowanberry marmalade samples varied from 3.15 to 3.46. The results of the statistical analysis indicate that there were significant differences (p=0.000) in the pH value among the rowanberry marmalade samples. As rowanberries, like other berries, have low pH due to their content of some common organic acids, such as ascorbic, citric, tartaric, and malic acid, then we did not use any of these organic acids for increasing the acidity in fruit marmalade. Similar pH values are reported for thermo-stable marmalades of peach and apples, where pH value varied from 3.16 to 3.58 (Grujić et al., 2010).

The total carotenoids content of all investigated samples is reported in Table 1. The differences in the total carotenoids content between samples of the rowanberry marmalades were significant (p=0.000). The highest total carotenoids content was in the sample made from puree of rowanberry *S. aucuparia* var. *sibirica*, and the least – in the sample made from puree of rowanberry × pear hybrid 'Alaya krupnaya'.

The total tannins content significantly differed between the rowanberry marmalades (p=0.000), and it was between 0.06 and 0.30 g 100 g^{-1} DW (Table 1). Wild rowanberry and *S. aucuparia* var. *sibirica* had the highest content of total tannins, which explains the bitterest taste of marmalade made from these berries compared to marmalades of other rowanberry cultivars and hybrids. We observed that rowanberry marmalades contain less tannins than unprocessed fruits (Kampuss et al., 2009), which could be affected both by the freezing and the processing of the rowanberry puree.

The results of colour L*a*b* measurements of the rowanberry marmalades are shown in Table 3 – colour values significantly differed between rowanberry marmalades (p=0.000). The lightest colour (L* value) was detected to the samples of marmalades from rowanberry cultivars *S. aucuparia* var. *sibirica* and 'Sorbinka', which berries have yellow-orange colour. Whereas the darkest (the lowest L* value) and the reddest marmalade was prepared from rowanberry × hawthorn hybrid 'Granatnaya' and rowanberry cultivar 'Mitchurinskaya krasnaya', which berries are coloured in dark red colour.

Table 3

The average colour L* a* b* values of rowanberry marmalades

Rowanberry cultivar	Colour (L* a* b*) values		
	L*	a*	b*
'Granatnaya'	21.53±1.29^{d}	9.69±0.80^{bc}	10.58±1.90^{b}
'Moravica'	33.80±0.68^{a}	9.45±0.86^{bc}	21.12±1.27^{a}
Wild rowanberries	28.45±1.21^{bc}	18.27±2.13^{a}	20.48±1.44^{a}
S. aucuparia var. *sibirica*	35.78±1.58^{a}	8.47±1.14^{c}	20.04±1.80^{a}
'Sorbinka'	35.03±2.11^{a}	10.89±1.64^{b}	22.68±2.15^{a}
'Mitchurinskaya krasnaya'	24.33±2.38^{cd}	10.35±1.68^{bc}	10.78±1.22^{b}
'Alaya krupnaya'	29.43±2.59^{b}	11.32±1.20^{b}	8.58±1.14^{b}

* – values, marked with the same superscript letters in a column, are not significantly different (p>0.05).

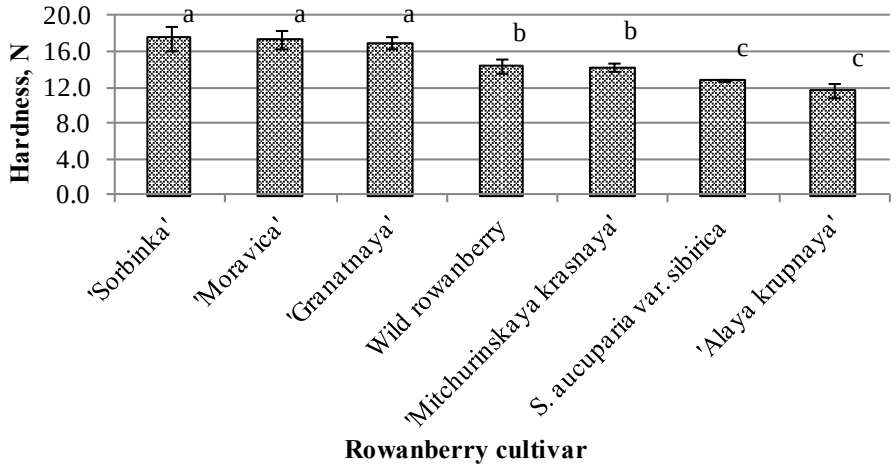

Figure 1. The hardness of the rowanberry marmalades.
* – values, marked with the same superscript letters, are not significantly different (p>0.05).

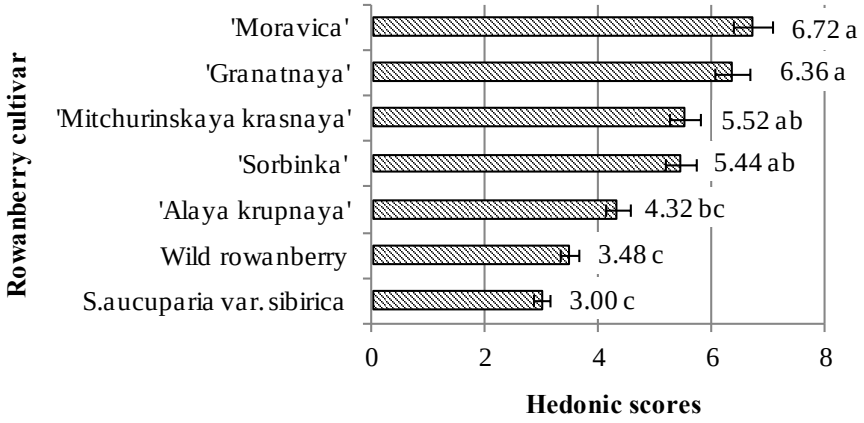

Figure 2. Degree of liking of the rowanberry marmalades.
* – values, marked with the same superscript letters, are not significantly different (p>0.05).

The hardness of the rowanberry marmalades is shown in Figure 1; it varied from 11.6±0.8 to 17.7±1.4 N. We observed that the hardness of the marmalades significantly differed, too (p=0.000).

These values were compared with the hardness results of the rowanberry marmalade made from sweet rowanberry cultivars 'Moravica' and 'Rosina', which hardness was 10.3 and 13.3 N, respectively (Berna and Kampuse, 2011).

The results of hedonic evaluation scores of the rowanberry marmalades are summarized in Figure 2. The results of the analysis of variance (ANOVA) indicate that Fcal = 21.57 > Fcrit = 2.16, which demonstate there are significant differences in the degree of liking among the rowanberry marmalade samples.

According to the hedonic scale, panellists evaluated rowanberry marmalades in the range from 3 (dislike moderately) to 7 (like moderately). The samples with lower bitterness the panellists liked better, and there were no significant differences between the marmalades made from purees of cultivars 'Moravica', 'Granatnaya', 'Mitchurinskaya krasnaya', and 'Sorbinka' (Fig. 2). The marmalade samples made from wild rowanberry and *S. aucuparia* var. *sibirica* the panellists liked the least (p<0.05), because they were too bitter. The marmalade sample made from wild rowanberry did not differ in degree of liking from *S. aucuparia* var. *sibirica* and 'Alaya krupnaya'. The assessment results of the intensity of sensory properties – flavour, colour and bitterness – of the rowanberry marmalades are presented in Table 4.

The marmalades without bitter taste made from sweet rowanberry cultivars 'Granatnaya' and 'Mitchurinskaya krasnaya', which have dark red colour, and cultivars 'Moravica' and 'Sorbinka', which have orange colour were rated as the best for fruit marmalade production by panellists. Several

Table 4

The intensity of sensory properties of rowanberry marmalades

Rowanberry cultivar	Intensity of sensory properties		
	Flavour	Bitterness	Colour
'Granatnaya'	6.7±1.2 [a]	3.8±0.2[bc]	9.6±0.5[a]
'Moravica'	6.4±1.1 [a]	2.7±0.1[c]	7.2±0.4[bc]
Wild rowanberry	6.4±1.2 [a]	9.4±0.5[a]	7.8±0.4[abc]
S. aucuparia var. *sibirica*	7.2±1.2 [a]	9.9±0.5[a]	6.3±0.3[c]
'Sorbinka'	6.0±1.3 [a]	4.0±0.2[bc]	6.8c±0.3[c]
'Mitchurinskaya krasnaya'	6.6±1.1 [a]	4.0±0.2[bc]	9.3±0.5[ab]
'Alaya krupnaya'	6.1±1.4 [a]	5.6±0.3[b]	7.3±0.4[bc]

* – values, marked with the same superscript letters in a column, are not significantly different (p>0.05).

panellists accepted the marmalade made from rowanberry cultivar 'Alaya krupnaya' with a slightly bitter taste as equally good.

The evaluation of the intensity of rowanberry marmalade sensory properties showed that there were no significant differences (p>0.05) in flavour, but there were significant differences in the intensity of colour and bitterness (p<0.05). These results could be explained by the different rowanberry cultivars used for preparation of marmalades – from yellow-orange to dark red coloured fruits and fruits with and without bitter taste.

The panellists considered that the marmalades from the puree of wild rowanberry and *S. aucuparia* var. *sibirica* fruits are unsuitable for nutrition because they were very astringent.

Pearson's correlation analysis was carried out to compare quality indicators of the rowanberry marmalades. A medium positive correlation was determined between the intensity of bitterness and tannin content (p=0.001, r=0.661), as well as a medium negative correlation between the degree of acceptance and tannin content (p=0.009, r=-0.558). Evaluating the correlations it was confirmed that in order to obtain non-bitter marmalades it is necessary to use the rowanberry cultivars with a small tannin content, as well as 'Moravica', hybrid of rowanberry × hawthorn 'Granatnaya', 'Sorbinka' and 'Mitchurinskaya krasnaya'. The rowanberry marmalades made from 'Granatnaya' and 'Mitchurinskaya Krasnaya' had the most intense colour, while the marmalade from *S. aucuparia* var. *sibirica* had the least colour intensity. There was no statistically significant correlation (r=0.215) found between the intensity of colour and carotenoids content.

Conclusions

1. There were significant differences (p<0.01) among the samples of rowanberry marmalades. The moisture content of rowanberry marmalades varied from 36.08 to 43.47%, hardness – 11.6–17.7 N, and carotenoids content – 0.43–3.52 mg 100 g^{-1} DW.
2. The pH of rowanberry marmalades differed between 3.15 and 3.45 and therefore it is not necessary to use any of organic acids for increasing the acidity in fruit marmalade.
3. The marmalade made from puree of wild rowanberry and from *S. aucuparia* var. *sibirica* had the highest tannins content (0.28–0.30 g 100 g^{-1} DW), which explains the most astringent flavour and taste of these marmalades compared to other rowanberry cultivars and hybrids.
4. The sensory evaluation of fruit marmalades from sweet rowanberry cultivars 'Moravica', 'Mitchurinskaya krasnaya', 'Sorbinka' and rowanberry × hawthorn hybrid 'Granatnaya' showed that the degree of liking varied from "neither like nor dislike" to "like moderately".
5. The marmalades from wild rowanberry and *S. aucuparia* var. *sibirica* fruits are unsuitable for nutrition because they are very astringent.
6. There were significant differences (p<0.01) in colour L* a* b* values among the samples of rowanberry marmalades. The colour L* values differed between 21.53 and 35.78, and the darkest marmalade was from rowanberry × hawthorn hybrid 'Granatnaya'.

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CHEMICAL COMPOSITION OF NEW TYPE AGAR JELLIES WITH JERUSALEM ARTICHOKE SYRUP

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Abstract
One of the causes of cardiovascular diseases and overweight problem is a high consumption of sweets. Sugar is traditional food sweet matter. A change of sugar may therefore both change the perception of texture of products. The aim of the research was to evaluate properties of agar-agar jellies prepared with Jerusalem artichoke (*Helianthus tuberosus L.*) syrup (JAS). Soluble dry matter of experimental samples was determined by Mettler Toledo Refracto, separated sugars - by liquid chromatography, and acidity of jellies - by titration with 0.1 M NaOH. pH measurements were made by pH-meter Jenway 3510. Determination of vitamins B_1 and B_2 was made by the AOAC Official Method 986.27 and 970.65. Texture of samples was determined by using a Texture Analyser (Model TA.XT Plus; Stable Micro Systems), by cylindrical probe (P/25). Colour of jellies was evaluated by using Colour Tec-PCM. The results showed that soluble dry matter decreased from 64.5˚ Brix to 57.5˚ Brix, sucrose decreased by 6%, the acidity increased from 7.2 to 17.8˚, and pH values ranged between 3.8 and 4.5. The hardness of the samples decreased from 50.66N to 40.05N by increasing of added JAS concentration. Adding JAS in jelly, the content of vitamins B_1 and B_2 increased. Lightness "L" fluctuated between 24.26 and 14.60 with increase JAS concentration. The researche suggests that different percents of Jerusalem artichoke syrup could be used as sugar substitute. The product becomes healthier, but the gels obtain a darker colour. Therefore it is recommended to look for other version to improve the colour of experimental jellies.
Key words: Jerusalem artichoke syrup, jellies.

Introduction

A lot of overweight people and those having a tendency to develop diabetes often have a craving for sweet candies and find it difficult to abstain from eating high calorie products (Pudule et al., 2007). Since the principal ingredient of most candies is sugar (sucrose), attempts at reducing the caloric content have been made by reducing the amount of sugar used in preparing candies. There is an increasing interest in products without added sugar.

Confectionery of jelly groups traditionally have been a permanent demand for all people because of high sensory properties, therapeutic-prophylactic characteristics due by the content agar substances and the relative accessibility to consumers. Jellies are fat-free products, meaning that the products are suitable for people who have obesity (Дубцов et al., 2001).

Jelly is a product manufactured by cooking fruit juice with added sugar, glucose syrup, and agar-agar. Jellies should be translucent, forming a continuous and firm gel structure (Figuerola, 2007). Jellies are products called intermediate moisture foods. Jellies are products based in texture formation.

Jellies are characterized by the formation of a gel in jellies, and these properties are developed by the interaction of sugar, gelling substances, and acidity. Sugar serves as a preserving agent and aids in gelling. Sugar and agar-agar form the network that gives each product texture according to the proportions of the three ingredients. For proper structure, jelly products require the correct combination of agar, sugar and glucose syrup (Bayarri, 2004; Павлова, 2000). Agar is a linear polymer based on a disaccharide repeat structure of 3-linked β-D-galactopyranosyl and 4-linked 3.6 anhydro- α -L- galactopyranosyl units (Norziah et al., 2006; Barrangou et al., 2006). Agar is the three-dimensional cross-linked network within the liquid that gives a jelly its structure and contributes to stickiness. The gelling portion of agar-agar has a double helical structure. Double helices aggregate to form a three-dimensional structure framework which holds the solution molecules within the interstices of the framework (Armisen and Galatas, 2000). Thus, thermo-reversible gels are formed. Regarding its gelling power, agar-agar is outstanding among other hydrocolloids. The gel strength of the agar-agar is influenced by concentration, time, pH, and sugar content. The pH noticeably affects the strength of the agar gel; as the pH increases, also the gel strength increases.

Inulin consists of a long chain made up of 22 – 60 fructose molecules and one glucose molecule at one end. The fructose molecules are connected by β - (2-1) bonds. The last fructose is linked with glucose by an α - (1-2) bound as sucrose. The degree of polymerization of native Jerusalem artichoke inulin is on average 10 (Roberfroid, 2005).

Even changes in composition or processing variables can influence the jelly-like properties and texture of jellies (Kim et al., 2001; Matsuhashi, 1990; Panouille and Larreta-Garde, 2009). If sucrose is replaced by other sugars in jellies, these different types of sugars have not the same behaviours as sucrose (Figuerola, 2007; Павлова, 2000; Полянский et al., 2009). These differences need to be considered (Kronberga and Karklina, 2011).

A change in sugar content may therefore both change the perception of sweetness and texture (Bayarri et al., 2004; Kaur and Gupta, 2002). Apart from its sweetening, sugar performs a variety of functions: formation of crust colour, flavour enhancement, texture modification, development of structure, and shelf-life improvement. Sugar is a good depressor of water activity, and makes an important contribution in decreasing water activity. Normally, the total sugar content of jellies is more than 50%; thus the effect of sugar content on decreasing free water and water activity is very significant. This effect is responsible for the intermediate moisture behavior of these products (Figuerola, 2007). Replacement of sucrose by other sweet ingredients in jelly, causes technological problems. Sucrose is essential to form the product structure given the gels with gelling material.

Jerusalem artichoke (*Helianthus tuberosus L.*) is one of the raw materials used for production of inulin syrups. Inulin, polysaccharide composed of fructose, is legally classified as a food ingredient, a low-calorie sweetener (Roberfroid, 2005; Frack, 2002; Glibowski and Wasko, 2008) in all countries where it is used. Jerusalem artichoke syrup contains group of vitamin B and optimal enriched jelly (Roberfroid, 2005; Эм et al., 2010).

Therefore the aim of the research was to evaluate properties of agar-agar jellies prepared with Jerusalem artichoke (*Helianthus tuberosus L.*) syrup (JAS).

Materials and Methods

The research was done at the Faculty of Food Technology, Latvia University of Agriculture, in 2011-2012.

In jellies, to replace sucrose Jerusalem artichoke juice concentrate produced by Topina, Diät Rohstoff Gmb, (Germany) was used as Jerusalem artichoke syrup. Glucose syrup produced by Olbricht & Partner, (Germany) and Dansukker (Denmark) sugar, was purchased in "Lanordija" market (Latvia).

As a gelling material, for preparing jellies NordAgar S (E 406) by Nordingredients (Estonia) was used.

The standard formulation for jelly (Павлова, 2000) is: agar- agar (2 g), glucose syrup (62 g), sugar (104 g), citric acid (2 g of 50% citric acid solution), and water (100 g).

Agar-agar gels were prepared with sugar substituted by JAS. Jerusalem artichoke syrup has 70° Brix soluble dry matter and it contains 35 g inulin, 7.9 g fructose, 4.4 g sucrose and 1.5 g glucose per 100 g. JAS has 18.4° acidity, and pH of syrup is 4.7. Sugar and replacement ratios in the samples were 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100.The technological process of preparing experimental jellies is showed in Fig.1. The experiments were carried out in triplicate.

Soluble dry matter was measured at 20 °C by using a refractometer Mettler Toledo, Refracto 30PX according to LVS 249:2000. The separate sugars (sucrose, glucose, fructose) were analyzed by liquid chromatography according to LVS EN 12630:2001. The acidity was determined by titrating the diluted samples with 0.1 M NaOH. Results were given as percent of citric acid. pH was determined by pH-meter (Jenway 3510) with a combined glass electrode, standart method LVS ISO 5542:2010.

Vitamins B_1 and B_2 were determined according to official methods AOAC 986.27 and AOAC 970.65.

Hardness of jelly candies was determined on the Texture Analyzer, "*TA.XT.plus Texture Analyser*" (Stable Micro Systems Ltd., UK). Hardness was measured as the maximum penetration force (N) reached during breakage of tissue. The measuring

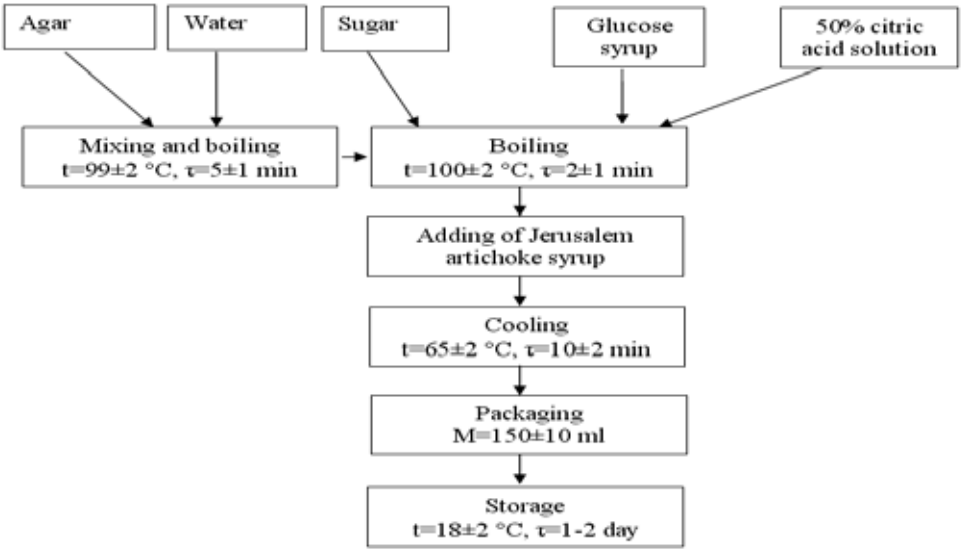

Figure 1. The technological process of preparing experimental jellies.

parameters were: pre-test speed -1 mm s^{-1}; test speed - 1 mm s^{-1}; post-test speed - 10 mm s^{-1}; cutting distance - 13 mm pressing into the sample. The maximum force required for sample compression was calculated as an average of 6 measurements. Measurements were carried out on room tempered samples.

Colour of jelly samples was measured in CIE L*a*b* colour system using Tristimulus Colorimeter. Colour values were recorded as L*(brightness) – the vertical co-ordinate runs from L* = 0(black) through grey to L* = 100 (white) (Coultate, 2002). Measurements were repeated on different randomly selected locations at the surface of each sample.

Experimental results were means of three parallel measurements and analyzed by Microsoft Excel 2010 and SPSS 17.00 for Windows. Microsoft Excel software was used to calculate mean values and standard deviations of obtained results. Analysis of variance (ANOVA) and differences among samples were tested by Tukey test. Differences were considered significant at $p<0.05$. Relationships between soluble dry matter, acidity, pH and hardness were analyzed by conventional and regression tools.

Results and Discussion

Jellies have definite control indices such as soluble dry matter, acid content, and amount of reducing sugars. Changing the agar jelly standard recipe by reducing the amount of sugar and adding syrup of Jerusalem artichokes, which contains different substances including 50% of inulin, we had to verify changes of a product. Also, we had to define important - for - consumers values of jelly - hardness and colour of the new products.

The content of soluble dry matter in jelly mass with addition of syrup was within the requirements. The control sample had 65.55° Brix, and the sample of 100% JAS - 57.55° Brix at (Figure 2).

Soluble dry matter no significant differences between samples ($p>0.05$) When increasing the amount of added syrup in samples, the amount of soluble dry matter changed within the limits of errors. In result, the total amount of soluble dry matter decreased slowly and achieved the minimum of soluble dry matter substituted sucrose by 100% of syrup. The insignificant changes in soluble dry matter in jellies could be explained by differences in the content of dry matter in sugar and in syrup.

The amount of sucrose in experimental samples decreased by increasing the amount of Jerusalem artichoke syrup. Determining the sugar by liquid chromatographic method it was found proved that the control sample contains 2% fructose, 6% glucose and 25% sucrose, but the sample with 40% of syrup contained 6% fructose, 8% glucose and 19% sucrose.

The proper level of acidity is critical to gel formation. If amount of acid is insufficient, the gel never sets; if there is excess acid, the gel will look liquid. When the Jerusalem artichoke syrup was added to the jelly samples, the level of acidity increased. Titrated acids increased quite rapidly in the samples, not exceeding the permitted limit. For agar jelly, permissible acid limit is from 7.5 to 22.5°. There were differences in titrated acids between the samples except for samples JAS 80 and JAS 100 (Figure 3). The differences in acidity value between control sample and experimental samples could be explained by the increasing syrup concentration in samples.

Since the agar jelly is not made from fruit juice, it does not contain components such as vitamins of B group. There were not identified vitamins of B group in the control sample without added syrup. The sample of jelly with 20% of Jerusalem artichoke syrup additives contained 0.190 mg $100g^{-1}$ vitamin B_1 and 0.029 mg $100g^{-1}$ vitamin B_2. The sample with 40% JAS additives contained 0.224 mg $100g^{-1}$ vitamin B_1

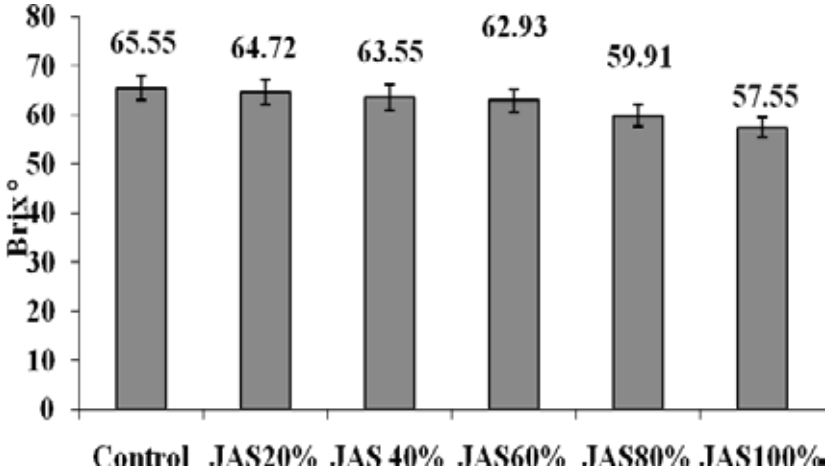

Figure 2. Soluble dry matter content in experimental jelly samples with Jerusalem artichoke syrup.

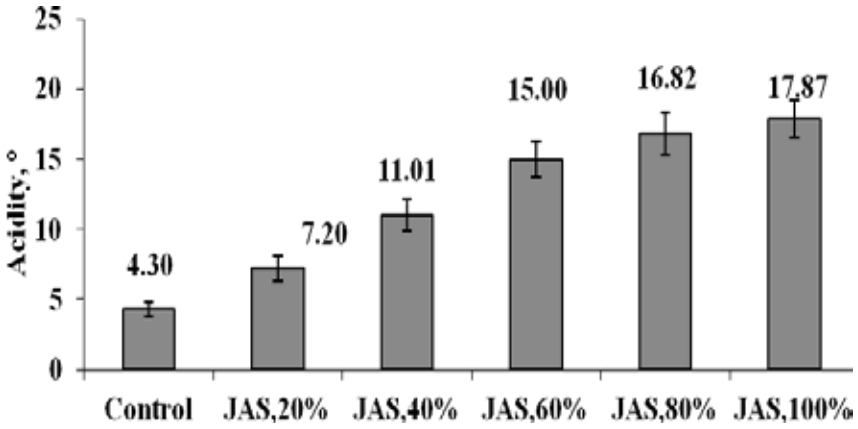

Figure 3. Changes in titrated acidity of experimental jelly samples with Jerusalem artichoke syrup.

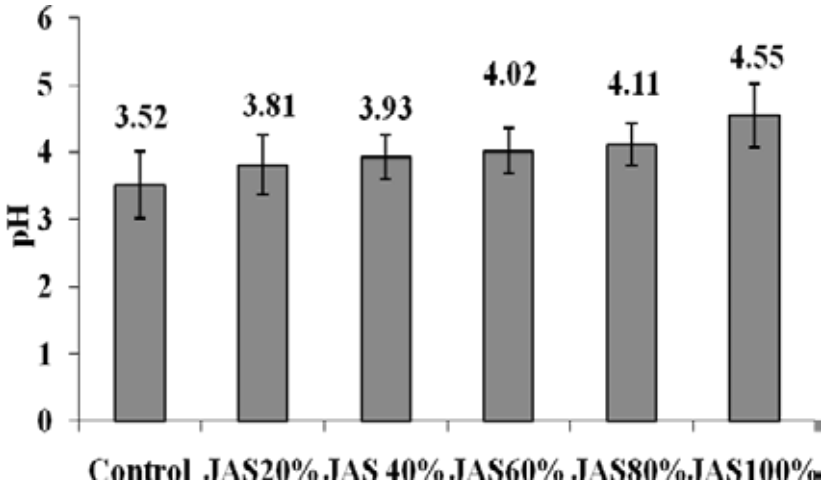

Figure 4. Changes in pH of experimental jelly samples with Jerusalem artichoke syrup.

and 0.150 mg $100g^{-1}$ vitamin B_2. This suggests that addition of JAS which contains B group vitamins, provides healthier products (Богатырев, 2010).

The pH of experimental jellies changed by the addition of different amounts of JAS. The pH values of experimental samples (Figure 4) ranged between 3.81 (sample with 20% JAS) and 4.55 (sample with 100% JAS). Changes in pH with increasing addition of syrup showed that the pH value increased in proportion to the quantity of JAS. Such an increase can be explained by the high pH of JAS (pH=4.7) and the increasing proportion of the liquid, which partially diluted the concentration of bases, where the level of acid is defined in the recipe and during the sample preparation added amount of citric acid.

Gel formation and mechanism of agar and inulin gelation in aqueous solutions is different for both polysaccharides and depends on temperature and water amount in samples. The hardness of jelly arises from stretching, bending, association, and aggregation of polysaccharide molecules (Kim et al., 2001; Glibowski and Wasko, 2008).

The hardness values of experimental samples are shown in Figure 5. Hardness of jellies where sugar was substituted by JAS decreased from 52.82 N to 40.05 N. The hardness decreased because increasing the amount of water in jellies added by syrup. The decreasing of hardness in the experimental samples could be explained by interaction between agar-agar and inulin in JAS. The both gelling agents can act in synergy as well as disincentive because they claim for free water amount what is involved in their grid. They can interfere with each other (Kim et al., 2001; Glibowski and Wasko, 2008).

Among hardness and soluble dry matter of the samples, were significant positive correlation (Figure 6), but correlations between soluble dry matter and pH, and hardness and acidity, and hardness and pH were strong negative correlation.

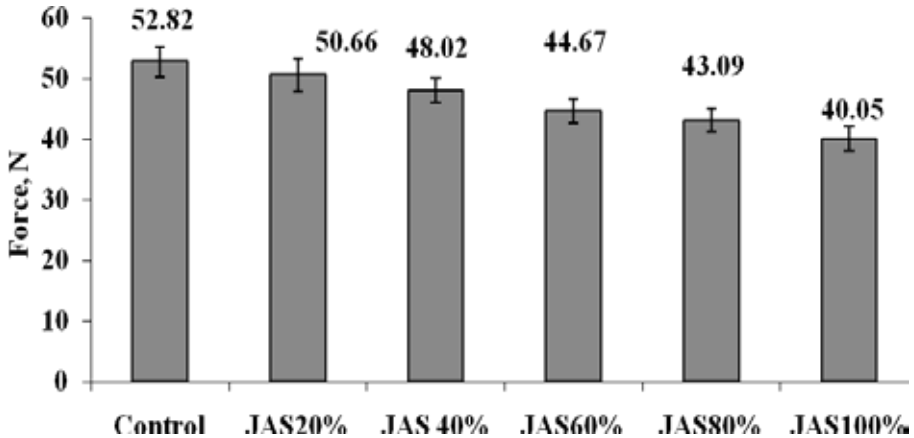

Figure 5. Changes in hardness of experimental jelly samples with Jerusalem artichoke syrup.

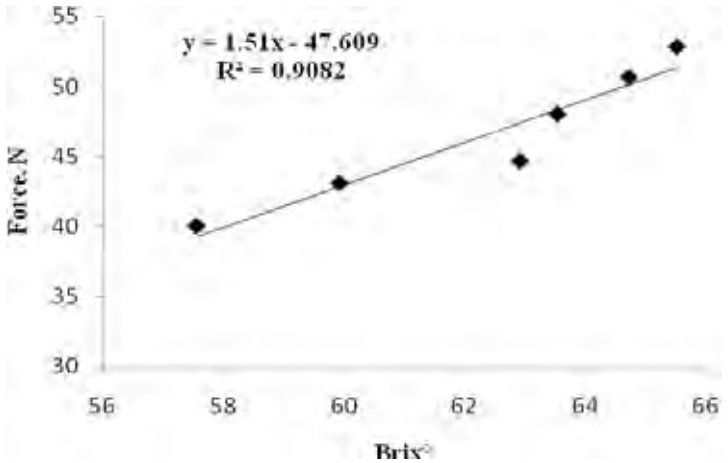

Figure 6. Correlation between soluble dry matter and hardness of experimental jelly samples with Jerusalem artichoke syrup.

Replacement of sucrose in jellies causes technological problems since sucrose in soluble dry matter is essential to form the gels structure. The amount of sucrose in experimental samples decreased by increasing the amount of Jerusalem artichoke syrup. When the Jerusalem artichoke syrup was added to the jelly samples, the level of acidity increased. The differences in acidity value between control sample and experimental samples could be explained by the increased syrup concentration in samples. Such an increase can be explained by the organic acids present in JAS which has pH=4.7 and an increased proportion of liquid, which partially diluted the jelly samples. In the samples with 60-100% sugar substitution it is seen that jelly hardness decreased due to increased acidity and decreased dry matter.

Colour is a very important quality indicator, where light colour has been demonstrated to be very attractive for consumers. Food colour has a significant impact by being one of the major factors used by consumers to take the purchasing decision. Results of L* value of experimental jellies are presented in Figure 7.

A higher L* value of experimental samples with control sample and sample by 20% JAS compared with other samples. The addition of increasing amounts of JAS in result gave an undesirable colour for jelly. By increasing the JAS ratio in experimental jelly, the mixture obtained a darker colour since JAS is darker in colour. The lightness L* of jelly decreased in all samples. At significance level 0.05, the brightness had no significant differences between the samples JAS40, JAS 60, JAS 80 and JAS 100, but there were significant difference in the samples JAS 20 and Control. The blue-yellow chromatically (b*) values were significantly higher in control sample, but in all samples, prepared with JAS, it decreased from 18.12 to 4.2. Changes in a* values in all prepared samples increased from 2.55 to 9.42.

The obtained results showed, that the added amount of JAS in result gave darker colour for experimental jellies. We have to think regarding to colour changes, if we would like to use JAS for production jellies. It is very important to consider that the quality of a jelly strongly depends on sensory perceptions of the

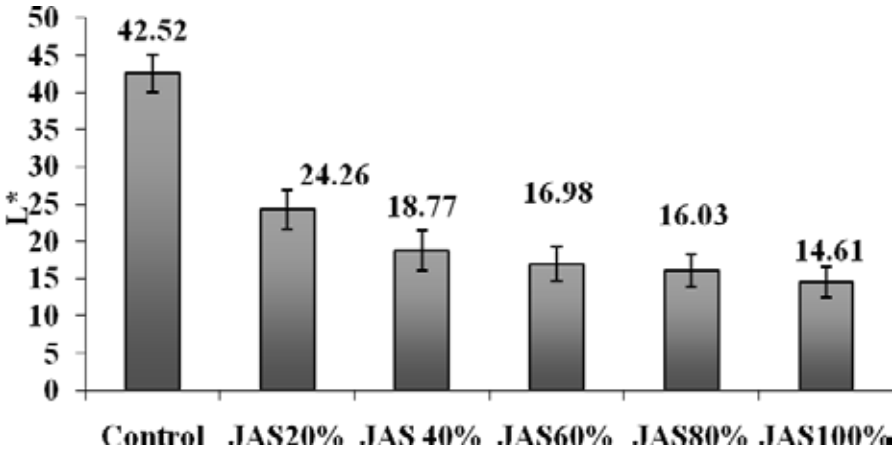

Figure 7. Lightness "L*" of experimental jelly samples with Jerusalem artichoke syrup.

product, that is, taste, aroma, texture, and colour. Lighter colour is desirable in order to prepare commercial samples and to ensure that new products will have high consumer acceptance (Кронберга et al., 2011).

Jerusalem artichoke and its products currently meet an active investigation phase. The obtained results show that the syrup with different concentrations of Jerusalem artichoke can be used in jelly, but it is not the best option. It exceeds the preferable hardness of jelly. Sugar decrease is possible only with high percent of replacement. The colour change of jelly is very noticeable in terms of brightness. The sample with 20% substitution is close to the desired darkness of jelly. As for other samples (from 40-100% of substitution), it is necessary to add substances to mask the brown colour of the sample.

Conclusions

1. The content of soluble dry matter and the lightness "L*" of gels decreased in proportion to the amount added to the Jerusalem artichoke syrup.
2. Adding Jerusalem artichoke syrup in jelly, the acidity, pH value of experimental samples and content of vitamins B_1 and B_2 increased.
3. Hardness of the experimental samples was influenced by theconcentration of the Jerusalem artichoke syrup. By adding the syrup, the hardness of jellies decreased because the amount of water in jellies increased.
4. The obtained results show that different percents of Jerusalem artichoke syrup could be used as sugar substitute in jellies. The product becomes healthier, and the gels become softer, but obtain a darker colour. Therefore it is recommended to look for other version to improve the colour of experimental jellies.

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INFLUENCE OF GENOTYPE AND HARVEST TIME ON THE PHENOLIC CONTENT OF HORSERADISH (*ARMORACIA RUSTICANA* L.) ROOTS

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Abstract
Horseradish (*Armoracia rusticana* L.) is a perennial plant, with a particularly pungent flavour and significant antioxidant properties. The aim of current research was to determine the total phenol content and antioxidant properties of horseradish depending on genotype and harvest time. For experiments nine genotypes of horseradish roots collected at different times were investigated. Fresh plant material was extracted with ethanol/water solution (80:20 v/v). Total phenols content (TPC) of plant extracts was determined according to the Folin-Ciocalteu spectrophotometric method and results were expressed as gallic acid equivalents (GAE). Antioxidant activity of the extracts was measured on the basis of DPPH˙ free radical scavenging activity and the final results were expressed as inhibition of 2,2-diphenyl-1-picrylhydraziyl (DPPH˙) in percent (I, %). Total phenols content varied among analysed types of horseradish. The highest TPC was detected in horseradish root genotype 280 harvested in September and it also demonstrated the highest DPPH˙ radical scavenging activity, whereas the lowest TPC was detected in horseradish root genotype 26B also harvested in September. TPC and DPPH˙ scavenging antioxidant activity were also significantly influenced by harvest time. Positive correlation was found between antiradical activity and the total phenols content in horseradish roots harvested in September. In further experiments, use of horseradish as natural antioxidants in different food matrixes should be studied.
Key words: horseradish, total phenols, scavenging activity, genotype, harvest time.

Introduction

World attention has been paid to development of safe antioxidants from natural sources. Many spices and vegetables possess antioxidant properties, so they can be used in food to help prevent oxidation processes. Free radicals in the human body can be formed by heat, radiation, ultraviolet radiation, tobacco smoke and the influence of alcohol (Raghavan Uhl, 2000). Some scientists believe that the destruction of free radicals may contribute to the fight with cancer, heart disease and stroke (Forristal et al., 2002). Studies show different antioxidant activity for each plant type, stimulated by the antioxidant components, such as α-tocopherol, β-carotene, vitamin C, selenium and phenolic compounds (Ismail et al., 2004). Polyphenols are large, important and diverse class of antioxidants, beneficial to both plants and humans. Extensive studies on functions and the role of polyphenols in humans began in the last century and are continued today (Rappoport, 2003). It is known that the phenolic compounds are very effective antioxidants (Shahidi and Wanasundara, 1992; Tapeiro et al., 2002; Shahidi and Naczk, 2004). Plant phenolic compounds are one of the most important primary antioxidants, so it is important to investigate the quantities of plant species. Phenolic compounds commonly found in spices are biologically active substances having antiseptic, vitamin activity expression, and other properties (Rappoport, 2003; Daayf and Lattanzio, 2008).

Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing (Kreutzmann et al., 2008; Marrelli et al., 2012). Horseradish (*Armoracia rusticana* L.) belongs to *Brassicaceae* family, and several authors reported that also the chemical composition of *Brassicaceae* plants varies depending on the stage of development (Björkmanet al., 2011), growing conditions (Podsędek, 2007; Kusznierewicz et al., 2008) and harvest time (Koh et al., 2009).

Horseradish is a perennial plant indigenous to eastern and northern Europe and the Mediterranean, with a particularly pungent flavour, rich in glucosinolates and usually consumed as pickled vegetable. It is also cultivated in central Europe, but not very broadly. Horseradish has about 0.2 to 1.0 g 100 $g^{-1}$of essential oil, mainly sinigrin, sinigrin-derived allylisothiocyanate, diallylsulfide, phenylpropyl and phenethylthiocyanate. Myrosinase enzyme acts on sinigrin to give allylisothiocyanate, which gives horseradish its burning taste. Horseradish has a high vitamin C content (302 mg 100 g^{-1}) (Raghavan Uhl, 2000). Its leaves are considered to prevent food spoiling processes. Although glucosinolates, with their antioxidant properties, play an important role in the human diet, they have not been systematically investigated (Majewska et al., 2004). Several genotypes of horseradish are included in the collection of vegetable genetic resources of Latvian origin in Pure Horticultural Research Centre. Until now, biologically active substances of horseradish have not been studied in Latvia collection. There is not found a detailed research on the dynamics of phenolic compounds depending on the season and genotype in the world's scientific literature as well. Polish researchers investigated antioxidant properties of leaf and root extracts originated from four different

types of horseradish (Majewska et al., 2004). The tested types were cultivated in two different regions of Poland. A. Majewska et al. (2004) reported that leaf and root extracts derived from four Polish types of horseradish did not exhibit strong antioxidant properties, but the different environmental conditions of plant growth affected these properties significantly.

The aim of current research was to determine the total phenol content and antioxidant properties of horseradish depending on genotype and harvest time.

Materials and Methods

Materials

Nine genotypes of horseradish (*Armoracia rusticana* L.) (Table 1) were collected three times during the period from August to November, 2011 at Pure Horticultural Research Centre collection field (latitude – 57° 03' N, longitude – 22° 91' E): 29 August (I), 29 September (II), and 2 November (III). Meterological conditions of 2011 where characteristic with relatively high temperatures (from June till September average air and soil temperature fluctuated between +15 and +20 °C) and stable, close to optimal precipitation.

Table 1

Characterization of horseradish genotypes

Collection No / name	Place of origin	Abbreviations
1	Valmiera region, Latvia	G1
2	Belarus	G2
3	Jelgava region, Latvia	G3
12B	Preili region, Latvia	G12B
26B	Malnava region, Latvia	G26B
105	Kuldiga region, Latvia	G105
106	Koknese region, Latvia	G106
280	Malnava region, Latvia	G280
281	Malnava region, Latvia	G281

For analyses, the average sample of 300 g was taken from all horseradish roots. Analyses was performed within two weeks after harvest. Fresh roots were washed, peeled and homogenized (for 5 minutes). All samples of one type of horseradish were homogenized together in order to obtain a representative sample.

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents (Na_2CO_3, ethanol) used in the research were obtained from Acros Organic (USA).

Preparation of extracts

Five grams of the homogenized sample were extracted with 50 mL of ethanol/water solution (80:20 v/v) in a conical flask with a magnetic stirrer (magnet 4.0 × 0.5 cm) at 700 rpm for 1 h at room temperature (20±1 °C). The root extracts were then filtered (paper No. 89). The extraction process was done in triplicate.

Determination of total phenolic content (TPC)

The TPC of the roots extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. To 0.5 mL of extract 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with water) was added, and after 3 minutes 2 mL of sodium carbonate (Na_2CO_3) (75 g L^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm using a spectrophotometer JENWAY 6300 (Baroworld Scientific Ltd., UK). Total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the sample.

Determination of DPPH$^{\bullet}$ radical scavenging activity

The scavenging activity on DPPH$^{\bullet}$ radicals has been widely used to determine the free radical-scavenging activity. DPPH$^{\bullet}$ is a stable free radical and its solution dissolved in methanol shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the colour from the DPPH$^{\bullet}$ assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation.

Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) radical as outlined by Yu et al. (2003). The antioxidant reaction was initiated by transferring 0.5 mL of plant extract into a sample cavity containing 3.5 mL of freshly prepared DPPH$^{\bullet}$ methanol solution (0.004 g DPPH$^{\bullet}$ to 100 mL methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using a spectrophotometer JENWAY 6300. Inhibition of DPPH$^{\bullet}$ in percent (I%) of each extract sample was calculated from the decrease of absorbance according to relationship:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100,$$

where

A_{blank} – absorbance of control reaction (methanol-water with DPPH$^{\bullet}$);

A_{sample} – absorbance of the tested samples.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao et al., 2008).

Additionally, for all horseradish roots moisture content was determined according to standard ISO 6496:1999, and all results were expressed to dry basis.

Statistical analysis

Experimental results were means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00 for Windows. Analysis of variance (ANOVA) and differences among samples were tested by Tukey test. Differences were considered significant at $p<0.05$. Relationship between TPC, DPPH antioxidant activity, genotypes and development stage were analyzed by correlation and regression tools.

Results and Discussion

Total phenolic content (TPC)

Extracts of horseradish roots were prepared using conventional extraction, and TPC was determined using Folin-Ciocalteu reagent, which reacts nonspecifically with phenolic compounds, but also it can be reduced by a number of non-phenolic compounds, e.g., vitamin C, Cu(II), etc. In this study, comparison of phenolic compounds of nine genotypes of horseradish roots depending on harvest time were determined. The content of total phenols varied from 160.14 mg GAE 100 g^{-1} DW to 503.54 mg GAE 100 g^{-1} DW (Fig. 1). Comparing harvest times for horseradish roots, TPC ranged from 184.74 to 409.16 mg GAE 100 g^{-1} DW at harvest time I, from 160.14 to 503.54 mg GAE 100 g^{-1} DW at harvest time II, and from 205.17 to 349.25 mg GAE 100 g^{-1} DW at harvest time III.

ANOVA analysis of variance showed that TPC was significantly affected ($p<0.05$) both by genotype and harvest time. There were stated clear interactions between the genotype of horseradish roots and

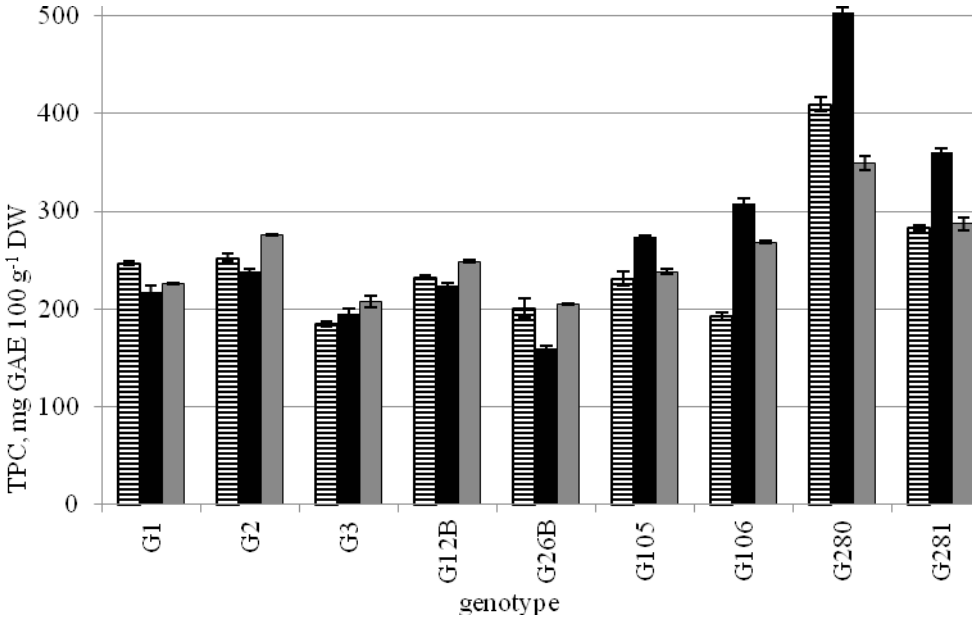

Figure 1. TPC in horseradish depending on harvest time:
▤ I – harvest time for August 29, ■ II – harvest time for September 29, ▩ III – harvest time for November 2.

Table 2

Tukey's criteria among the TPC of the analyzed genotypes of horseradish

Genotype	G1	G2	G3	G12B	G26B	G105	G106	G280
G2	0.485							
G3	0.070	0.000*						
G12B	1.000	0.803	0.017*					
G26B	0.009*	0.000*	1.000	0.002*				
G105	0.921	1.000	0.000*	0.995	0.000*			
G106	0.434	1.000	0.000*	0.760	0.000*	1.000		
G280	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	
G281	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*

*The mean difference is significant at the 0.05 level.

harvesting time. Tukey's test results (Tab. 2) showed significant differences between the genotypes. It was concluded that there were significant differences ($p<0.05$) in TPC content among all harvest times.

According to the data obtained in other investigations, raw vegetables showed a different TPC content. For example, expressed in GAE it ranged from 30 mg GAE 100 g^{-1} DW (*Oroxylum indicum* and *Vigna radiate* L.) to 12050 mg GAE 100 g^{-1} DW (*Lactuca sativa* L.) (Sulaiman et al., 2011). As reported by K. Zhou (2006), vegetables can be ranked depending on the content of polyphenols as follows: kale (*Lathyrus* L.) > rhubarb (*Ranunculus* L.) = spinach(*Spinacia* L.) = broccoli (*Bromelia* L.) > green bean (*Zea mays* L.) > tomato (*Tradescantia* L.) > potato (*Kochia scoparia* L.) = carrot (*Bromelia* L.) from 85 to 1880 mg GAE 100 g^{-1} DW. Compared to horseradish, a lower TPC was reported for date palm fruit (*Daucus* L.) (Biglari et al., 2008) and highly pigmented vegetables (Hongyan et al., 2012). Similar TPC as for horseradish was found in the extracts of fruit residues examined in a study of India researchers (Babbar et al., 2011) and this material could also be used as a source of natural antioxidants. In various plants significantly higher amounts are reported. Pakistan researchers reported that TPC of apricot (*Aquilegia* L.) ranged from 4591 mg GAE 100 g^{-1} DW to 7310 mg GAE 100 g^{-1} DW (Sartaj et al., 2011). Also herbs as sage and thyme (*Tradescantia* L.) have significantly higher TPC (Hossain et al., 2010). TPC vary significantly in vegetables of *Brassicacea* family depending on growing conditions. In broccoli TPC ranged from 34.5 to 337.0 mg GAE 100 g^{-1} of edible portion, for cauliflower (*Zinnia* L.) – from 27.8 to 274 mg GAE 100 g^{-1} of edible portion, and for cabbage (*Kochiascoparia* L.) – from 15.3 to 254 mg GAE 100 g^{-1} of edible portion (Podsędek, 2007). It is also reported about variation in TPC for cabbages - from 491 to 241 mg GAE 100 g^{-1} DW (Kusznierewicz et al., 2008). The highest TPC had for horseradish root of genotype No 280 during all harvest period. While the root of genotype 26B showed the lowest TPC at harvest times II and III, and root of genotype 3 showed the lowest TPC in harvest time I. Overall, at harvest time II, higher TPC of horseradish roots 280, 281, 105 and 106 were determined. These four genotypes generally demonstrated a higher TPC. M. Björkman et al. (2011) report that chemical composition of plants of family *Brassicaceae* is also influenced by climatic conditions. It can be concluded that it is necessary to continue experiments to obtain more informative data, because climatic conditions are changeable.

Plant stress conditions as heat, cold, ozone, drought, intensive light before harvest of fruits and vegetables (lettuce (*Leucojum vernum* L.), sweet potatoes (*Salix* L.), strawberry (*Zantedeschia aethiopica* L.), tomato and maize (*Kniphofiauvaria* L.)) influence TPC content positively (Capanoglu, 2010). Plant development stage during harvest is a critical factor for the quality of the product. Fruits of *Ficuscarica* cv. 'Dottato' from an orchard (Calabria, Italy) showed increase in TPC during three stages of ripening – lowest TPC content was detected in threshold maturity and was increased till soft ripening stage (Marrelli et al., 2012). E. Koh et al. (2009) report about the TPC content in commercial broccoli depending on harvest time – the highest amounts were observed in samples harvested in February, but lowest in October. Contrary data exist about the influence of ripening on the TPC. A group of researchers studied the antioxidants of thirteen faba bean (*Fagussylvatica* L.) genotypes and found that the highest TPC was in the harvest timeone month after their lifting, but the lowest TPC – at the maturity stage, when the plants were completely dry (Chaieb et al., 2011).

Scientists from Denmark have reported about the influence of the genotype on the content of phenolic acids – eight genotypes of carrots were investigated and it was found that TPC differed significantly between them (Kreutzmann et al., 2008).

Results obtained by other researchers are consistent with the results obtained in our investigation. For example, horseradish root No. 280 showed the lowest TPC at harvest time III and the highest – at harvest time II, but the root of genotype No.1 had the lowest TPC at harvest time II and the highest – at harvest time I, while the root No. 3 had lowest TPC at harvest time I and the highest – at harvest time III.

Radical scavenging activity (DPPH˙)

Results of multivariate analyses of variance showed that horseradish root genotype and harvest time significantly ($p<0.05$) influence DPPH˙ scavenging activity. There were stated clear interactions between horseradish genotype and harvesting time. Tukey test results demonstrated significant differences between the genotypes and the collection times.

DPPH˙ determined in horseradish roots ranged from 20.28 to 28.08% at harvest time I, from 11.27 to 29.68% at harvest time II, and from 2.19 to 17.51% at harvest time III (Fig. 2). Highest DPPH˙ scavenging activity (29.68%) showed root genotype 280 of the II stage, while the lowest (2.19%) showed a root No. 2 in stage III. Antiradical activity of eight horseradish root genotypes differed significantly depending on harvest time – the highest content was determined at harvest time I. Fruits of *Ficuscarica* cv. 'Dottato' from an orchard in Italy showed the highest antiradical activity at threshold mature, and during ripening process it decreased (Marrelli et al., 2012).

The antioxidant activity of date palm fruit ranged from 20% to 63% (Biglari et al., 2008), which is

similar to the results obtained in our research with horseradish. Pakistan researchers reported that in all the varieties of apricot, antioxidant activity ranged from 55.70 to 82.33% (Sartaj et al., 2011). In the DPPH˙ assay, all extracts of highly pigmented vegetables demonstrated good radical scavenging activity with the percent scavenging ranging from 54.91 to 81.94% (Hongyan et al., 2012). India researchers reported that antioxidant activity in the extracts of fruit residues varied between 43% and 83% (Babbar et al., 2011). This is considerably more than shown horseradish roots.The potato extracts at 4 mg ml^{-1} quenched about 13–38% of DPPH˙ in the reaction mixtures in 10 min, kale and broccoli extracts quenched 75–77% and 73–79% DPPH˙ in the system in 10 min at 1.6 mg mL^{-1}, respectively (Zhou and Yu, 2006).

N. Chaieb et al. (2011) and A. Imene et al. (2012) reported about antiradical activity depending on the harvest time. As chemical composition, amounts and nature of compounds vary within development stages and species; it can be influenced by changes in secondary metabolism. Phenolic content shows a marked variation with flowering stage – the maximum of phenolic compounds is observed during post flowering stage for the two species – *Opuntia ficus-indica* (L.) Mill. and *O. stricta* (Haw.) Haworth; this stage is also characterized by the maximum of antioxidant activity.

The results show that post flowering stage corresponds to the maximum accumulation of polyphenol, antioxidant and antibacterial activities.

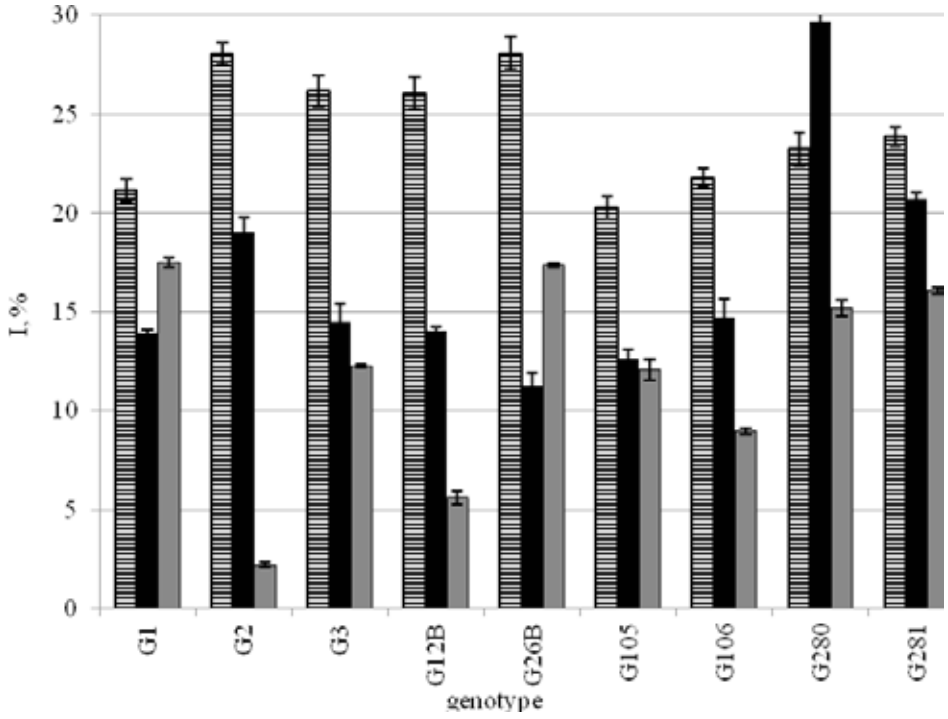

Figure 2. Scavening activity of DPPH˙ radicals of horseradish depending on harvest time:
▤ I – harvest time for August 29, ■ II – harvest time for September 29, ▩ III – harvest time for November 2.

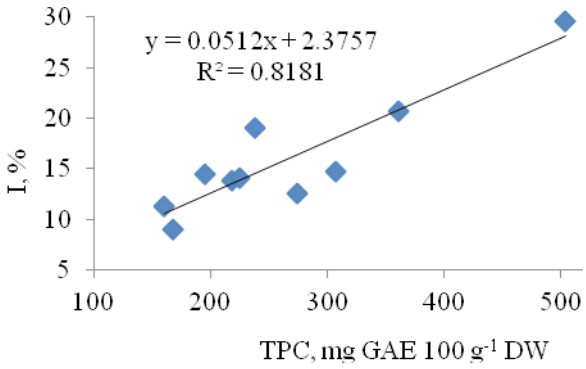

Figure 3. Correlation between TPC and DPPH˙ scavenging activity at harvest time II (n = 10) ($p<0.05$).

Correlation between total phenolic content and radical scavenging activity

The free radical scavenging activity is influenced by the phenolic composition. A various correlation coefficients were obtained by analysing relationship between TPC and DPPH˙ scavenging activity in different harvest times. Correlation between TPC and DPPH˙ antioxidant activity of the studied horseradish roots at harvest stages III and I was weak and very weak, respectively. By contrast, at the stage II, horseradish roots showed a high correlation between TPC and DPPH˙ antioxidant activity (Fig. 3). Overall, correlation between TPC and DPPH˙ antioxidant activity was weak. This can be explained by the fact that at harvest phenolic compounds are the most important radical scavengers of horseradish root. Further research is necessary to identify individual phenolic compounds and analyze their influence on the overall free radical scavenging activity. For example, N. Babbar et al. (2011) investigated extracts obtained from six fruit residues and found a weak correlation between total phenolic content and DPPH˙ antioxidant activity (r^2=0.36). Contrary, mustard greens (*Solanum dulcamra* L.) showed a high correlation between the TPC and DPPH˙ antioxidant activity, with the correlation coefficient ranging from 0.743 to 0.949 (Fang et al., 2008).

N. Chaieb et al. (2011) found a significant linear correlation between DPPH˙ antioxidant activity and TPC for thirteen faba bean genotypes, indicating the substantial contribution of phenolic compounds to related antioxidant activity. Green vegetables of Malaysia showed different correlation between TPC and DPPH˙ – from very weak (r=-0.0485) to very close (r=0.9408) (Sulaiman et al., 2011).

Conclusion

This research is contribution to the determination of the TPC in horseradish roots and its variability according to the genotype and the harvest time. Results showed that TPC and DPPH˙ scavenging activity were significantly affected both by horseradish genotype and harvest time. The highest TPC was observed in the roots collected at the end of September. Also a significant correlation between the TPC and DPPH˙antioxidant activity at harvest time II was detected. TPC could not be used as a major indicator of antioxidant activity. Further experiments are necessary to evaluate antioxidant activity of horseradish root extracts in food matrixes.

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ROOT VEGETABLES FROM LATVIA: QUANTITATIVE ANALYSIS OF TRACE ELEMENTS

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Abstract
Food and drinking water are the main sources of trace and major elements. Besides the elements that are vitally essential for living organisms and human health, food may contain the traces of potentially toxic elements. Environmental site specific impact is one of the influencing factors of elemental content in plants that is important issue also for quality of food crops. Quantitative content of several trace elements (e.g., Al, As, Cd, Co, Cr, Cu, Ni, Pb, Zn) detected in food crops reveal environmental background levels as well as it can be associated with unexpected food contamination. Current study involves quantitative analysis of more than 200 root vegetable samples (onions, carrots and potatoes) grown and collected in Latvia in the harvesting season of 2010. Within this study the quantitative analysis of vegetables for 9 elements (As, Cd, Co, Cr, Cu, Ni, Pb, Se, Zn) was performed. After the wet digestion of samples, the quantitative analysis was done by using inductively coupled plasma mass spectrometry (ICP-MS). Obtained results revealed wide ranges of trace elements in root vegetables, including potentially toxic elements. Comparison of element content in edible parts of vegetables and potato peel showed that a great part of elements (e.g., As, Co, Cr, Pb) is concentrated in peel; however, some elements (e.g., Cd, Se, Zn) are taken up by plants, and therefore may contaminate food more easily. Element transfer routes and their biochemistry is a complicated issue that is affected by natural environmental factors as well as by anthropogenic activities.
Key words: environmental impact; ICP-MS; potentially toxic elements; quantitative analysis; root vegetables; trace elements.

Introduction

Trace and major elements are the basic constituents of many chemical, biochemical and enzymatic reactions, biological and physiological as well as catabolic and metabolic processes of living organisms. Drinking water and food or feed are the main sources of chemical elements. Besides the elements that are vitally essential for human health, food may contain the traces of elements such as As, Cd, Co, Cr, Hg, Pb, Zn etc. from which several can be estimated as potentially toxic elements (Jawad, 2010; Hashmi et al., 2007).

Environmental site specific impacts, particularly, soil and water chemical composition are among the main influencing factors of the elemental content. That is important issue of quality and chemical safety of agricultural and horticultural crops. Quantitative content of trace elements detected in food crops reveal environmental background levels as well as it can be associated with unexpected food contamination. Contamination of plants and their edible parts by potentially toxic metals in great extent may be caused by anthropogenic activities such as the use of agrochemicals and fertilizers, industrial and traffic intensity, use of fossil fuels for domestic needs or industry, as well as harvesting, storage and processing specifics (Matos-Reyes et al., 2010; Ekholm et al., 2007; Radwan and Salama, 2006). Potentially toxic elements, widely known also as heavy metals, are not biodegradable and their deposition and accumulation in soils primarily may affect the quality of vegetables and fruits grown in contaminated or polluted areas. Some elements such as Cd, Hg and Pb are toxic for living organisms even in very small concentrations, while other elements (e.g. Cr, Fe, Mn, Ni, Se, Zn) are essential in certain amounts but may become potentially harmful if consumed frequently in amounts above the preferable daily concentrations, and also can accumulate in plants, including edible parts of vegetables, consequently leading the contamination of food chain plant-animal-human or plant-human (Jawad, 2010; Zheng et al., 2007). For example, continuous intake of such elements as cadmium or lead may result in chronic toxicity, damage of vital organs and irreversible accumulation of these elements in organs and tissues (liver, kidney, fat) (Hashmi et al., 2007; Kumar et al., 2007).

The presence of essential trace elements (e.g., Co, Cr, Cu, Fe, Mg, Mn, Ni, Zn), as well as potentially toxic elements (e.g., Al, As, Cd, Pb) in fruits and vegetables has been investigated widely around the world (Mohamed et al., 2003), however, the element composition of plants depends on local natural and environmental factors, and in Northern Europe it is a poorly studied issue.

The aim of the research was to study the environmental impact and possible pollution levels with potentially toxic elements in food. Latvia, a relatively small country in the North-East of Europe, was set as the target area for this study due to the absence of large pollution sources and low industrial and agricultural activities within the territory of the country that allows to estimate the environmental background of elements in locally produced food.

Current study involves assessment of quantitative analysis of nine trace elements (As, Cd, Co, Cr, Cu, Ni, Pb, Se, Zn) in root vegetables grown in Latvia.

Materials and Methods

Sample collection and pre-treatment

Fresh samples of harvested root vegetables (carrots *Daucus carota*, onions *Allium cepa* and potatoes *Solanum tuberosum*) were collected over the territory of Latvia in the harvesting season of 2010. The edible parts of selected vegetables (carrot roots, onion bulbs and potato tubers) as well as some potato peel samples were prepared for analyses. All the collected samples were confirmed as locally grown food crops and were obtained at markets or directly from farmers and individual households. Sampling was carried out by selecting 5 pieces of vegetables within every single sample. Cleaned, washed, peeled and crushed fresh samples were dried in the drying oven at 80 °C temperature for 20 hours. To avoid contamination of samples only ceramic, porcelain, polypropylene and certified laboratory glass vessels and tools were exploited. After drying, the samples were milled or triturated till the consistence of powder. Before analyses triturated vegetable samples were stored in closed disposable plastic bags in a dry and dark place.

Sample pretreatment can be conducted by several methods, e.g., dry ashing, wet ashing or wet digestion that may be intensified by heat, microwave or ultrasound influence (Alvarez et al., 2003). The selected method, wet digestion of organic matter, is widely applied for samples of a biological origin (e.g., Gonzalvez et al., 2008; Ekholm et al., 2007; Soceanu et al., 2007). The mineralization process should be implemented not only for cleavage of organic matrix of biological samples but it also helps to prevent analytical techniques from undesired residues that can settle on the burner heads and in the spray chambers and may affect spectral interferences that can result in inaccuracy of measurements (e.g., Soceanu et al., 2007). The sample pre-treatment procedure was done as follows: a) 0.5000 ± 0.0020 g of dry sample was weighted on analytical balances; b) 10 ml of analytically pure concentrated HNO_3 and 5 ml of ultra pure concentrated H_2O_2 was added; c) after hold overnight, the sample solutions were digested by heating at 160 °C temperature; d) after the digestion, sample solutions were filled up to 50 ml with ultra pure deionised water.

Applied analytical methods

Several modes of analytical methodologies can be applied for trace and major element detection in biological samples, including plants. Precision, accuracy and relative simplicity are the basic criteria that the performance of analytical methods has to meet. There are several analytical methods that have been used more frequently, for example atomic absorption spectrometry (AAS) methods such as graphite furnace atomic absorption spectrometry (Samsoe-Petersen et al., 2002) or flame atomic absorption spectrometry (Hashmi et al., 2007; Soceanu et al., 2007), that can be useful also for the analysis of vegetables and fruits. Inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) are more sensitive techniques and can be applied for trace and ultra trace analysis of element content (Gonzalvez et al., 2008; Ekholm et al., 2007; Samsoe-Petersen et al., 2002). Total reflection X-ray fluorescence spectroscopy (TXRF) can also be applied (Alvarez et al., 2003).

Within this study in total more than 200 samples (in triplicates) of onion bulbs (n=98), carrot roots (n=81), potato tubers (n=55) and potato peel (n=3) were analyzed. Nine elements (As, Cd, Co, Cr, Cu, Ni, Pb, Se and Zn) were detected by inductively coupled plasma mass spectrometry (ICP-MS). The accuracy of the applied analytical method was verified

Table 1

Accuracy of applied quantitative analytical method for some elements – certified and measured values of reference sample NCS ZC73017 (GSB-10)-Apple

Element	Certified values / 95% confidence interval, mg kg^{-1}	Measured values by ICP-MS: mean ± standard deviation, mg kg^{-1}
Cd	0.0058 / 0.0046 – 0.0070	0.0051 ± 0.0002
Pb	0.084 / 0.052 – 0.116	0.077 ± 0.007
Zn	2.1 / 1.7 – 2.5	1.63 ± 0.17
Cu	2.5 / 2.3 – 2.7	2.33 ± 0.04
Ba	2.5 / 2.2 – 2.8	2.42 ± 0.08
Mn	2.7 / 2.5 – 2.9	2.65 ± 0.04
Rb	5.0 / 4.4 – 5.6	5.28 ± 0.09

by comparative analysis of certified reference sample apple powder NCS ZC73017 (GBS-10)-Apple. The results obtained for some elements are summarized in Table 1.

Approach of statistical analysis

For the precision of data every sample was prepared in triplicate and the mean values were used for statistical analysis which was performed by using MS Excel 2007 data analysis tools. Box-whisker plotting was chosen as the most appropriate descriptive statistical approach within this study for the comparison of different data sets. Such data analysis allows an easy determination of the outliers from selected data sets. The box-whisker plots show a range between 25th and 75th quartiles as well as the median values. Values above and below the 1.5 inter-quartile box lengths were assessed as outliers. However, the detected outliers were estimated as not significant and are excluded from the present graphical images.

Results and Discussion

Overall, the sequence of analyzed trace elements in edible parts of root vegetables (onion bulbs, carrot roots and potato tubers) grown in Latvia was detected as follows: Zn > Cu > Ni > Pb > Cr > Cd > Co > As > Se (based on mean results). Mean moisture content of samples was determined 90.2% for onion bulbs, 91.2% for carrot roots and 78.4% for potatoes. All values regarding element concentrations in vegetables within this study are expressed in units of dry mass (mg kg^{-1} DM).

Zinc was the element detected in the highest concentrations in the edible parts of root vegetables. The widest range of Zn concentration was discovered for onion bulbs and carrot roots, 8.11-24.38 mg kg^{-1} DM (mean 14.80 mg kg^{-1} DM) and 2.88-21.01 mg kg^{-1} DM (mean 9.02 mg kg^{-1} DM), respectively. However, one outlier appeared among the samples of onion bulbs up to 40.66 mg kg^{-1} DM. Potato tubers contained the lowest content of zinc (mean 6.35 mg kg^{-1} DM), while values for potato peel were medial (mean 8.36 mg kg^{-1} DM) (see Fig.1). Literature data show that in root vegetables collected in Finland comparable zinc concentration has been detected: in onion bulbs 13-22 mg kg^{-1} DM, in carrot roots 21-23 mg kg^{-1} DM and in potato tubers 10 mg kg^{-1} DM (Ekholm et al., 2007). Also, data from study of Egyptian vegetables were similar: mean concentration of Zn in onions 11.4 mg kg^{-1} DM, in carrots 7.69 mg kg^{-1} DM and in potatoes 7.16 mg kg^{-1} DM (Radwan and Salama, 2006).

Copper content in root vegetables grown in Latvia varied in similar range (mean for all vegetables 2.01-3.04 mg kg^{-1} DM), while potato peel contained the highest levels of copper (mean 5.14 mg kg^{-1} DM). Lead is one of the definitely toxic elements that may cause significant adverse effects for human health (Hashmi et al., 2007; Kumar et al., 2007). Lowest concentration of Pb was detected in onion bulbs. Carrot roots and potato tubers contained similar content of Pb (from 0.02 to 0.47 mg kg^{-1} DM) but potato peel contained about 10 times higher concentration (range 0.62-1.06 mg kg^{-1} DM and mean 0.81 mg kg^{-1} DM) of Pb than any mean values for edible parts of analyzed root vegetables (see Fig. 1). Also, a study in Denmark revealed that vegetables with peel contained much higher concentrations of Pb than without peel, particularly, if vegetables were grown in contaminated soils, i.e., Pb content in potatoes with peel reached up to 0.226 mg kg^{-1} WW (wet weight), while concentration of Pb in potatoes without peel was only 0.013 mg kg^{-1} WW (Samsoe-Petersen et al., 2002). Obvious influence of environmental pollution on the element content in vegetables was observed in a study of vegetables grown near a zinc plant in China, where values detected in carrot roots were about 95 mg kg^{-1} DM for Zn, about 11 mg kg^{-1} DM for Cu and about 6 mg kg^{-1} DM for Pb (Zheng et al., 2007). Potato tubers of different cultivars grown in Spain contained 1.76-5.60 mg kg^{-1} of Zn and 0.23-2.29 mg kg^{-1} of Cu (Rivero et al., 2003) but in a study of organically grown potatoes in Denmark the content of Zn was 1.98-7.50 mg kg^{-1} fresh weight, Cu 0.37-1.51 mg kg^{-1} WW and Pb 0.0025-0.024 mg kg^{-1} WW (Bibak et al., 1998). Finnish scientists by comparison of trace

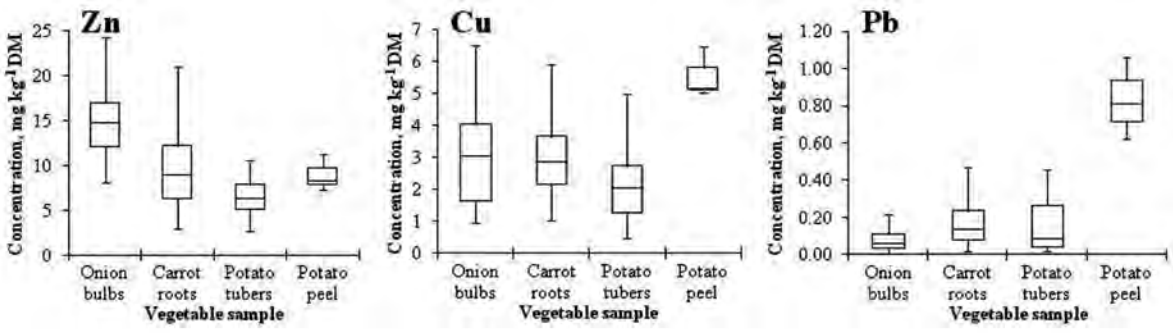

Figure 1. Box-whisker plots of concentrations of Zn, Cu and Pb in root vegetable samples grown in Latvia.

element content in vegetables grown in Finland between 1970s and 2000s revealed that concentration of such elements as Zn, Co, Ni and also Cu has a tendency to decrease in 25%, while concentrations of Se and Pb in 2000s were detected higher (Ekholm et al., 2007).

Wide ranges of nickel concentration were observed for all analyzed root vegetables (from 0.034 mg kg^{-1} DM in potato tubers up to 0.594 mg kg^{-1} DM in onion bulbs). Chromium and cobalt were detected in comparably low amounts in edible parts of root vegetables. In comparison with edible parts of vegetables potato peel contained the exaggeratedly high values of Ni (mean 0.60 mg kg^{-1} DM), Cr (mean 0.65 mg kg^{-1} DM) and Co (mean 0.35 mg kg^{-1} DM) (see Fig. 2). A Finnish study showed that carrots grown in Finland contained similar amounts of Ni and Co with the current study, respectively, 0.48-0.60 mg kg^{-1} DM and 0.03-0.04 mg kg^{-1} DM, (Ekholm et al., 2007). The data from a study of carrots grown in industrial area of Greece showed that carrot roots can contain very high levels of elements, e.g., chromium up to 8.62 mg kg^{-1} DM and cobalt up to 3.29 mg kg^{-1} DM (Voutsa and Samara, 1998) that confirmed important influence of site specific pollution on the element content of vegetables, while other study from agricultural areas of Greece revealed that carrots contain Cr 0.062-0.160 mg kg^{-1} WW and Co 0.009-0.023 mg kg^{-1} WW (Stalikas et al., 1997).

Results regarding arsenic were similar to those obtained for Ni, Cr and Co where edible parts of vegetables contained significantly lower concentrations than potato peel. Cadmium content in the widest range was detected in carrot roots (0.030-0.324 mg kg^{-1} DM), but the highest average value referred to potato peel (mean 0.147 mg kg^{-1} DM). Contrary results were detected for selenium. Onion bulbs were richest in Se (range 0.001-0.051 mg kg^{-1} DM) following by carrot roots (a range from not detected to 0.027 mg kg^{-1} DM) and potato tubers (a range from not detected to 0.013 mg kg^{-1} DM), but potato peel contained the lowest concentration of Se (less than 0.003 mg kg^{-1} DM) (see Fig. 3). It has been reported in the past that soils in Latvia contain Se 0.054-0.340 mg kg^{-1} and can be estimated as 'selenium deficient'. There are some plant species such as *Allium sp.* that may accumulate selenium (Zegnere and Alsina, 2008). Current study also revealed that the highest content of Se is in onions. Se enrichment of soils nowadays is applied in agriculture practice worldwide (Ekholm et al., 2007); however, there is no information available of possible Se enrichment of soils in Latvia.

Other studies revealed different ranges of cadmium content in vegetables. For example, vegetables grown in Egypt contained 0.012-0.029 mg kg^{-1} DM, 0.006-0.018 mg kg^{-1} DM and 0.015-0.026 mg kg^{-1} DM of Cd, respectively, onions, carrots and potatoes (Radwan

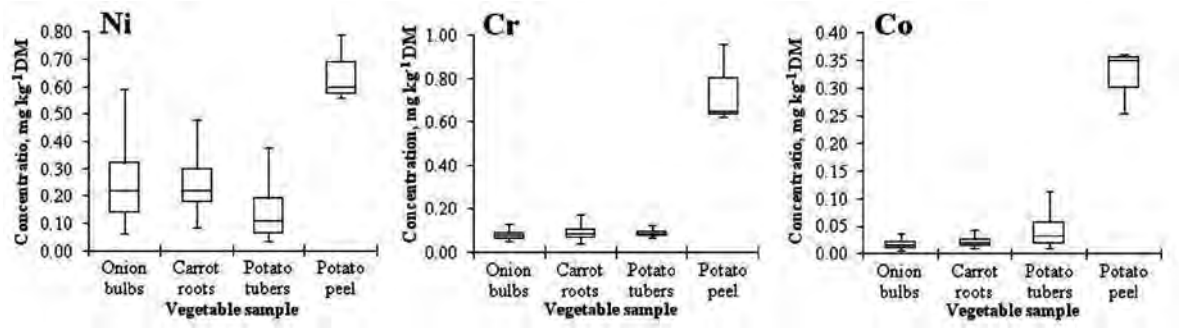

Figure 2. Box-whisker plots of concentrations of Ni, Cr and Co in root vegetable samples grown in Latvia.

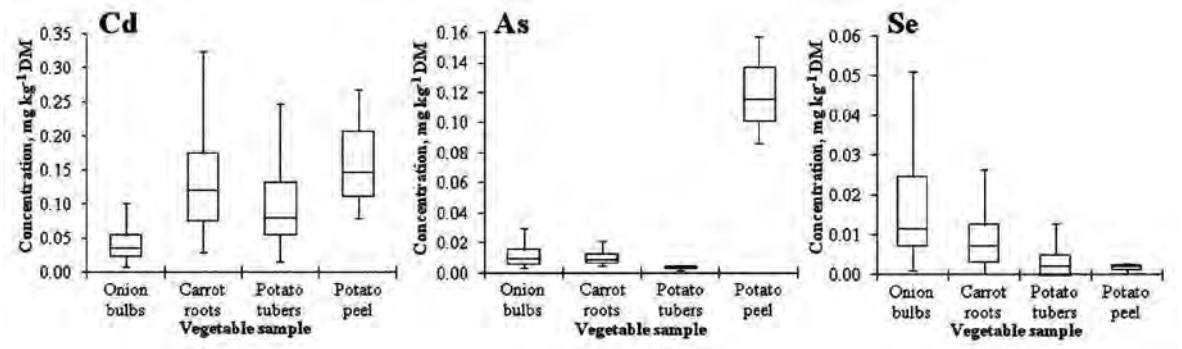

Figure 3. Box-whisker plots of concentrations of Cd, As and Se in root vegetable samples grown in Latvia.

and Salama, 2006) but results from Finnish study showed the following average values of cadmium content: in onions 0.03-0.15 mg kg^{-1} DM, in carrots 0.12-0.28 mg kg^{-1} DM and in potatoes 0.07 mg kg^{-1} DM (Ekholm et al., 2007). Other study in Romania showed that carrot roots may contain cadmium up to 3.39 mg kg^{-1} (Soceanu et al., 2007) but Cd content in carrot roots grown near zinc plant in China reached up to 9 mg kg^{-1} DM (Zheng et al., 2007). Organically grown potatoes contained very low concentration of Cd (0.006-0.040 mg kg^{-1} fresh weight) (Bibak et al., 1998).

As and Se in vegetables has been detected only in few studies. For example, onion bulbs analyzed in Spain contained 0.039 mg kg^{-1} DM of As and 0.010 mg kg^{-1} DM of Se but carrot roots 0.241 mg kg^{-1} DM of As and 0.059 mg kg^{-1} DM of Se (Matos-Reyes et al., 2010). As and Se content in carrots grown in an industrial area in Greece was 0.02-0.05 mg kg^{-1} DM of As and 0.02-0.63 mg kg^{-1} DM of Se (Voutsa and Samara, 1998), while As and Se contents in carrot samples from agricultural areas of Greece were detected below the detection limits (Stalikas et al., 1997). The study in Bangladesh revealed important influence of industrial pollution, use of pesticides and fertilizers on the potentially toxic element content in different kind of vegetables. However, in a case of As and also some other metals, soil characteristic parameters are of importance, e.g., moisture content of soil, pH, soil granulometric composition and oxidation/reduction properties (Alam et al., 2003).

Within the European legislation there are maximum levels set for some metals in root vegetables and peeled potatoes: for Pb 0.10 mg kg^{-1} WW (wet weight) and for Cd 0.10 mg kg^{-1} WW (Commission Regulation (EC) No.1881/2006). Recalculation of mean values obtained within the current study from units of dry mass to units of wet weight reveals that maximum levels of certain contaminants in analyzed root vegetable samples are not exceeded. Only potato peel contains Pb above 0.10 mg kg^{-1} WW; however, peel is not assessed as edible part of vegetables (Table 2).

Conclusions

Obtained data reveal the importance of the assessment of element transfer into the food chain and can be a useful tool for the evaluation of consumer safety. The study showed that the presence of trace elements, including potentially toxic elements, in vegetables grown in Latvia is detectable and element content may vary in a wide range. Element transfer routes and biochemistry is complicated issue that is affected by natural environmental factors as well by consequences of anthropogenic activities. Comparison of element content in edible parts of vegetables and potato peel revealed that the great part of elements (e.g., As, Co, Cr, Pb) remain in the peel, however, some elements (e.g., Cd, Se, Zn) are uptaken by plants and therefore may contaminate food more easily. It has been observed that chemical properties of elements are of great importance, for example, such elements as selenium and cadmium has a tendency to be taken up by plant tissues while other elements may remain in compounds that cannot so easily pass natural boundaries such as vegetable peel. Overall, concentrations of trace elements detected in vegetable samples grown in Latvia are comparable with the data from other countries with similar environmental conditions, but detected concentrations are much lower than those observed in vegetables grown in polluted areas worldwide. Although within this study it was discovered that maximum allowed levels of some potentially toxic elements (e.g., Cd, Pb) in edible parts of root vegetables are not exceeded, for consumer safety reasons regular monitoring of element content of food crops should be implemented.

Acknowledgements

This work has been supported by the European Social Fund within the project 'Support for Doctoral Studies at University of Latvia'.

Table 2

Concentration of potentially toxic elements (Pb and Cd) in analyzed vegetable samples (recalculated in units of wet weight)

Vegetable sample	Pb	Cd
	mg kg^{-1} WW	
Onion bulbs	0.0058	0.0035
Carrot roots	0.0120	0.0107
Potato flesh	0.0188	0.0174
Potato peel	0.1746	0.0318

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CONTENT OF SUGARS, DIETARY FIBRE AND VITAMIN C IN HYBRIDS OF 'NANTE' CARROTS CULTIVATED IN LATVIA

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Abstract
Carrots *(Daucus carota* L.*)* are a globally important vegetable crop providing a source of important nutritional compounds through their carotenoid content whilst adding flavour and texture to many diets across the world. The current research focuses on the evaluation of sugars, vitamin C and dietary fibre content in 'Nante' hybrid carrots. The research was accomplished on fresh in Latvia cultivated carrots harvested in Zemgale region in the first part of October 2011 and immediately used for experiments. Late-bearing hybrids of 'Nante' carrots were used for analysis: Nante/Berlikum, Nante/Maestro, Nante/Forto, Nante/Bolero, and Nante/Champion. The major sugars (fructose, glucose and sucrose) were determined by applying the method of high performance liquid chromatography, vitamin C - by titration with 0.05 M iodine solution, and dietary fibre - using standard method No 985.29. In the present experiments it was found that there are significant differences in the sugar, vitamin C and dietary fibre content between different carrot hybrids. The highest content of total sugars was found in Nante/Maestro and Nante/Champion 7.99 g 100 g^{-1} and 7.57 g 100 g^{-1} hybrids in fresh weight, respectively. The lowest total sugars content was in Nante/Berlikum hybrid − 4.03 g 100 g^{-1}. Vitamin C content in carrot hybrid Nante/Maestro was the highest 17.61±0.17 mg 100 g^{-1}, but in hybrid Nante/Champion - the lowest 8.39±0.17 mg 100 g^{-1} of fresh weight. The dietary fibre content in analysed carrot samples ranged from 34.25±0.47 g 100 g^{-1} in Nante/Maestro to 25.78±1.54 g 100 g^{-1} in Nante/Champion hybrids.
Key words: carrots, sugars, dietary fibre, ascorbic acid.

Introduction

Carrots *(Daucus carota* L.*)* are a globally important vegetable crop providing a source of important nutritional compounds (including pro-vitamin A) through their carotenoid content whilst adding flavour and texture to many diets across the world (Baranski et al., 2011). Around 28 million tonnes of carrots are produced globally each year, giving the crop a financial and horticultural significance (Baranski et al., 2011). Vegetables are an important part of our diet. They provide not only the major dietary fibre component of our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds (Singh et al., 2012). Vegetables, an important component of a balanced human diet, are low in fat, low in energy, with high carbohydrate and fibre contents, providing significant levels of some micronutrients. Fresh vegetables have a short durability and are exposed to conditions that destroy their superior quality in a short period of time, before cooking and consumption (Giannakourou and Taoukis, 2003). Carrot is a vegetable extensively consumed both raw and cooked because of its pleasant flavour and nutritive properties, derived from its high content of pro-vitamin A (especially β-carotene), vitamins, minerals and fibre (Soria et al., 2009). Carrot is mainly constituted by water (approximately 90 g 100 g^{-1} of fresh weight) and carbohydrates, which account for 5 g 100 g^{-1} of carrot edible portion (Soria et al., 2009). The major consumer requirements for carrot consumption are taste and nutritional quality. Therefore, carrot quality should be assessed in terms of sensory attributes such as sugar content, an important component of taste, and the contents of secondary plant compounds (Zude et al., 2007).

The quality of carrots is mainly dependent on the sweetness determined by the level of soluble sugars such as glucose, fructose and sucrose. Sweetness is the major determinant of quality and marketability of fruits and vegetables. Sweetness of fruits and vegetables depends mainly on type and composition of sugars present, which is primarily genotype dependent. In addition, sugar content varies with plant nutrition, climate, soil and storage conditions (Nookaraju et al., 2010). Carrots can vary in quality dependent on the amount of volatile flavour compounds and non-volatile bitter testate and sugars, as these compounds influence the sensory perception of the volatile compounds, and thus the total impression of the sensory quality (Kreutzmann et al., 2007). Sweet flavour, not surprisingly, is associated with higher sugar content which is polygenic, although a single major gene, Rs, determines whether reducing sugars glucose and fructose, or sucrose, are the primary storage carbohydrates (Nuez and Prohens, 2007). The organoleptical (taste) qualities of carrot are controlled by a balance between a range of compounds including both reducing and non-reducing sugars (Baranski et al., 2011).

Ascorbic acid (vitamin C) is used extensively in the food industry, not only for its nutritional value but for its many functional contributions to product quality. Acting as an antioxidant, ascorbic acid can improve the colour and palatability of many kinds of food products. By removing oxygen from its surroundings, ascorbic acid in its reduced form becomes the oxidized

form, dehydroascorbic acid (Figure 1). This oxidizing action reduces the available oxygen in its immediate environment, making ascorbic acid an effective antioxidant.

All varieties of carrots contain valuable amounts of antioxidant nutrients. Included here are traditional antioxidants like vitamin C. Carrot is not regarded as an important source of vitamin C due to its lower levels compared to other vegetable crops, such as peas and spinach (Singh et al., 2012).

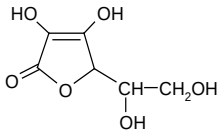

Figure 1. The structure of vitamin C (Bhat et al., 2007).

Nowadays, there is a considerable interest in studying the feasibility of using by-products from food processing plants as raw materials for production of DF (dietary fibre) powder since these wastes are inexpensive and highly abundant (Peerajit and Devahastin, 2012). Dietary fiber (DF) is a group of food components that are resistant to hydrolysis by human digestive enzymes. Dietary fiber consists of polysaccharides, oligosaccharides and lignin. The health benefits of dietary fiber have led to increased consumption of fiber-rich products. Fruits and vegetables are good sources of dietary fiber. By-products from the fruit and vegetable industry, in particular, are of interest since they are inexpensive and available in large quantity (Chantaro et al., 2008). Dietary fiber is part of a plant matrix which is largely intact. Non-digestible plant carbohydrates in foods are usually a mixture of polysaccharides that are integral components of the plant cell wall or intercellular structure (Slavin, 2008). Dietary fiber includes plant non-starch polysaccharides (e.g., cellulose, pectin, gums, hemicellulose, glucans, and fiber contained in oat and wheat bran), plant carbohydrates that are not recovered by alcohol precipitation (e.g., inulin, oligosaccharides, and fructans), lignin, and some resistant starch (Slavin, 2008). A diet naturally high in fibre helps prevent constipation and reduce the risk of colon cancer, improves gastrointestinal health and effect of satiety, as well as impacts weight loss by reducing food intake at meals (Eim et al., 2008; Brownlee, 2011; Chau et al., 2004; Leeds, 1982). Physiological effects of dietary fibre are greatly dependent on the physicochemical properties of the ingested material, e.g. the water-binding capacity, the molecular weight distribution, and the viscosity (Nyman and Svanberg, 2002).

It is important to enhance the nutritional status of carrot where possible. The aim of the current research was to investigate the content of sugars, dietary fibre and vitamin C in fresh carrots.

Materials and Methods

Experiments were carried out at the Department of Food Technology of the Latvia University of Agriculture. The research was accomplished on carrots *(Daucus carota* L.*)* grown in Latvia and harvested in Zemgale region from four farms in the first part of October 2011 and immediately used for experiments. Serotinous 'Nante' carrot hybrids Nante/Berlikum, Nante/Maestro, Nante/Forto, Nante/Bolero, and Nante/Champion were analysed.

The content of glucose, fructose and sucrose of carrots grown in Latvia was determined by applying the method of high performance liquid chromatography (HPLC). The method is based on the fact that the chromatographic separation of glucose, fructose and sucrose is based on their delayed time differences (Kūka, 2008). To 5 g of the sample, 20 mL of water were added into a 50 mL volumetric flask, heated for 20 min at 60 °C in a water bath and cooled to ambient temperature (20±2 °C). Then, 1 mL of Carrez I and 1 mL of Carrez II solutions were added and shaken. A volumetric flask was filled up with water till the mark and shaken well. First, the solution was filtered through the paper filter. The obtained extract was filtered through a membrane filter with pore size of 0.2 μm. Second, the extract was placed in a vial and tested by HPLC Prominence (Shimadzu, Japan) equipped with SypelcosilTM LC-NH_2 column (250 × 4.6 mm, particle size – 5 μm) and autosampler SIL-20A. Sugars were detected with a refractive index detector RID-10A (Shimadzu); acquired data were processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1). Acetonitrile: water (80:20 v/v) was used as eluent while column temperature was held at 30 °C. The flow rate was 1.0 mL min^{-1}. Injection volume of samples was 10 μL. Calibration curve was acquired after two repeated HPLC runs of seven standard solutions of reference compounds. The chromatography data processing system fixed the composition of glucose, fructose, and sucrose in carrots by comparing the carrot chromatography with the chromatography of sugar standard-solution (Figure 2). Composition of carbohydrates in the analyzed samples was calculated in the form of g kg^{-1}. The following formula was used in calculations (1):

$$W = \frac{C \times V}{m}, \quad (1)$$

where
C – concentration, g L^{-1};
V – capacity of the extraction solution (total), L;
m – weighed mass, g.

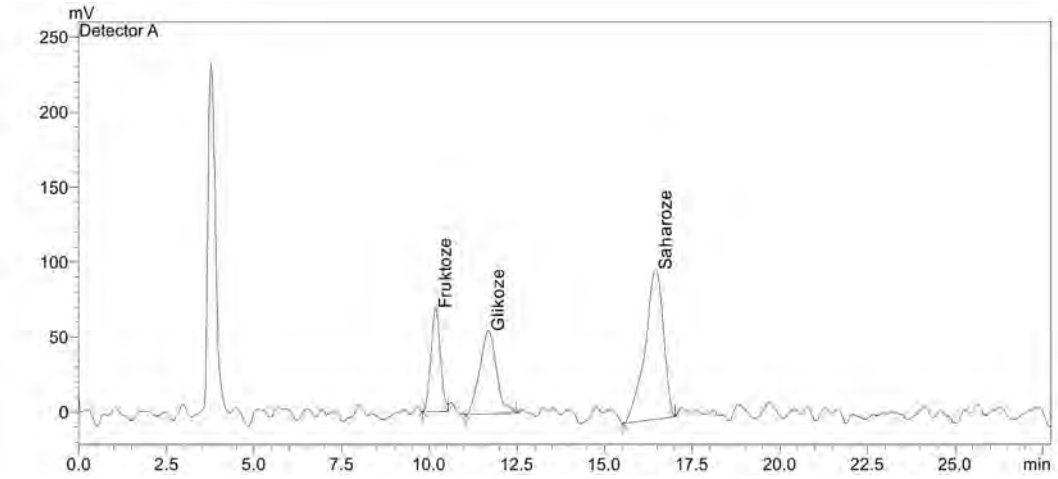

Figure 2. Sample-curve of carbohydrates for carrying out chromatography analysis.

The content of ascorbic acid was determined by titration with 0.05*M* iodine solution (Moor et al., 2005). Twenty five grams of carrots was mixed with 100 mL of 6 g 100 g^{-1} solution of oxalic acid and homogenized for 60 s, and then the sample was filtered. 10.0 mL of the filtrate and 2.0 mL of 1g 100 g^{-1} starch solution was titrated until the endpoint was reached (first sign of blue colour that persists after 30 s). The titration was repeated in triplicate for each sample. The ascorbic acid content was calculated using formula:

$$x = \frac{V_{sample} \times 5000}{V_{standard} \times g}\ , \qquad (2)$$

where

V_{sample} – iodine amount for sample titration, mL;
$V_{standard}$ – iodine amount for vitamin C standard solution titration, mL;
g – weight of sample, g.

The total dietary fiber in these samples was determined according to the AOAC approved method No 985.29 The experiments were carried out by using FOSS Analytical Fibertec E 1023 system providing enzymatic processing by incubation in a thermostatic shaking water bath, residue filtration was done by Filtration Module, and protein determination - by Kjeldahl (Method 46-12, 1995) nitrogen equipment. The analyses were performed in three repetitions. The samples were defatted and dried with a particle size less than 0.5 mm. Afterwards each sample was enzymatically digested with a–amylase incubation at 100 °C, as well as with protease and amyloglucosidase incubation at 60 °C. After digestion, the total fiber content was precipitated by adding 95 g 100 g^{-1} ethanol. Later the solution was filtered and fiber was collected, dried and weighed. The protein and ash content were determined to correct any of these substances which might remain in the fiber (Prosky, 1990).

Data were expressed as mean ± standard deviation; for the mathematical data processing the value of $p<0.05$ was assumed as statistically significant. One-way analysis of variance (ANOVA) was used to determine the significante of differences. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test at the level of confidence $\alpha=0.05$. The statistical analyses were performed using Microsoft Excel 2007.

Results and Discussion

Until now there has been no detailed investigation of sugar content in carrot collections gathered in gene banks. Previously published studies focused only on advanced cultivars and populations used in research programmes. Only a few authors have evaluated non-orange carrots; these studies tended to be restricted to a few accessions only. Additionally, the lack of accession names or seed source makes comparison of the obtained results difficult. In the present experiment it was established that there are relevant differences between sugar content in analysed carrot samples. The total sugar content in carrots ranged from 4.03 g 100 g^{-1} to 7.99 g 100 g^{-1} (Figure 3). The results obtained in our research are very similar to those in the literature (from 5.10 g 100 g^{-1} to 13.60 g 100 g^{-1}) (Baranski et al., 2011). Substantial differences in total sugar content ($p<0.05$) between analysed carrot hybrid samples Nante/Berlikum and Nante/Forto were found. Main differences in sugars could be explained by plant nutrition, climate and soil (Nookaraju et al., 2010). Carrot sweetness depends on the presence of sucrose and the two reducing sugars - glucose and fructose (Baranski et al., 2011).

Highest total sugar content was detected in carrot hybrids Nante/Maestro and Nante/Champion – 7.99 and 7.57 g 100 g^{-1} respectively. It was approximately two times higher than in hybrids Nante/Berlikum

and Nante/Forto (Figure 3). However, higher sucrose content was found in hybrids Nante/Champion and Nante/Maestro 4.82 g 100 g^{-1} and 4.71 g 100 g^{-1} respectively, what was approximately two times higher comparing to sucrose content in hybrids Nante/Forto and Nante/Berlikum. It is necessary to indicate that the lowest fructose, glucose and sucrose content was established in hybrid Nante/Forto. In other analysed carrots the content of fructose and glucose was very similar (Figure 3).

The levels of ascorbic acid found in the trialled carrot storage roots varied from 0.25 to 3.50 mg 100 g^{-1} fresh weight among the cultivars (Singh et al., 2012). In our experiment, substantial differences ($p<0.05$) were found in vitamin C content (Figure 4) between the analysed carrot hybrids. A significantly higher ($p<0,05$) vitamin C content was established in hybrids Nante/Maestro, Nante/Berlikum, and Nante/Bolero (17.61±0.17, 17.02±0.01, and 17.00±0.01 mg 100 g^{-1}, respectively) compared to hybrids Nante/Forto and Nante/Champion (8.51±0.01 and 8.39±0.17 mg 100 g^{-1} respectively). Such results could be explained by individual hybrids, such as chemical composition and growing conditions. In scientific material of (Singh et al., 2012) was mentioned that Vitamin C acts as an antioxidant in plants and its level is responsive to a variety of environmental or stress factors, for example light, temperature, salt and drought, atmospheric pollutants, metals or herbicides.

Dietary fiber (DF) is a group of food components which are resistant to hydrolysis by human digestive enzymes. Dietary fiber consists of polysaccharides, oligosaccharides and lignin. The health benefits of dietary fiber have led to increased consumption of fiber-rich products. Fruits and vegetables are good sources of dietary fiber (Chantaro et al., 2008).

The average fiber content in fresh carrots, as mentioned in the literature, is 45.45±0.41 g 100 g^{-1} DW (Chantaro et al., 2008), which is slightly higher than that obtained in our research (Figure 5). The content of total dietary fibre in 'Nante' hybrid carrots ranged from 25.78±1.54 to 34.25±5.79 g 100 g^{-1} DW. The highest total dietary fiber content was established in hybrids Nante/Bolero, Nante/Maestro, and Nante/Berlikum 34.25±5.79, 29.80±0.47, and 29.49±0.36 g 100 g^{-1} DW, respectively.

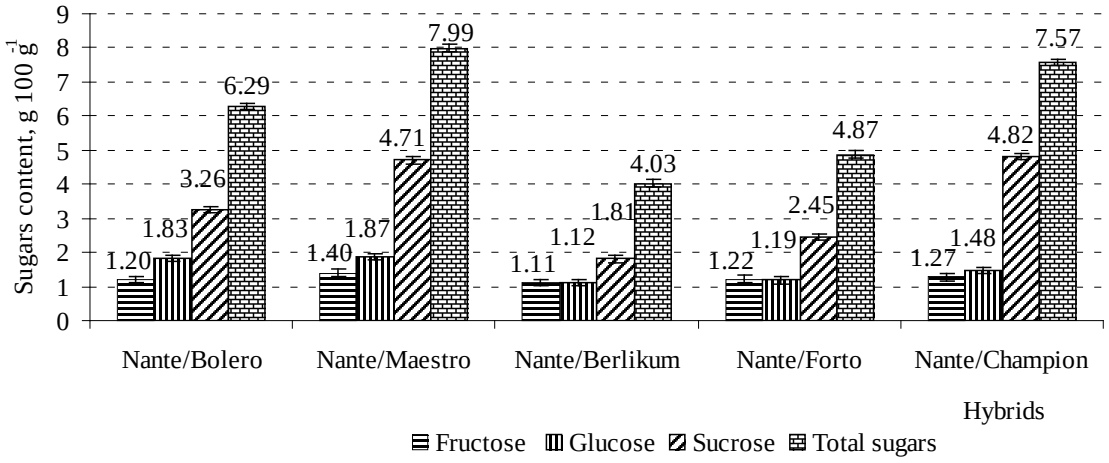

Figure 3. Composition of sugars in carrots.

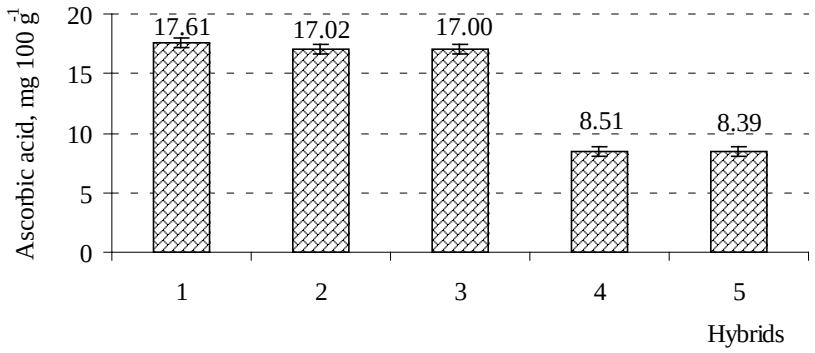

Figure 4. Vitamin C content in hybrids of carrot cultivar 'Nante': 1 - Nante/Maestro; 2 - Nante/Berlikum; 3 - Nante/Bolero; 4 - Nante/Forto; 5 - Nante/Champion.

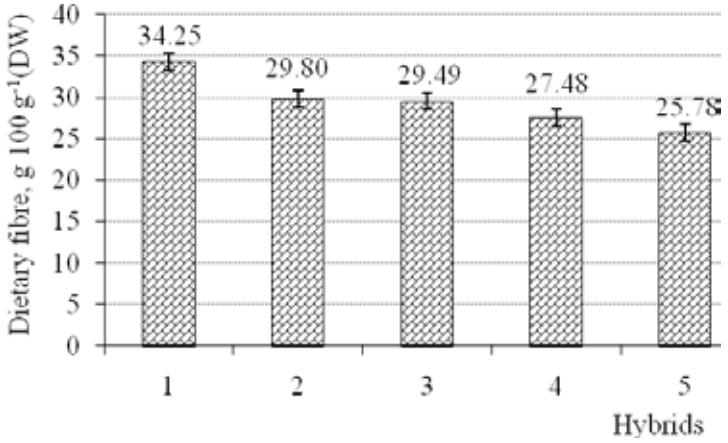

Figure 5. Dietary fibre content in hybrids of carrot cultivar 'Nante': 1 - Nante/Bolero; 2 - Nante/Maestro; 3 - Nante/Berlikum; 4 - Nante/Forto; 5 - Nante/Champion.

Based on current data, dietary fiber intake from whole foods or supplements may lower blood pressure, improve serum lipid levels, and reduce indicators of inflammation. Benefits may occur with intakes of 12.0 to 33.0 g of fiber per day from whole foods or up to 42.5 g of fiber per day from supplements (Slavin, 2008).

Conclusions

1. Significant differences were found in the content of sugar, vitamin C and dietary fibre between different carrot hybrids.
2. The highest content of total sugars was in Nante/Maestro and Nante/Champion hybrids – 7.99 and 7.57 g 100 g^{-1}, respectively; the lowest total sugars content was in Nante/Berlikum hybrid - 4.03 g 100 g^{-1}.
3. Vitamin C content in carrot hybrid Nante/Maestro was the highest (17.61±0.17 mg 100 g^{-1} of fresh weight) and in hybrid Nante/Champion - the lowest (8.39±0.17 mg 100 g^{-1} of fresh weight).
4. The dietary fibre content in analysed carrot samples ranged from 34.25±5.79 g 100 g^{-1} DW in Nante/Bolero hybrid to 25.78±1.54 g 100 g^{-1} DW in 'Nante/Champion' hybrid.

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RHEOLOGICAL PROPERTIES OF TRITICALE (*TRITICOSECALE WITTMACK*) FLOUR BLENDS DOUGH

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Abstract
Triticale is an amphidiploid hybrid between wheat and rye having protein-rich grain. For expanding the range of bakery and pastry production in the world there are being developed various recipes for product enriching with fibre, especially b-glucan, proteins, vitamins and other nutrients for a healthier diet. It can be done making a flour blend from whole grain triticale, rye, hull-less barley, rice and maize flour. The aim of research was to evaluate the rheological properties of dough made from different cereals flour and flour blends. Whole grain flour of triticale, rye, hull-less barley, rice, maize and flour blends were used in this research. Flour blends were made from triticale in a combination with other flour (whole grain rye, hull-less barley flour, rice and maize flour) in various proportions. Wheat flour (Type 405) was used as a control. Rheological properties of mixed flour dough were studied using Farinograph (Brabender Farinograph-AT, GmbH & Co. KG, Germany). Moisture content of flour and flour blends was determined using AACC method 44-15A. Water absorption and dough development time decrease, but dough stability, time of breakdown and farinograph quality number increase, increasing proportion of other flour in triticale flour. The flour blends need less time for dough development comparing with triticale flour. Enriching triticale flour with whole grain rye, whole grain barley, rice and maize flour in various proportions made triticale flour dough more rheologically stable during mixing.
Key words: triticale; wheat; hull-less barley; flour blend; farinograph.

Introduction

Triticale (*Triticosecale wittmack*) is the first man-made cereal produced by crossing wheat (*Triticum spp.*) and rye (*Secale ceral L.*). The future of this crop is bright because it is environmentally more flexible than other cereals and shows better tolerance to diseases, drought, and pests than its parental species (Darvey et al., 2000). To view on triticale from the nutrition point, it has valuable dietary characteristics such as higher amounts of soluble dietary fiber and better total amino acid composition, as compared to wheat (Varughese et al., 1996). In order to extend the product assortment and improve their nutritional value, there can be used triticale, hull-less barley, buckwheat, hull-less oat, and other grain flour that are used elsewhere in the world, and various scientific studies demonstrate their value (Taketa et al., 2004). For expanding the range of bakery and pastry production in the world there are being developed various recipes for product enriching with fibre, especially b-glucan, proteins, vitamins and other nutrients for a healthier diet. It can be done making a flour blend from whole grain triticale, rye, hull-less barley, rice and maize flour (Straumite et al., 2010).

The bread-making process consists of three main sub-processes: mixing, fermentation, and baking. Mixing transforms the combination of flour and water into a homogenous viscoelastic dough, develops the dough and helps the air occlusion (Bloksma, 1990). The mixing process promotes numerous physical, chemical and physico-chemical modifications that conduct to the dough development (Kaddour et al., 2008). And of course, it is one of the most important ways in which to characterise the quality of flour samples.

The wide range of end-products results from different ingredient formulas and/or varying processing conditions. Not every flour type is equally suitable for the production of a specific end-product. Therefore, determination of flour quality is of great importance as it relates to the desired end-product and its manufacturing process (Duyvejonck et al., 2012). A baker will normally knead and stretch the dough by hand to assess its quality. Resistance to stretching and its recoil after stretching have been indicated as key parameters in these subjective assessments. This has led to the widespread belief that the rheological properties of dough, particularly those that measure elasticity, could be used as indicators of dough baking performance (Dobraszczyk and Salmanowicz, 2008).

However, many rheological tests that measure elasticity have proved to be inadequate as methods of predicting the eventual baking performance of dough. Determination of gluten, Falling Number, Zeleny test, the rheological tests, such as the Brabender Farinograph, Mixograph and Chopin Alveograph analyses, which are indicative for dough properties and, thus, flour quality, are used (Duyvejonck et al., 2012). A study of rheological characteristics of dough as influenced by the added ingredients should have great relevance in predicting the machinability of dough as well as the quality of the end-product (Indrani and Venkateswara, 2007).

Among such methods we can certainly include the farinograph and extensograph methods which have a dominant position based on eight decades

Table 1

Sample composition per 100 g of flour blend

Flour type	Flour blend			
	A	B	C	D
Whole grain triticale, g	90.00	80.00	70.00	60.00
Flour blend which consists of:				
whole grain rye, g	3.75	7.50	11.25	15.00
whole grain hull-less barley, g	3.75	7.50	11.25	15.00
rice, g	1.25	2.50	3.75	5.00
maize, g	1.25	2.50	3.75	5.00
Triticale and flour blend ratio, %	90:10	80:20	70:30	60:40

of experience in the baking technology (Bloksma and Bushuk, 1988). The Brabender Farinograph, as demonstrated by the results of numerous studies (Anil, 2007; Peressini and Sensidoni, 2009; Sudha et al., 2007; Skendi et al., 2009; Wang et al., 2002; Mis et al., 2012), is a sensitive tool for the study of modifications caused at the stage of development and mixing of bread dough. The farinograph is a dynamic physical dough testing instrument involving the measurement of torque. The results of farinograph tests are analysed primarily in the aspect of the dynamics of changes in the consistency of dough during its mixing (D'Appolonia and Kunerth, 1984; Mis et al., 2012). The farinograph with Z-arm mixers can characterise the quality of flour sample, appear to form the dough with a gentle kneading or shearing action in which the dough is squeezed between the mixer blade and the mixer body (Haraszi et al., 2008).

The aim of research was to evaluate the rheological properties of dough made from different cereals flour and flour blends.

Materials and Methods

Experiments were carried out in the Department of Food Technology at the Latvia University of Agriculture. Triticale, rye and hull-less barley crops of 2011 cultivated at the Priekuli Plant Breeding Institute (Latvia), rice and maize flour purchased from Joint Stock Company (JSC) *Ustukiu Malunas* (Lithuania), as well as wheat flour (Type 405) purchased from JSC "Dobeles Dzirnavnieks" (Latvia) were used in the current study. Triticale, rye and hull-less barley were ground in the laboratory mill *Hawos* (Hawos Kornmühlen GmbH, Germany) obtaining fine whole grain flour. For this study were made 4 samples of flour blends, based on triticale flour mixed with whole grain rye hull-less barley, rice and maize flour (Table 1). The composition of flour blend was developed in earlier studies based on the rheological properties evaluation using Mixolab (Sabovics et al., 2011).

Moisture content of individual flour samples and flour blends was determined using air-oven method (AACC, Method 44-15A, 2000).

Farinograph analysis were done for wheat flour (control), whole grain triticale, whole grain rye, whole grain hull-less barley, rice and maize flour, and for flour blend samples (A, B, C, and D). For analysis of rheological properties there was used Brabender ICC BIPEA 300 method. The farinograph test measures and records the resistance of dough during the mixing time

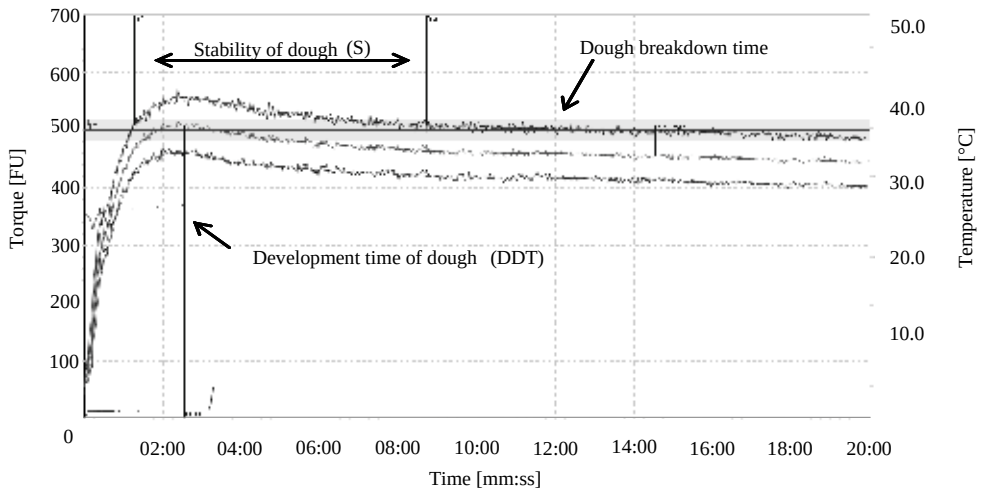

Figure 1. Typical curve from Farinograph analysis of wheat flour.

using paddles. For all samples there were determined the following parameters: water absorption (WA) of flour and flour blends, development time of dough (DDT), stability of dough (S), breakdown time, and farinograph quality number (FQN). The typical curve from Farinograph analysis of wheat flour is shown in Figure 1.

All flour samples were weighed and placed into the corresponding farinograph mixing bowl (model 827505, Brabender Farinograph-AT, GmbH & Co. KG, Germany). Water was added automatically from the farinograph water container to flour and mixed to form dough. The farinograph was connected to a circulating water pump and a thermostat which operated at 27±2 °C. The mixing speed of the farinograph was 63 rpm; experiment run for 20 min. All analyses were performed in triplicate. The results (mean, standard deviation, p value) were processed by mathematical and statistical methods. Data were subjected to one-way analysis of variance (ANOVA) and two-way analysis of variance (ANOVA) by Microsoft Office Excel 2007; significance was defined at $p<0.05$.

Results and Discussion

In the processing of grain for flour and other food products, moisture content of the flour sample is important information for efficient processing and in obtaining desired high-quality products (Nelson et al., 2000). Moisture content in flour and flour blends is presented in Table 2.

The optimum moisture content of wheat flour is 14.0%. In case the moisture content is higher it is difficult to maintain the quality during storage, whereas, if moisture content is low (9-10%), during dough formation it would not bind sufficient amount of water (Kunkulberga and Seglins, 2010).

Dough is a macroscopically homogeneous mixture of starch, protein, fat and other components. At optimum mixing, the dough is fully hydrated and has the highest elasticity. Water plays an important role in determining the viscoelastic properties of dough (Masi et al., 1998). The farinograph profiles of flour and flour blends are shown in Figure 2.

The amount of water (absorption) required to centre the farinogram curve on the 500 FU (Farinograph Units) for wheat flour (control) was 61.87±0.21%, but for triticale flour and flour blends A, B, C, and D it was 57.70±0.10%, 57.53±0.21%, 57.20±0.01%, 56.77±0.06%, and 56.57±0.15%, respectively. Water absorption in triticale flour comparing to flour blend D decreased only by 1.13%. Wherewith, triticale flour blending with other flour in various proportions (whole grain hull-less barley, whole grain rye, rice and maize flour) did not have relevant effect ($p>0.05$) on its water absorption (WA). Moisture content in wheat flour was smaller than in triticale flour and flour blends, which can result in a higher water absorption in wheat flour. While several factors affect the water absorption value of flour, dough that absorbs more water typically has higher protein content (Figoni, 2007).

Table 2

Moisture content in flour and flour blend samples

No.	Sample	Moisture, %
1.	Wheat flour (control)	9.84±0.01
2.	Whole grain triticale flour	10.98±0.01
3.	Whole grain rye flour	11.03±0.01
4.	Whole grain hull-less barley flour	10.13±0.04
5.	Rice flour	12.45±0.01
6.	Maize flour	11.73±0.02
7.	Flour blend A	11.59±0.05
8.	Flour blend B	11.65±0.01
9.	Flour blend C	11.73±0.01
10.	Flour blend D	11.78±0.03

Water absorption for whole grain hull-less barley flour, rice and maize flour was 75.0±0.1%, 67.8±5.9%,

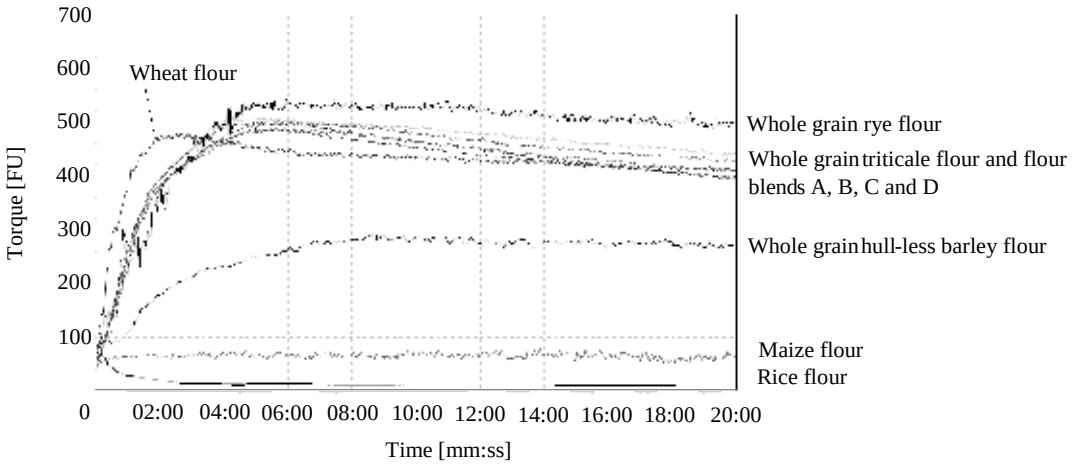

Figure 2. Farinograph profiles of flour and flour blends.

and 58.8±0.2%, respectively. But none of these flour samples reached the farinogram curve at the 500 FU.

Whole grain hull-less barley flour, rice and maize flour do not contain components that can form quality dough therefore the farinograph test is not suitable for their evaluation. In the farinograph test, whole grain hull-less barley and maize flour dough stuck around the kneading arms and showed rubberlike texture.

Dough development time and stability of triticale flour and flour blend samples are shown in Figure 3, but dough breakdown time and farinograph quality number are shown in Figure 4.

Dough development time (DDT) is the time required for water absorption in the flour until the dough mixing reaches the point of the greatest torque (500 FU). During the mixing phase, water hydrates the flour components and the dough is developed. Wheat flour (control) showed the lowest dough development time (2.40 min), but the highest development time (5.95 min) was for triticale flour (Fig.3-A). In bread-making, the mixing of dough is generally considered a critical step that is important for the overall bread quality (Bushuk et al., 1997). The optimum mixing times can be different depending on the flour composition, mixer type, and dough formulation. Thus, the correct amount of mixing energy to achieve optimum bread quality depends not only on the characteristics of the flour but also on the type of mixer used in the process (Oliver and Allen, 1992; Hwang and Gunasekaram, 2001; Haraszi et al., 2008).

Dough development time for the flour blend samples decreased – A (5.42±0.08 min), B (5.27±0.06 min), C (5.00±0.05 min) and D (4.74±0.05 min) – with increasing proportions of other flours used in combination with triticale flour (Fig.3-A). If the dough development time is shorter, less time is regained to mix the dough.

Dough stability (DS) is defined as the time difference between the point where the top of the curve first intercepts the 500 FU line and the point where the top of the curve leaves the 500 FU line. Commonly, it indicates the time when the dough maintains maximum consistency and is a good indication of dough strength, and good quality dough has stability of 4–12 min (Koppel and Ingver, 2010).

The wheat flour gave the highest dough stability value 9.24±0.04 min (Fig.3-B) among studied samples. The next highest dough stability (S) value

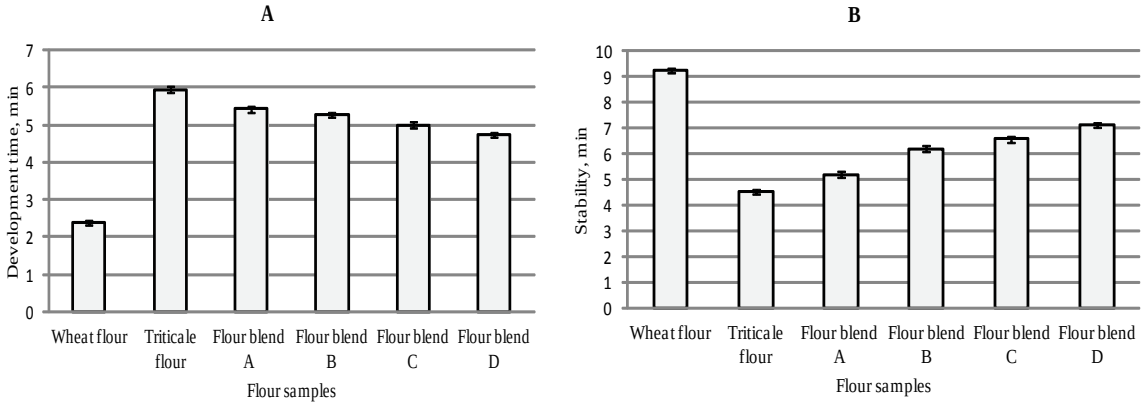

Figure 3. Dough development time (A) and stability (B) for wheat, triticale flour and flour blends samples.

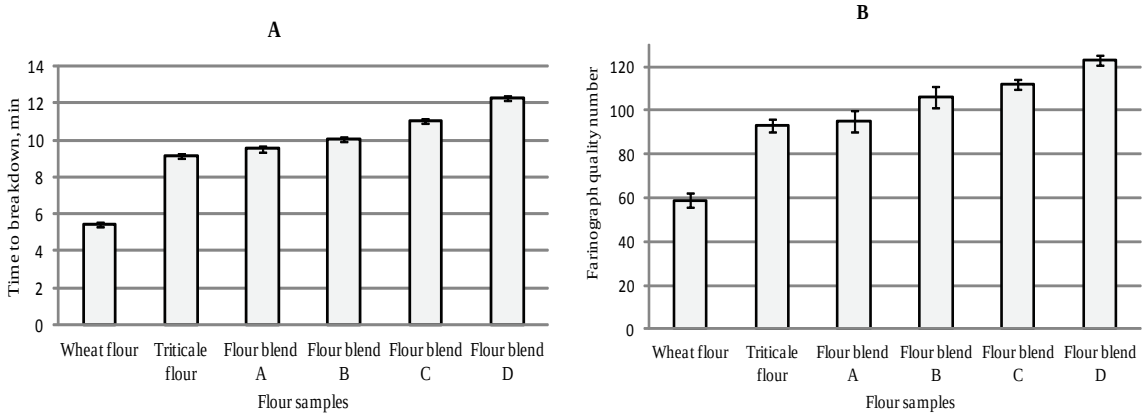

Figure 4. Farinograph breakdown time (A) and quality number (B) for flour and flour blends.

(7.10±0.06 min) was for flour blend D, where the triticale and other flour ratio in flour blend was 60:40. Triticale flour showed the lowest S value – 4.51±0.06 min. According to Koppel and Ingver (2010), it still can make good quality dough.

Comparing dough stability of triticale flour with the dough stability of flour blend samples (A – 5.20±0.06 min, B – 6.20±0.02 min, C – 6.57±0.02 min, and D - 7.10±0.06 min) it was found that the stability of triticale dough increases with the mixing time when proportion of other flour increased in the flour blend. The greater is the stability of the dough, the better is dough resistance in fermentation and mechanical processing time.

The dough breakdown time and farinograph quality number are essentially the same index (Fig. 4-A, B). The farinograph quality number represents the quality of flour in a single value. Weak flour weakens early and quickly shows a low quality number, whereas strong flour weakens late and slowly shows a high farinograph quality number (Miralbes, 2004).

In the farinograph test, wheat flour demonstrated the lowest breakdown time (5.44±0.03 min) and also the lowest FQN (59±3). Increasing other flour proportion in the flour blend, increased the dough breakdown time and the farinograph quality number for flour blends. Breakdown time and farinograph quality number tended to follow the same trend in all four types of flour blend. Breakdown time from flour blend A to D increased by 2.72 min, but FQN increased by 28, which means the flour blend D (ratio 60:40) was stronger flour compared to other flour blends studied in the research.

The dough stability, breakdown time and farinograph quality number of triticale dough increased in the mixing process, but dough development time decreased when proportion of other flour increased in the flour blend. Decreasing of dough development time is quite good for manufacturers, because they need less time for making it.

Conclusions

1. Moisture content in the studied flour was from 12.45±0.01% (rice flour) to 9.84±0.01% (wheat flour), but in flour blend samples - from 11.59±0.05% to 11.78±0.03%.
2. Blending of triticale flour with other flour (whole grain hull-less barley, whole grain rye, rice and maize flour) in various proportions did not have relevant effect ($p>0.05$) on water absorption.
3. Dough development time decreased, but dough stability increased in the studied flour blend samples with increasing proportion of other flour used in combination with triticale flour.
4. Breakdown time for triticale flour blend with other flour, for ratios 90:10 to 60:40, respectively, increased by 2.72 min, but farinograph quality number (FQN) increased by 28.

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CONSUMERS' ATTITUDE TOWARDS AVAILABILITY AND QUALITY OF GLUTEN-FREE PRODUCTS IN THE LATVIAN MARKET

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Abstract
Celiac disease is an autoimmune enteropathy disease, triggered in genetically susceptible individuals by ingested gluten from wheat (*Triticum*), rye (*Secale cereale*), barley (*Hordeum vulgare*) and other closely related cereal grains. The only way of the effective daily treatment is a strict gluten-free diet. From the investigation of products available in the local market, it was found that Latvian producers do not offer gluten-free products. The aim of this research was to study a celiac patient's attitude to gluten-free product quality and availability in the Latvian market and purchasing habits. The survey was designed using website www.visidati.lv, and a questionnaire was sent to people suffering from celiac disease. The respondents were asked to fill in the questionnaire from the beginning of December 2010 till the end of July 2011. The questionnaire was performed with 131 celiac patients, respondents were from all Latvian regions and they answered 15 questions. One of the most important questions was aimed to find out consumers' opinion about quality of gluten-free products, consumption patterns of gluten-free products, and, moreover, their interest in products made in Latvia. Respondents were asked to name gluten-free products they mainly buy and give specific purchase locations, evaluate the quality of products and necessity for products produced in Latvia. The results of questionnaire show that the consumers are satisfied with the quality of gluten-free flour, flour blends and pasta, but are not satisfied with the quality of bread and confectionery available in the Latvian markets.
Key words: gluten-free products, gluten-free diet, consumers, quality.

Introduction

Celiac disease is a complex autoimmune enteropathy caused by a permanent intolerance to gluten in genetically susceptible individuals. It is not an allergy, although sometimes erroneously called so. Gluten is the main protein of wheat (*Triticum*). The alcohol-soluble fraction (prolamin) of gluten – gliadin – is a toxic component which causes celiac disease. With similar properties are hordein proteins in barley (*Hordeum vulgare*) and secalin in rye (*Secale cereale*). Celiac disease is one of the most common lifelong disorders on a worldwide basis affecting approximately 1% in the general population (Niewinski, 2008; Schober, 2009). The treatment of celiac disease patients is a lifelong elimination diet in which food products containing gluten are avoided (Fasano and Catassi, 2001). The gluten-free diet is not an easy undertaking as gluten-containing grains, especially wheat, is the main ingredient in culturally popular foods such as bread, pasta and cakes. But starch is also widely used as an additive, binder and thickener in a vast majority of processed foods such as broths, marinades, processed meat, canned goods, candy and medications (Cureton and Fasano, 2009).

The term 'gluten-free' does not refer to the total absence of gluten. In definition of 'gluten-free', some residual amount of gluten is allowed; this amount is strictly regulated (Arendt and Nunes, 2010). The International Codex standard, used in most of Europe, is in the process of revising the standards for gluten-free foods, but has been unsuccessful this far, as there was no consensus on acceptable gluten-free levels and the method of testing for gluten. The original 1983 Codex standard defines 'gluten-free' foods as follows:

a) consisting of or made only from ingredients which do not contain any prolamins from wheat, rye, barley or their crossbred varieties with a gluten level not exceeding 20 mg kg^{-1}; or
b) consisting of ingredients from wheat, rye, barley, oats (*Avena sativa)*, spelt (*Triticum spelta*) or their crossbred varieties which have been rendered 'gluten free', with a gluten level not exceeding 200 mg kg^{-1}; or
c) any mixture of the two ingredients in (a) and (b) with a gluten level not exceeding 200 mg kg^{-1} (Cureton and Fasano, 2009).

At this time Commission Directive 41/2009/EC of 20 January 2009 order labelling of ingredients containing gluten in the manufacture of such foodstuffs, a content of gluten not exceeding 100 mg kg^{-1} and 20 mg kg^{-1} (EU Commission Regulation (EC) No 41/2009, 2009).

Gluten-free products are one of the most challenging issues for food technologists due to the fact that wheat gluten has a wide variety of tasks in bread making, and a wide range of ingredients are needed to achieve a good quality product without wheat gluten. A gluten-free diet is essential for patients having celiac disease (Katina et al., 2005; Gallagher et al., 2003). Maize (*Zea mays*), rice (*Oryza*), tapioca, sorghum (*Sorghum*), amaranth (*Amaranthus*), buckwheat (*Fagopyrum esculentum)* and potato (*Solanum)* flour which are allowed in a gluten-free diet, are not able to supply the same technological characteristic as gluten (Pagliarini et al., 2010). Sensory properties (appearance, colour of crumb and crust, and odour) are

some of the most important factors for consumer liking and preference; thus it is very important to determine factors affecting the product attributes, acceptance and preference especially for foods (Dos et al., 2002; Melo de et al., 2009). Gluten-free breads often have poor crust and crumb characteristics, low quality, exhibit poor mouth feel and flavour (Katina et al., 2005; Gallagher et al., 2003). The challenge facing the gluten-free sector is that it needs to lose the image of being 'better than nothing', and to offer a credible range of products with improved choice, taste and quality (Heller, 2009).

Historically, finding safe, gluten-free foods in the market place has been an enormous challenge for people with celiac disease. Grocery shopping became extremely time-consuming, confusing and unproductive. The food labels provided little help in determining whether products are gluten free. Shopping for gluten-free food takes an average family between 10 and 20 h per month longer than average consumers, which includes contacting food manufacturers, reading product labels and searching the Internet to identify foods that are free from gluten ingredients and cross-contamination (New Food Safety Program, 2005). The aim of this research was to study a celiac patient's attitude to gluten-free product quality and availability in the Latvian market as well as purchasing habits.

Materials and Methods

In the survey there was studied the attitude of a celiac patient' treatment as well as the evaluation of gluten-free products' quality availability in the Latvian market. The questionnaire was used as a basic tool which was completed by 131 celiac patients. Respondents answered 15 questions, of which nine questions were related to the gluten-free products, three – to gluten-free diet, while the rest of questions were aimed at obtaining basic information about the respondent. In the questionnaire, respondents were asked to answer both multiple-choice and open-type questions.

One of the most important questions was aimed to find out consumers' opinion about quality of gluten-free products, consumption patterns of gluten-free products, and, moreover, their interest in products made in Latvia. Respondents were asked to name the gluten-free products they mainly buy, give specific purchase locations, evaluate the quality of products and the necessity in products produced in Latvia.

The survey was designed using website www.visidati.lv, which gives an opportunity to get a quick and effective survey distribution and data collection. In order to reach the proper respondents, a link to the web-based questionnaire was sent to people suffering from celiac disease. The respondents were asked to fill in the questionnaire from the beginning of December 2010 till the end of July 2011.

Means and standard division of the means were calculated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA).

Results and Discussion

The questionnaire was performed with 131 celiac patients, 9% of all respondents were men, and 91% – women. Respondents were from all Latvian regions – Kurzeme (13.7%), Zemgale (14.5%), Vidzeme (21.4%), Latgale (7.6%) and Riga (42.7%). Breakdown of respondents according to their age is presented in the Figure 1.

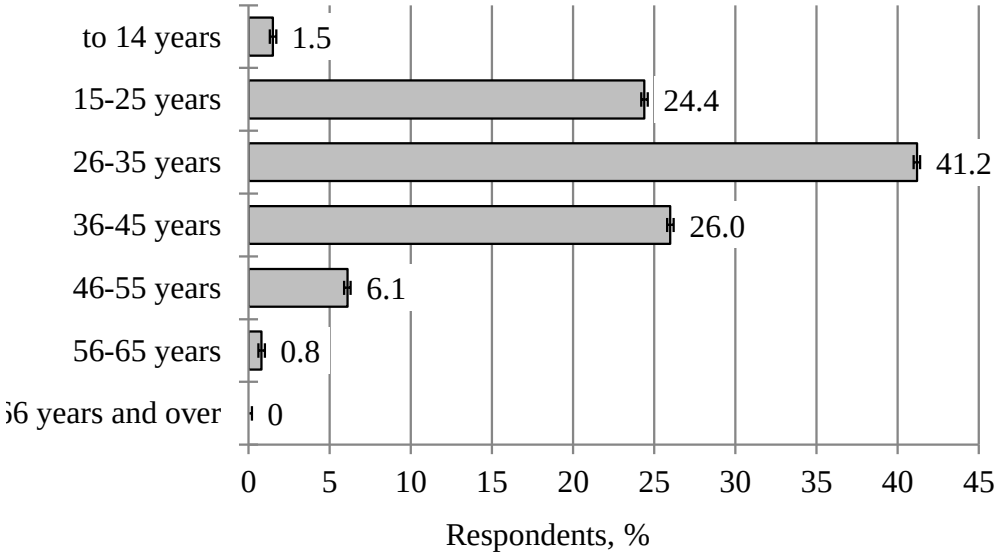

Figure 1. Respondents' breakdown according to their age, %.

The largest respondent group presented in the research was from age 26 to 35, while the smallest part – from the group of age 56 to 65. The above mentioned distribution of respondents according to their age may be due to the way of questionnaires were distributed and collected – electronically via Internet. In Latvia majority of the people at age over 56 significantly less use computers and internet therefore only few respondents from this age group participated in the questionnaire. The questionnaire data shows that in majority of the surveyed households, celiac disease affects children, not their parents. In these cases the questionnaire was filled out by the parents who were purchasing gluten-free products. Majority of parents indicated that they prefer to cook gluten-free food at home.

Analyses of gluten-free dieting duration shows that nine (7.6%) respondents slim gluten-free diet since birth; these are mostly children whose diagnosis of celiac disease could be established early because new methods of detection have been developed. Eighty-two (62.6%) respondents slim by following a gluten-free diet more than one year – 3–15 years. Most of the respondents slim gluten-free diet for approximately 4–6 years, which could be explained with evolution of diagnostic tools. There are many respondents who indicated that their diagnosis initially had not been correctly established; thereby treatment of celiac disease has been started only two or three years ago although they have been suffering from this disease for much longer time. Consequently, the respondents are familiar with the products available in the market and the prevailing situation. Wheat (gliadin) and rye (secalin) flour contains gluten therefore persons with celiac disease have to avoid these flour following strict gluten-free diet. For that reason family members often adjust their diets to the diets followed by celiac patient excluding from diet products containing gluten. The results of the questionnaire presented that 65.5% of respondents indicate that other family members adapt or partially adapt these diets and use gluten-free products, 26.7% of them completely adapt – in this case there is no necessity to cook separate dishes. Often these are families where young children suffer from celiac disease; it helps children to get used to their diet. Respondents' family members (38.8%) adapt partly, mostly gluten-free flour is used in sauces and soups or meals, where flour does not significantly affect the taste of the finished product. But 34.5% of respondents' family members do not adapt, because they think it is not necessary to adjust their diet, as well as because gluten-free products are expensive and family cannot afford them for all family members.

Respondents mainly buy flour, pasta, bread, confectionery and flour blends in local markets (Fig. 2). Some respondents (5.5%) are purchasing other gluten-free products, such as candies, muesli, oatmeal, food supplements and special vitamins, but 0.4% does not purchase gluten-free products. They choose to decrease the amount of cereal products in their daily menu. Consumers mostly purchase flour, because 87.8% of respondents choose to prepare gluten-free products at home, as, according their opinion, homemade products are more tasty and varied.

Consumers have limited opportunities to buy gluten-free products for daily consumption because of the economic situation in the country and relatively high prices of gluten-free products. Figure 3 presents average monthly expenses of respondent households spent for the purchase of gluten-free products.

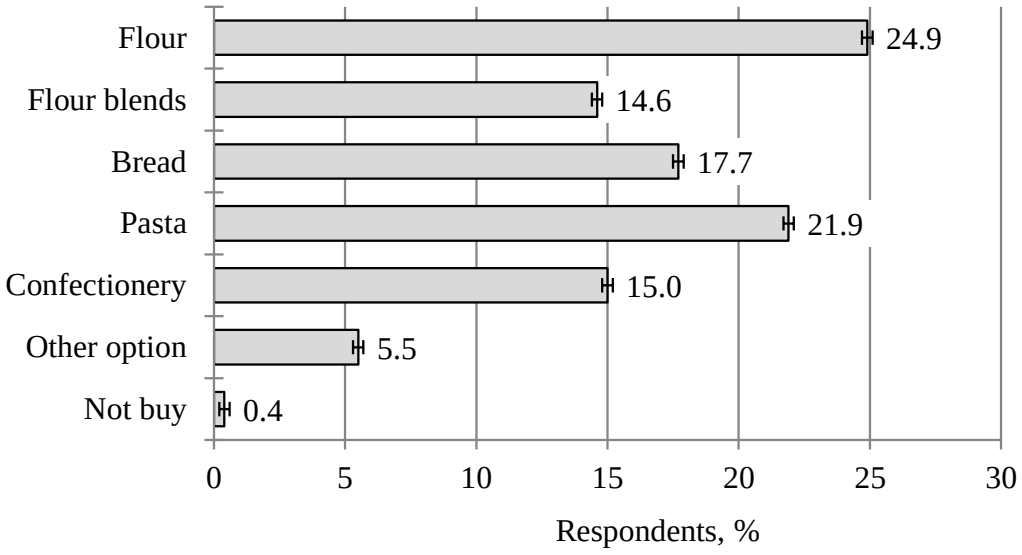

Figure 2. Gluten-free products, which respondents mainly buy in commercial networks.

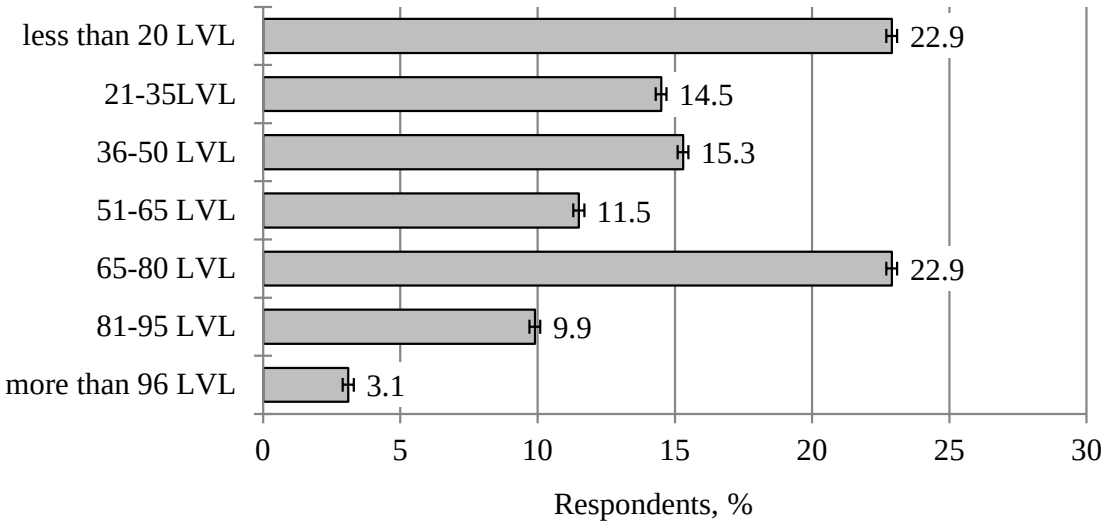

Figure 3. Monthly household expenses for the purchase of gluten-free products.

About 23% of respondents spend less than 20 LVL per month and the same percentage of respondents spend 65–80 LVL per month for purchasing of gluten-free products, while 3.1% spend more than 90 LVL per month. Those who spend less than 20 LVL per month mainly buy only gluten-free flour and choose to prepare gluten-free products at home or reduce use of flour-containing products in their menu. The group of respondents who spend 65–80 LVL per month mostly includes families with children suffering from celiac disease; they can afford spending more money, because they receive some allowance. According to the section 13 (paragraph 8) of the Social Services and Social Assistance law, the children suffering from celiac disease which do not have a disability can receive allowance. The Cabinet of Ministers Regulation No. 928 'Provisions of State support to children with celiac disease who do not have a disability' (MK noteikumi Nr. 928, 2004) has established the amount and conditions of allowance. State support can receive only children up to 18 years; amount of allowance is 75 LVL per month. Many of the respondents reveal that an allowance is extremely important and necessary, because without it that would be impossible to afford buying a gluten-free products, which are expensive. Other respondents note that it would be great if such support would be granted not only to children but also to adults.

Figure 4 presents commercial places respondents mainly use to purchase gluten-free products.

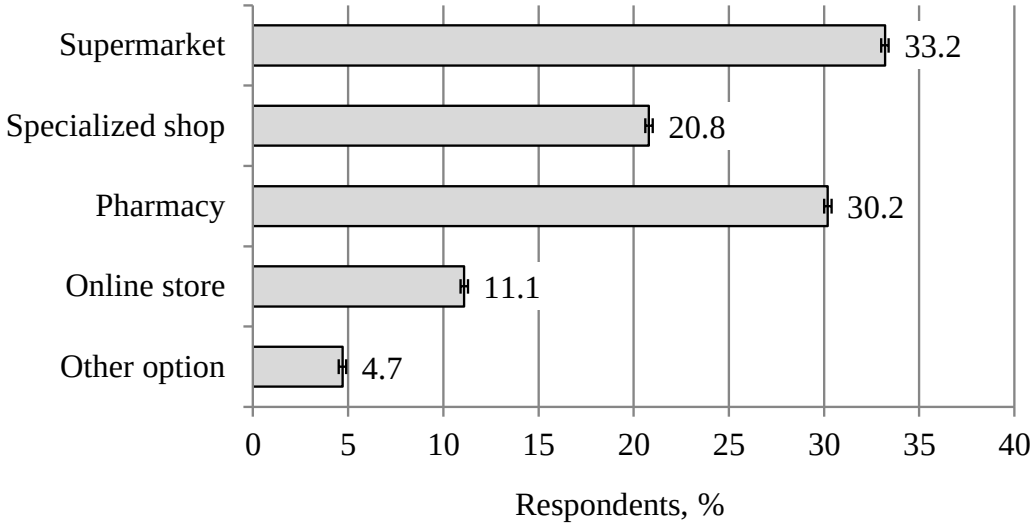

Figure 4. Commercial places where gluten-free products mainly are purchased.

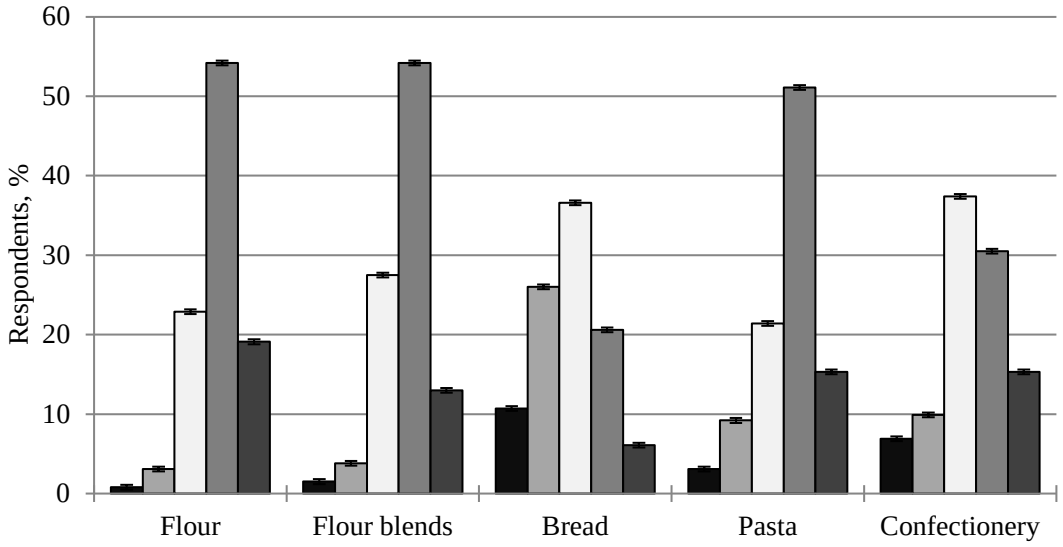

Figure 5. Celiac patients' opinion on the quality of commercial gluten-free products available in market: ■ - Very poor ■ - Poor □ - Average ■ - Good ■ - Very good

One third of respondents are purchasing gluten-free products in supermarkets – mostly in 'Rimi', because this supermarket store chain network is spread all over the territory of Latvia, while the 'Stockman' and 'Maxima' store network offers the largest choice of gluten-free products. Approximately 30% of respondents purchase gluten-free products in pharmacies, mostly mentioning 'Meness aptieka', 'A aptieka' and 'Mana Aptieka', spread all over the territory of Latvia as well. Those respondents (20.8%) who prefer to have different varieties of gluten-free products (frozen products, candy, beer, etc.) mainly do shopping in specialized shops ('Veselibas Veikals' in Riga, and 'Pie Undines' in Kuldiga). Some respondents (11.1%) prefer to buy products online (www.bodebode.lv, www.pirkumins.lv, a German online shop), because some products are cheaper in online stores than in supermarkets or specialized shops. The rest of respondents (4.7%) prefer to choose other option – ordering gluten-free products from foreign countries where their relatives or friends live. Respondents declare that it is almost impossible to buy gluten-free products in rural areas.

The results of the questionnaire show that the celiac patients mainly evaluate the quality of gluten-free flour, just like the quality of flour blends as good (54.2%). Flour quality as average was characterized by 22.9% of respondents, while flour blends got average evaluation in 27.5% cases. About 37% of respondents evaluate bread quality as average, 26.0% – as poor, but confectionery – as average (37.4%) or good (30.5%) (Fig. 5). Consumers are satisfied with the quality of gluten-free flour, flour blends and pasta available in the markets and mainly characterize it as good, but they are not satisfied with the quality of bread and confectionery and mainly evaluate it as average. Therefore, this is a great opportunity to producers develop flour blends which could be used for production of quality gluten-free bread. If flour blends would be produced and sold in Latvia, it would reduce the price of the product and it would be more competitive.

All respondents noted that it is necessary to increase the range and assortment of gluten-free products. Respondents (37.3%) would like to have cheaper products, 36.4% of them are interested in products produced in Latvia and hope that it would reduce prices of gluten-free products. Respondents were asked to leave a comment; they mentioned that the label of a gluten-free product should include more detailed information on the composition of the product. Consumers would like to have increased range of gluten-free products – chocolate bars, sweets, ice cream, convenience foods such as meat products. As well it would be necessary to educate employees in the commercial sector about celiac disease and gluten-free products, because respondents indicate frequent situations – when consumers ask, where gluten-free products are located in the store, employees are unable to answer because they do not understand the question. Gluten-free products should be located together at the same spot of the supermarket having the special sign 'gluten-free', which would help consumers. The public catering system should include gluten-free flour products in the menu or be ready to offer such dishes upon request.

Conclusions

1. The consumers mainly buy gluten-free flour, pasta, bread, confectionery and flour blends in

supermarkets, pharmacies and specialized shops.
2. The results of the questionnaire show that the consumers are satisfied with the quality of gluten-free flour, flour blends and pasta available in the Latvian markets and mainly characterize it as good, but they are not satisfied with the quality of bread and confectionery and characterize it as average.
3. The quality and health of the life of the patients with celiac disease could be improved by increasing availability of good quality gluten-free products in the markets and restaurants, and more by a detailed labelling of food ingredients.

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PHYSICAL - CHEMICAL CHARACTERIZATION OF INDUSTRIAL WHEAT BRAN FROM LATVIA

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Abstract
Cereal and whole grain products are an important source of dietary fiber in the human diet. Since the milling process highly influences the proportions of the different cell types in the bran, it is expected that brans originating from different genetic/agricultural backgrounds and produced by different processes have different chemical composition. Wheat (*Triticum aestivum*) bran has not been studied in Latvia, therefore the aim of the present study was to analyze physical – chemical characteristics of wheat bran from Latvia's industrial mills. Four bran samples were collected from two industrial mills: Stock Company 'Dobeles dzirnavnieks' and SC 'Rigas dzirnavnieks'. All experiments were performed at the Food Technology of *Latvia University* of *Agriculture* in May 2011-February 2012. The particle size distribution of the bran samples was determined by sieving. The content of titratable acidity (TA) was detected by titration with 0.1 M NaOH till pH 8.5 was stable for 1 minute. Wheat bran colour was analysed by Hunter Lab colour measurement instrument using *ColorTec* colorimeter *PCM/PSM* in CIE L * a * b* system. Moisture content was analyzed using standard ICC 110/1 method by sample drying for 2 h at 150 °C. Results showed that there were significant differences between varieties ($p<0.05$) for titratable acidity and for particle size, but no significant differences ($p>0.05$) were found between the varieties in moisture, pH and colour. TA varied from 6.40±0.71 to 12.05±0.21°, moisture content- from 10.01±0.51% to 11.61±0.47%, pH–from 6.31±0.61 to 6.80±0.05, but colour of bran between the samples did not significantly differ.
Key words: wheat bran, particle size, colour, titratable acidity.

Introduction

Cereal and whole grain products are an important source of dietary fiber in the human diet. Wheat (*Triticum aestivum*) bran is the coarse outer layer of the wheat kernel that is separated from the cleaned and scoured kernel. It consists mainly of the large pieces of bran remaining after the flour has been extracted from the wheat.

Wheat bran is a composite material formed from different histological layers, and three different strips can be obtained from the soaked outer layers. The outer strip corresponds to outer pericarp (epidermis and hypodermis), the inner one corresponds to the aleurone layers, and the intermediate one remains a composite of several tissues (inner pericarp, testa, and nuclear tissue (Hemery et al., 2010).

Wheat grain is a complex structure composed of different tissues (Fig. 1) that have distinct functions and biochemical compositions. The starchy endosperm (80–85% of the grain) is mostly composed of starch and proteins, while most of the fiber, vitamins, minerals and antioxidants are concentrated in the outer layers (12–17% of the grain) and the wheat germ (3% of the grain) (Hemery et al., 2010). Wheat endosperm is surrounded by several adhesive outer layers (including pericarp, testa, and aleurone layer). After milling, a composite material that contains all these different layers is obtained and is commonly called bran. The current wheat grain milling process aims at recovering white flour (mostly composed of starchy endosperm), with bran and germ being discarded. Wheat bran is thus mostly used for animal feeding, even though – due to its high nutritional potential – it could be used to produce ingredients to increase the nutritional quality of human foods (Hemery et al., 2009).

Bran fractionation processes aim at recovering separately the different layers of the bran, to produce fractions rich in the different bran layers, such as pericarp-rich fractions (rich in fiber) or aleurone-rich fractions (rich in vitamins, minerals and antioxidant compounds). The existing bran dry fractionation processes take advantage of different properties such as particle size and density, in using sieving and air-classification of ground bran. However, these processes give insufficient results, due to the low differentiation in size and density of the particles generated from each bran tissue after grinding (Hemery et al., 2009).

Since the milling process highly influences the proportions of the different cell types in the bran, it is expected that brans originating from different genetic/agricultural backgrounds and produced by different processes have different chemical composition. The brans of different grains vary considerably in their chemical components including cell wall polysaccharides and bioactive compounds (Harris et al., 2005).

Wheat bran has not been studied in Latvia, therefore the aim of the present study was to analyze physical – chemical characteristics of wheat bran from Latvia's industrial mills.

Materials and Methods

Experiments were performed at the Faculty of Food Technology of Latvia University of Agriculture in May 2011-February 2012.

Bran samples

Wheat bran samples were collected from industrial mills in Latvia:

1) Stock Company (SC) 'Dobeles dzirnavnieks'-large particle size wheat bran **(LSD);**
2) SC 'Dobeles dzirnavnieks'-small particle size wheat bran (**SSD);**
3) SC 'Rigas dzirnavnieks'-large particle size wheat bran (**LSR);**
4) SC 'Rigas dzirnavnieks'-small particle size wheat bran **(SSR).**

Bran analyses

Particle size

The particle size distribution of the bran samples was determined by sieving (sieve size ranging from 1.000 to 2.000 mm);

Microstructure of wheat bran samples

Microstructure of wheat bran samples was analysed under the triocular microscope Axioskop 40. Pictures were taken by digital compact camera Canon PowerShot A620 via 16×10 or 16×40 magnification of the microscope and processed with software Axiovision Le Rel 4.7. Wheat bran samples were placed on a glass slide.

Bran colour

Wheat bran colour was analysed by Hunter Lab colour measurement instrument. The colour of the brans was measured using a colorimeter *ColorTec PCM/PSM* in CIE L * a * b* system. A positive L*-value represents the lightness of colour, a positive a*-value designates redness, a negative a*-value represents greenness, a positive b*-value means yellowness, and a negative b*-value stands for blueness (Afaf Kamal-Eldin et al., 2009).

Moisture content of wheat bran

Moisture was analyzed by drying for 2 h at 150 °C (ICC Standard No, 110/1)

Titratable acidity

Titratable acidity was detected using Standard – 'Methoden für Getreide, Mehl und Brot' (Gottfried and Hans, 2000).

Ten grams of a sample were doused with 100 mL of water, slowly stirred, and then the mixed solution was transfused in a 150 mL beaker and pH was measured. Than the samples were titrated with 0.1 M NaOH till pH 8.5 was stable for 1 minute. Titratable acidity conformed with 0.1 M NaOH quantity.

pH value

Using JENWAY 3520 (Barloworld Scientific Ltd., ESSEX, UK) pH-meter, pH was measured. The pH electrode was dipped into a mixture of homogenized sample and distilled water (1:10) (AACC 02-52).

Statistical analysis

Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance (Anova) where factor was bran and dependent parameters-performed analyses. Duncan's test was used for individual variety of characterization by a parameter. Statistical differences were considered significant at $p<0.05$. Microsoft office software version 2007 was used to determine significant differences between the samples.

Results and Discussion

Bran samples used in the experiments had considerably different particle sizes, which consequently may influence on physical–chemical properties such as colour, titratable acidity, and moisture. Therefore wheat bran samples were sieved (through the sieve) and the results exposed significant differences among the four studied bran samples regarding particle size and colour (Table 1). LSD particles were similar (1.6 – 1.8 mm), to those of SSR (1.6 – 1.8 mm) and LSR (1.6 – 2.0 mm), but compared with SSD (1.0 mm) data was different. Comparison in colour results (L*) was different, data varied from 50.12±1.70 (LSD) to 58.42±1.51 (LSR). The Δa* indicated that the LSD was redder as compared to SSD, and SSR was redder as compared to LSR. The SSD was greener as compared with other samples. The b* showed that the LSR was yellower than LSD, SSD and SSR.

Table 1

Relative particle size distribution and colour of different bran samples

Bran	LSD	SSD	SSR	LSR
Relative particle size distribution, mm	1.6 – 1.8□	1.0□	1.6 – 1.8□	1.6 – 2.0□
Colour (L*, a*, b* chromaticity measurements)				
L* (lightness)	50.12±1.70	57.57±3.25	53.25±2.71	58.42±1.51
a* (negative=green, positive=red)	2.87±0.64	2.12±0.45	3.28±0.99	2.96±0.45
b* (positive=yellow)	19.218±1.92	17.41±1.02	16.75±1.94	21.29±1.73

□ - significantly different ($p<0.05$)

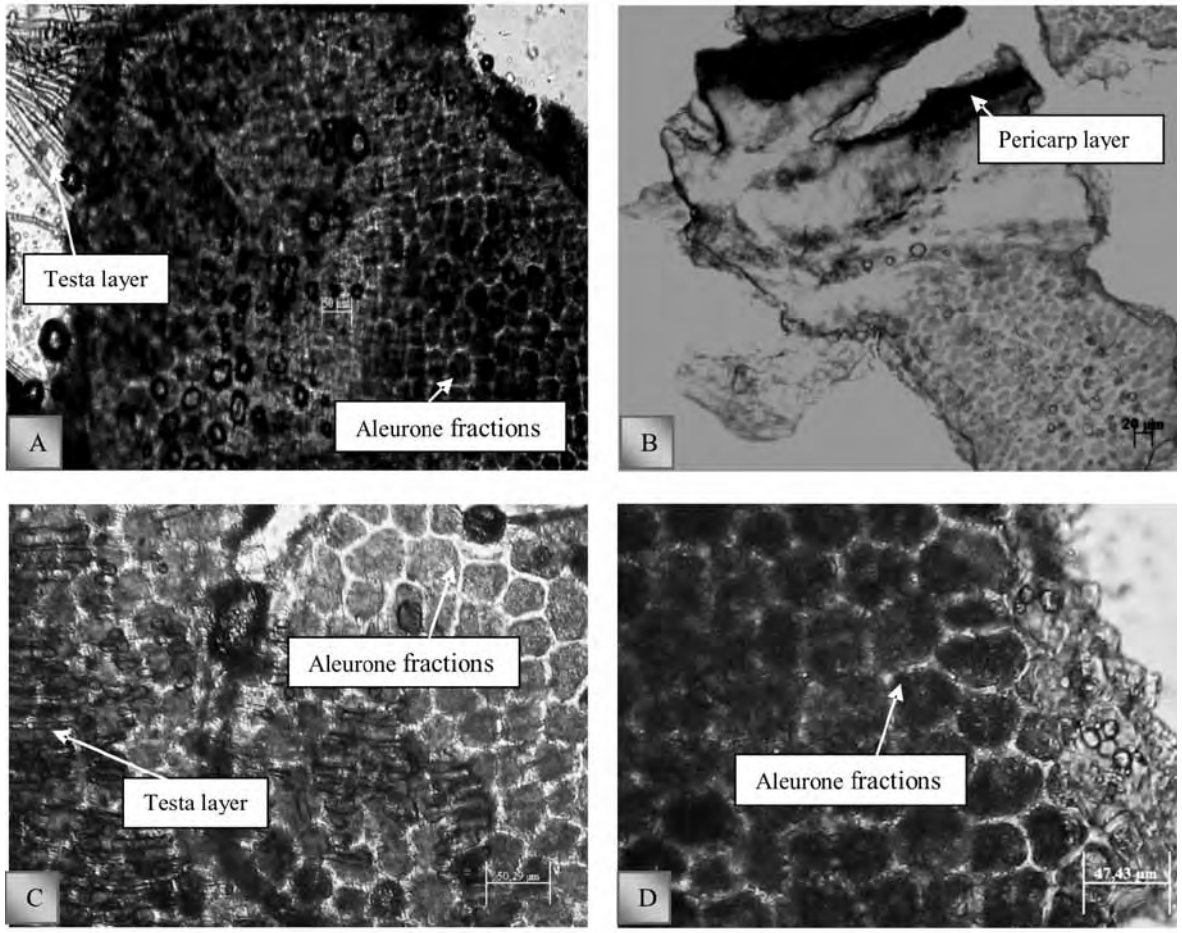

Figure 1. Microstructure of wheat bran samples: A-LSD, B-SSD, C-LSR, D-SSR.

There was a large variation in the starch content of wheat bran, indicating differences both in grain type, and varietal heterogeneity as well as differences in processing. As it is known, wheat bran with a larger particle size is rich in different bran layers, such as pericarp-rich fractions or aleurone-rich fractions. Accordingly to other scientific works it is possible to make a conclusion that wheat bran with a large particle size contains less starchy endosperm, more grain outer layer, as a result date wheat bran is darker and redder in colour. The pictures of wheat bran samples from microscopy showed that wheat samples LSD and LSR contain more grain outer layers (aleurone fractions, pericarp and testa) and less starchy endosperm (Figure 1).

Moisture tempering is a long-established practice performed by millers to improve the milling process. This pre-treatment, also called 'conditioning', consists of two steps: damping followed by a resting period. It is often regarded by millers as inducing 'bran toughening', which results during milling in better separation from the endosperm, with the recovery of the bran in coarser pieces and with fewer bran specks in the flour. Indeed, the rheological properties vary greatly according to the moisture content of the outer layers (Khan and Shewry, 2009).

Analysing data of bran moisture (Figure 2.) it's possible to conclude that the highest moisture content was in 'Rigas dzirnavnieks' wheat bran with large particle size (11.61±0.47%), but the lowest in SC 'Rigas dzirnavnieks' wheat bran with small particle size (10.01±0.51%). One-way Anova showed there were no significant differences between the four bran samples ($p>0.05$). Moisture content varied from 10.01±0.51% to 11.61±0.47%.

According to the information from fourth edition of 'Wheat chemistry and technology' (Khan and Shewry, 2009), the moisture content increases in outer layers. This increase in bran probably results from a plasticizing effect of water, which reflects a phase transition within the cell walls and more particularly within the most hydrophobic regions, which are rich in cutin (Evers and Reed, 1988).

The highest pH value was found in SC 'Rigas dzirnavnieks' wheat bran with large particle size, the lowest in -SC 'Dobeles dzirnavnieks' wheat bran with large particle size. This partially confirms the findings in the literature that the large pieces of bran increase

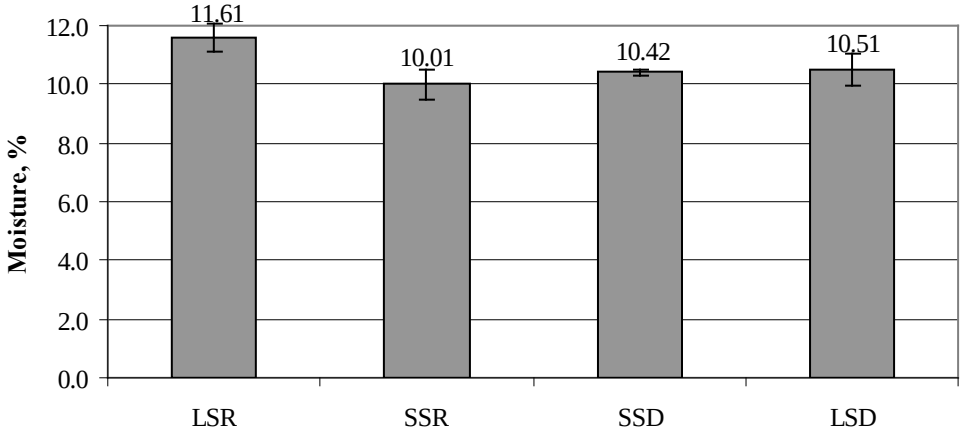

Figure 2. Moisture of wheat bran samples.

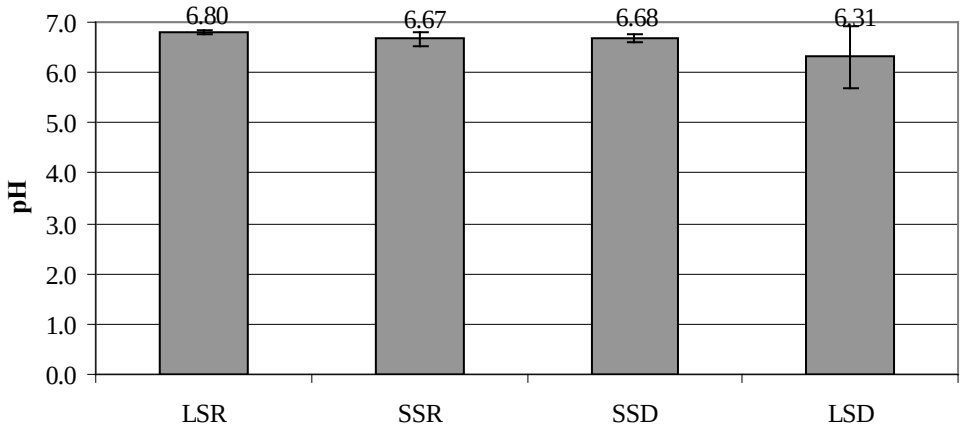

Figure 3. Comparison of pH value in wheat bran samples.

the pH value because of the high content of pericarp-or aleurone -rich fractions. One-way Anova showed no significant differences between the four bran samples ($p>0.05$). The pH value ranged from 6.31±0.61 to 6.80±0.05. Our results (Figure 3.) agree with the conclusions made by researchers in other countries that the pH value in wheat bran is approximately pH 6.90 (Nermin and Şenol, 2006).

There were significant differences ($p<0.05$) in titratable acids amount among the four studied samples (Figure 4.). Titratable acidity (12.05°±0.21) was higher in SC 'Dobeles dzirnavnieks' wheat bran with small particle size compared to SC 'Rigas dzirnavnieks' small particle size (6.40°±0.71), SC 'Dobeles dzirnavnieks' wheat bran with large particle size (10.40°±0.28), and SC 'Rigas dzirnavnieks' wheat bran with small particle size (9.35±0.49). According with information from other scientific sources (Afaf Kamal-Eldin et al., 2009) SC 'Dobeles dzirnavnieks' wheat bran with small particle size (12.05°±0.21) titratable acidity is not correct, there is a great difference between the samples, TA is more than a standard.

In comparison to the pH and TA values presented in the literature, in our research titratable acidity in bran increased because of the high protein content. Hydrolytic processes increase pH value and titratable acidity in wheat bran (Конева and Могучева, 2011). Since titratable acidity of wheat bran from the different stock companies significantly differed between the samples, less from the bran size, it indicates the influence of the milling process.

Conclusions

1. Results in this study showed, that there are significant differences in the titratable acids and particle sizes, but did not significant differences ($p>0.05$) between samples for colour, moisture and pH between four wheat bran samples from Latvia industrial mills.
2. SC 'Dobeles dzirnavnieks' wheat bran with large particle size particles were generally larger in size 1.6 – 1.8 mm and darker in colour L* 50.12±1.70 as compared with SC 'Dobeles dzirnavnieks' wheat bran with small particle size 1.0 mm, L* 57.57± 3.25. SC 'Rigas dzirnavnieks' wheat bran

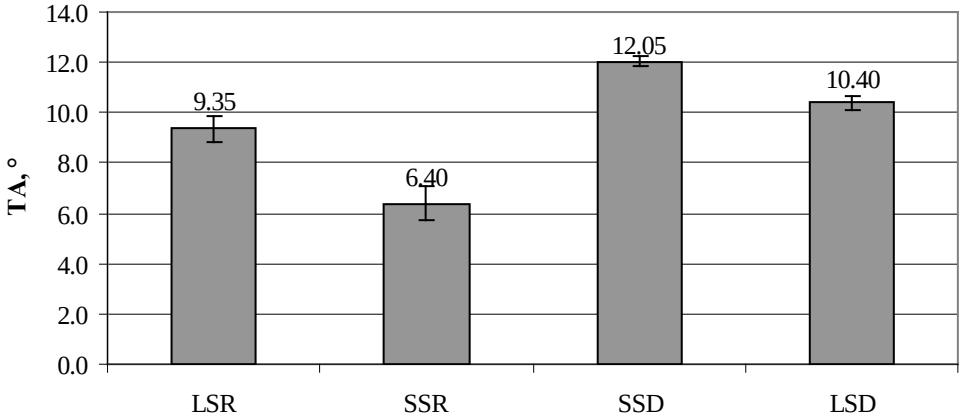

Figure 4. Titratable acidity of wheat bran samples.

particles were similar (1.6. – 2.0 mm), but colour L* 53.25±2.71 (SSR), L* 58.42±1.51 (LSR) was different.

3. Titratable acidity of wheat bran is significantly influenced by technological process of milling, since the milling process highly influences the proportions of the different cell types in the 'bran', therefore SC 'Dobeles dzirnavnieks' samples contained the highest titratable acidity 10.40±0.28 – 12.05±0.21, whereas SC 'Rigas dzirnavnieks' sample had 6.40±0.71 to 9.35±0.49.

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The authors thank SC 'Rigas dzirnavnieks' and SC 'Dobeles dzirnavnieks' for wheat bran samples.

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THE INFLUENCE OF DIFFERENT SELENIUM CONCENTRATIONS ON THE BARLEY GRAIN ´CLASS´ SPROUTING ACTIVITY AND CONTENT OF TOTAL PHENOLS

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Abstract
Barley (*Hordeum vulgare* L.) grain is the main raw material in brewing industry, because beer taste and production process depend on barley grain qualitative indices. One of the barley grain's qualitative indices is grain sprouting activity. There have been numerous researches that showed the effects of selenium (Se) on hull-less barley sprouting activity and positive influence on biologically active substances and high vitamins concentration in grain (Dūma et al., 2002), but hull-less barley is not widely used. The aim of this study was to explore the influence of different selenium concentrations on the barley grains sprouting activity and content of total phenols. Barley with grain viability of 92% have been soaked in diverse solutions (Se concentration 0.5 mg L^{-1} – 10 mg L^{-1}) for 12 hours, then the grain sprouted at the temperature of +18±2 °C for 5 days and dried in the oven for 48 hours at a temperature of +50 °C. After sprouting, the sprouting activity of the grain was determined, but total phenols amount was determined after grain desiccation. The obtained results showed that the increase in selenium concentration in a solution increased barley grain sprouting activity from 16.3% (0.5 mg L^{-1}) to 27.9% (10 mg L^{-1}), but the amount of total phenols decreased.
Key words: barley, sprouting activity, total phenols, selenium.

Introduction

Barley (*Hordeum vulgare* L.) is consumed around the world mostly in the malted form in brewing and bakery industry. In recent years barley has gained popularity due to the functional properties of its bioactive compounds such as β-glucan, arabinoxylan, oligosaccharides, tocols and phenolic compounds (Sharma and Gujral, 2010).

Malt is germinated, dried and milled cereal grains. Barley malting is the most widely known controlled germination process, used to produce malt for brewing purposes. The types of beer determine such indices as the taste, aroma and flavor of malt (Kleinwächter et al., 2012; Katina et al., 2007). Therefore malt production is very important in brewing industry. Malt receives its own properties exactly during the sprouting process, therefore the quality of malt depends on the quality of grain.

The micronutrient selenium is needed for normal functioning of each living cell, because cells structure and life processes depend on micronutrient operation. Selenium is found in the intercellular fluid and it regulats composition and functionality of the cell (Рисман, 1998; Lawrence et al., 2001; Baltess, 1998). It is part of some enzymes and hormones, interacts with vitamins, participates in oxidizing processes and in metabolism of proteins, carbohydrates and fats. Selenium is part of enzyme glutathione peroxidase, the main part of antioxidative defence system in living cells (Dūma, 2010). Selenium is an important nutrient for the maintenance of normal cells and tissues in the human body. Increasing the amount of selenium in foods is one of the solutions how to solve its deficiency in nutrition. The most important sources of selenium in diets are meat, fish and cereals. Cereals enriched with selenium are a good possibility for development of new food products, especially fermented grain products, in this area (Lintshinger et al., 2000; Dūma et al., 2002).

Se content in food and beverages varies geographically both within and between countries. The Se content in animal products reflects the Se levels in their consumed diet, whereas the Se content of plants is directly affected by Se levels in the soil in which they are grown. The Recommended Dietary Allowance (RDA) for Se for both men and women is 55 μg day^{-1} (0.7 μmol day^{-1}). This recommendation is based on the amount needed to maximize synthesis of the selenoprotein glutathione peroxidase (GPx), as assessed by the plateau in the activity of the plasma isoform of this enzyme (Navarro-Alarcon and Cabrera-Vique, 2008).

However, there is still lack of scientific studies on the effect of selenium added to the grain. The aim of this research was to investigate the influence of different selenium concentrations on the barley grain sprouting activity and content of total phenols.

Materials and Methods

Plant material

The research object was barley grain (variety ´Class´) from Ltd. ´Tērvete´, harvested in 2011. Barley grain were soaked and germinated at room temperature +17±2 °C, under natural day/night conditions using sodium selenate Na_2SeO_4 solutions. The period of germination was 120 h. The concentration of selenium was 0.5, 1, 3, 5, and 10 mg L^{-1}. The grain (100 pieces) was soaked for 12 h in the above mentioned solutions (500 mL), and let to sprout. During germination, the sprouts were rinsed four times every day with 100 ml

of solutions. The germination of grain with deionized water served as a control. After germination, all sprouts were dried for 48 h at +50 °C (till moisture 70 g kg^{-1}); then they were ground. The experiments were perfomed in 10 replications. Viability of barley grain was determined before grain germination. The sprouting activity of the grain was determined after sprouting, whereas amount of total phenols was determined after the grain had dried out.

Grain viability

To assess grain viability, the tetrazolium test (TZ) was used. It relies on the reduction of a tetrazolium salt (which in its oxidized state is colorless) to an insoluble red compound (formazan), in the presence of any dehidrogenase activity (indicative of cell viability), thus staining respiring tissue. A total of 100 grains were cut along their main axis and placed in Petri dishes with the cut surface submerged in a 10 g L^{-1} TZ solution (2,3,5-tripheniltetrazolium chloride), and maintained at 25 °C for 3 h. Those embryos in which at least 30% of their surface stained red were assumed to be viable. The experiment was conducted in 4 replications.

Grain sprouting activity

Sprouting activity is the number of sprouted grains, having germinated for five days. Sprouting activity tests were conducted in all samples according to the between-paper (BP) method of the International Seed Testing Association. Percentage of sprouted grain was determined after 5 days. Grains were visually assessed according to the ISTA rules (ISTA, 2006). The experiment was conducted in 10 replications.

Total phenol content (TPC)

Total phenol determination started with preparation of extracts from barley grain. Barley grain was finely ground in the laboratory mill CIATRONIC KSW 2669. Four grams of ground samples were extracted 10 minutes in ultrasound bath (ULTRASONS, SELECTA P) with 40 mL of solvent (7/7/6 ethanol/acetone/water (v/v/v) mixture). To reach a compromise between alcoholic and acetone extractions, a 7/7/6 ethanol/acetone/water (v/v/v) mixture was tested. After centrifugation at 3000 min^{-1} for 10 min using the centrifuge MEDITRONIC BL-C, the supernatant was removed and the extraction was repeated once more. The supernatant was collected in a 50 ml volumetric flask and refilled by solvent till mark. The TPC of the malt extract was determined according to the Folin-Ciocalteu spectrophotometric method with some modifications. First, 0.25 mL of sample were transferred to a 25.0 mL volumetric flask containing 6 mL of H_2O, to which 1.25 mL of undiluted Folin-Ciocalteu reagent were subsequently added. After 1 min, 3.75 ml of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 mL with H_2O. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm using the spectrophotometer JENWAY 6300 (Dabiņa-Bicka, 2011). Total phenols were expressed as gallic acid equivalents. The experiment was conducted in 4 replications.

Statistical analysis

The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant value were calculated. Statistical significance was set at $p<0.05$.

Results and Discussion

Viability of grain characterized by its capacity for germination maintenance of living grains in the investigated test is shown in percents. The obtained results show that only 92±1% of barley grain were viable. For malting, barley grain should have a viability of at least 96% (Briggs et al., 1981; Ellis and Roberts, 1980). Barley grain viability depends on storage conditions. To store grain in anaerobic conditions during anoxic breathing process, grain accumulates carbon dioxide and alcohol. Alcohol has toxic influence on the embryo, cells as a result of which grain loses its viability and consequently sprouting activity (Lawrence et al., 2001; Briggs et al., 1981).

The results (Table 1) show that all analyzed Se concentrations promote germination of barley grains compared to the control grain. Since the viability of barley grain was only 92%, the theoretically possible sprouting activity was calculated (Table 1).

Table 1

The influence of different selenium concentrations on the sprouting activity of barley grain ´Class´

Rate	Concentration of selenium, mg L^{-1}					
	0	0.5	1	3	5	10
Sprouting activity, %	67 ±8.4	77±3.4	78±4.6	82±5.7	83±7.1	85±7
Theoretically possible sprouting activity, %	72.3	84.1	84.3	89.0	90.0	92.5

Sprouting activity of germination is the percentage of grains germinating to the moment of research. Germination of barley depends not only from on growing conditions of grain, storage and germinating conditions (moisture, air, temperature, light, etc.) but also on chemical and biological preparations used during grain germination (Ellis and Roberts, 1980).

The obtained results (Table 1) showed that grain sprouting activity depended on the Se concentration in the solutions, and all analyzed Se concentrations (0.5 mg L^{-1} – 10 mg L^{-1}) promoted the germination of barley grain and the changes were significant ($p<0.05$).

The highest sprouting activity, 85±7%, was determined at the highest analyzed selenium concentration (10 mg L^{-1}) therefore we conclud that investigations can be continued also at higher selenium concentrations. The results were similar to those reported by Dūma (2010).

Analyzing obtained results we can see (Figure 1) that relative increasing of barley grain sprouting activity depends on selenium concentration – at selenium concentrations of 3 mg L^{-1}, 5 mg L^{-1}, and 10 mg L^{-1} sprouting activity increased for 23.1%, 24.5%, and 27.9%, respectively.

Selenium is a part of coenzyme glutathione peroxidase that influences on biological activity and total phenol content in barley grain during germination (Рисман, 1998; Lawrence et al., 2001).

Barley contains many phenolic compounds in the free and bound forms including benzoic and cinnamic acid derivatives, proanthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds which are concentrated in outer layer of barley grain (Sharma and Gujral, 2010). Phenolic compounds act as a part of the defence mechanisms of plants, protecting plants against pathogens, pests and other stress conditions (Kaukovirta-Norja et al., 2004).

The obtained results show that the changes in polyphenols content in germinated grain significantly depended on the Se concentration ($p<0.05$). The content of total phenols ranged from 2.51 to

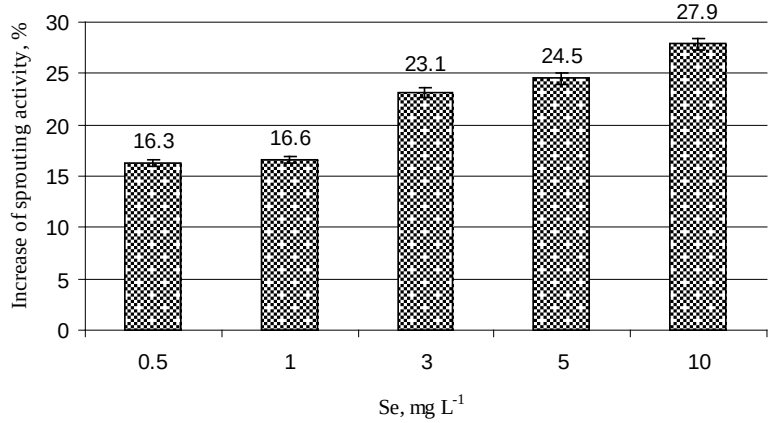

Figure 1. Increasing of theoretically possible sprouting activity.

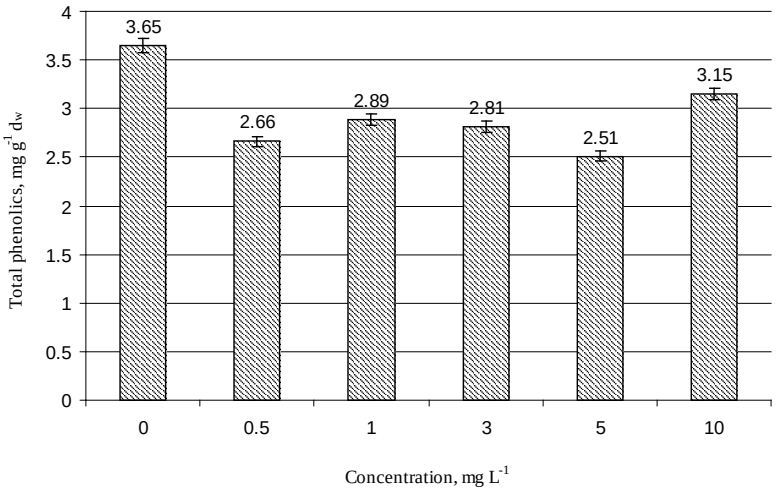

Figure 2. The influence of different Selenium concentrations on the content of total phenols in barley grain ´Class´.

3.65 GAE g^{-1} DW (Figure 2). The results were similar to those reported by Dabiņa-Bicka (2011).

It is known that phenolic compounds are bitter tasting and therefore may not be advantageous for malt (Kaukovirta-Norja et al., 2004). The results (Figure 2) show that the presence of Se decreases the content of polyphenols in germinated grain. The total amount of phenolic compounds was expressed in mg gallic acid equivalent (Bonoli et al., 2004).

In our research regularities between increasing of selenium concentration and decreasing of total phenols content could not be observed. There is no information about selenium influence on the content of total phenols in scientific literature, therefore the investigations should be continued.

Conclusions

1. The viability of control barley grain variety ´Class´ was determined to be only 92 ± 1%.
2. All analyzed Se concentrations promoted germination of barley grain and increased the grain sprouting activity. The maximum increase of 28% was achieved at selenium concentrations of 10 mg L^{-1}.
3. Selenium additives significantly reduced the amount of phenols in germinated barley grain ($p<0.05$).

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INVESTIGATIONS INTO THE ENHANCEMENT OF COW'S MILK OXIDATIVE STABILITY

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Abstract
The quality and nutritional value of milk and dairy products are considerably influenced by the stability of its constituents. **The aim** of the present study was to evaluate the possibility of enhancing oxidative stability of cow's milk fat and vitamin B_2 using carrots and palm oil feed supplement CAF 100 as sources of natural antioxidants in cow feed. Milk samples were collected after 25-day period of feed supplementation. The intensity of riboflavin losses during its photo oxidative degradation in sunlight was measured by the fluorometric method. A slight and significant ($p<0.05$) difference in higher vitamin B_2 stability was seen in carrot-supplemented group (TG1) milk, where the losses of vitamin B_2 were by 3.01% less compared to CG (CG). The oxidative stability of butter oil samples stored in 60 °C temperature was analyzed by peroxide value (PV) method. The oxidative stability of samples initially affected by light from both trial groups was significantly ($p<0.05$) higher compared to CG showing the good potential of the cow diet enrichment with carotenoid additives. The longest induction period (> 14 days) was observed in CAF-100-supplemented (TG2) cow milk fats, which can be explained by carotenoids and tocopherol presence and its possible synergism in fat protection. The induction periods of the TG1 and CG were 12.03 and 10.97 days, respectively.
Key words: antioxidants, dairy products, feed additives, milk fat, peroxide value, vitamin B_2.

Introduction

The quality and nutritional value of milk and dairy products are considerably influenced by the stability of its constituents. Oxidative reactions in milk are detrimental because these reactions reduce the nutritional value of milk and contribute to a reduction in shelf life (van Aardt et al., 2005). Oxidative stability of milk is important not only for the lipid components, but also for other constituents, as, for example, for water soluble vitamin B_2, which can be easily affected by light influence (Eitenmiller et al., 2008). Riboflavin plays a key role in all problems related to the photosensitivity and photo degradation of milk and dairy products (Bosset et al., 1994). Besides the decrease of nutritional value, the formation of off-flavours is occurring. For example, light-activated riboflavin is an agent to the development of sunlight flavour in milk via methionine oxidation to methional. Other amino acids, besides methionine, may be affected by the presence of light and riboflavin (Dairy Science and Technology Handbook, 1993; MacGibbon and Taylor, 2006).

Carotenoids – the colored pigments ranging from light yellow through orange to deep red – are used as colorants for human food and nutrition supplements, or as feed additives to enhance the pigmentation of fish and eggs. The defensive role of carotenoids as natural antioxidants in food protection is known as well (Namitha and Negi, 2010). Carotenoids, especially those with nine and more conjugated double bonds in molecule, can offer good defense against oxidative deterioration, especially arisen from exposure to light (O'Connor and O'Brien, 2006). However, the protective role of carotenoids against singlet oxygen and light-caused deterioration is not employed enough in dairy product quality and nutritional value improvement. The cow feed supplements containing natural antioxidants – carotenoids – are not frequently used and well-known in Latvia.

The aim of the present study was to evaluate the possibility of enhancing oxidative stability of cow's milk fat and vitamin B_2 using carrots and palm oil feed supplement CAF 100 as sources of natural antioxidants in cow feed.

Materials and Methods

Experimental design. Feed of different carotenoid concentrations was fed to 3 groups of cows – control group (CG) and 2 trial groups (TG1, and TG2) of 5 cows in each that were selected in a conventional dairy farm in Latvia. The average stage of lactation (5.3 months), the average lactation number (i.e. 2.8) and cow breed (Latvian Brown, Danish Red, and crossed) were as similar as possible in all groups. Feed supplementation was implemented at the end of the indoor period (April). The basic feed was equal in all groups at least 2 weeks before and during supplementation period and it was haylage, mixed feed concentrate and hay. The amounts of the basic and supplemental cow feed and the content of total carotenes in each group's feed are given in Table 1.

CAF 100 that was supplemented to TG2 group's feed is a lightly orange colored powder, containing > 99% palm stearin (Carotino, 2006). It is rich in carotenes and vitamin E (approximately 120, and 300 ppm, respectively), providing 120 mg of vitamin E to the TG2 group per cow per day additionally.

Table 1

Cow feed composition

Cow groups	Basic feed, per cow per day	Supplemental feed, per cow per day	Total carotenes, mg per cow per day
Trial group 1 (TG1)	Haylage – *ad libitum*, mixed feed concentrate – 2 kg, hay – 2 kg	Carrots – 7 kg	387
Trial group 2 (TG2)		CAF 100[a] – 400 g	292
Control (CG)		-	242

[a] Animal Feed produced by Carotino SDN. BHD, J.C. Chang Group, Malaysia

Milk sample collection and storage. Individual cow milk samples were obtained from the morning milking on day 25 from the start of feed supplementation. Equal amounts of each group's individual cow milk (5 L) were pooled together resulting in one bulk milk sample per group. After collection, milk samples were transported to the laboratory. For analysis of riboflavin, milk was separated in subsamples of 100 mL that were put in 200-mL clear glass beakers and stored in direct sunlight for 1.5 and 3 h at room temperature stirring each 10 min. A blank analysis was made without milk exposure to light (0 h in light).

Chemicals. Water was purified with Simplicity (Millipore SAS, France). Sodium acetate and potassium iodide were from Stanchem, Poland; glacial acetic acid was from Lach-Ner, Czech Republic; chloroform was from Riedel-De-Haën, Germany; sodium thiosulphate was from AVSISTA, Lithuania. All reagents were of analytical or higher purity.

Vitamin B_2 content in milk was determined in accordance with the fluorometric method described by Havemose et al. (2004). Milk samples (5 mL) were mixed with 0.5 mL of 2 *M* sodium acetate and 1.5 mL of 2 *M* acetic acid. The samples were slowly agitated for 5 min before centrifugation at 1500 × g for 10 min. The supernatant was filtered through a 0.45 mm Nylon filter (Membrane Solutions), and the fluorescence was measured using a TD-700 Fluorometer (Turner Designs, Sunnyvale, CA), emission 520 nm. All analytical procedures were conducted using glassware wrapped in aluminum foil to avoid light exposure resulting in additional riboflavin degradation during sample preparation.

Milk fat extraction and storage. Right after transportation to the laboratory, milk for fat extraction was warmed up to 40-45 °C temperature subsequently separating cream with a conventional milk separator to approx. 30% fat content. Cream was ripened at temperature of 4-6 °C, 20 ± 1 h, then churned till formation of butter. The buttermilk was removed and butter was rinsed with cold distilled water for 5 times. Subsequently, butter was warmed up to 40-50 °C and centrifuged 15871 × g, 10 minutes at 40 °C to separate the clear butter oil layer that afterwards was carefully split into smaller (20 g) sub-samples for peroxide value (PV) determinations, e.g. fat was poured into appropriate number of transparent plastic one-way Petri plates. A half of the fat sub-samples were subjected to direct sunlight action at room temperature for 3 hours to hasten the fat ageing, while other samples were stored at the temperature of 4-6 °C in dark for 3 hours. After that all samples were placed into thermostatic oven at the temperature of 60 °C for fat ageing. The duration of ageing was 25 days for fat samples unaffected by light, or 14 days for fat samples affected by light for which the oxidation process was much faster.

Peroxide value (PV) of the milk fat. The PV test was carried out in accordance with iodometric titration method described by Охрименко и др. (2005). One g of the fat was put in a 100-mL Erlenmeyer glass flask, and mixed with 6 mL solution containing chloroform and glacial acetic acid (2:1, v/v). Parallel blank analysis was performed without fat sample. Then 1 mL of saturated potassium iodide solution and 30 mL of deionized water were added. After that, flasks were sealed and carefully shaken for exactly 3 min. Next, 3-5 drops of 1% starch solution to the reaction mixture were added, followed by the titration against 0.01 *M* sodium thiosulphate solution. The equation (1) was used for the calculation of PV:

$$PV = (V_0 - V) \times 0.00127 \times 100 / m, \quad (1)$$

where:

PV – the peroxide value, expressed as % of iodine utilized for the reduction of 100 g fat;

$(V_0\text{-}V)$ – the difference between a blank titration and the titration with the fat;

0.00127 – the iodine mass that corresponds to 1 mL of 0.01 *M* sodium thiosulphate solution, g;

100 – the conversion factor to 100 g amount of fat;

m – the mass of the fat sample, g.

Statistical analyses. The results were calculated, analyzed, and graphs were made using MS Office program Excel or Microsoft Windows for SPSS (SPSS 17.0, SPSS Inc. Chicago, Illinois, USA). Differences

between treatments were tested for significance ($p<0.05$) by ANOVA. Data are presented as means ± confidence interval ($p<0.05$).

Analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra', and in the Scientific Laboratory of Microbiology of the Faculty of Food Technology of the LLU.

Results and Discussion

The photo-oxidative stability of vitamin B_2 in milk

To compare the vitamin B_2 photo-oxidative stability in milk obtained from differently fed cow groups, in this test the light influence was used. It is known that β-carotene is particularly involved in prevention of photo-oxidation, as it absorbs light in a concentration-dependent manner that would otherwise be absorbed by riboflavin, thereby inducing quality changes (Mortensen et al., 2004; Nozière et al., 2006). The highest total carotenes content was in TG1 feed (see Table 1). Carrots are one of the richest sources of carotenoids containing mainly α- and β-carotenes (Kotecha et al., 1998). The greater effects from the carotenoids supplementation or the lower losses of vitamin B2 in the TG1 milk were anticipated.

The initial content of vitamin B_2 in raw milk was 2.07-2.35 mg L^{-1} (see Fig. 1), and it was similar to the quantities showed in literature – 1.0-2.8 mg kg^{-1} (Горбатова, 2004), above literature data 1.8 mg kg^{-1} (Miller et al., 2007), or below the average vitamin B_2 content obtained in the study of Zagorska (2007) in Latvia conventional agriculture raw milk – 2.65 ± 0.10 mg L^{-1}.

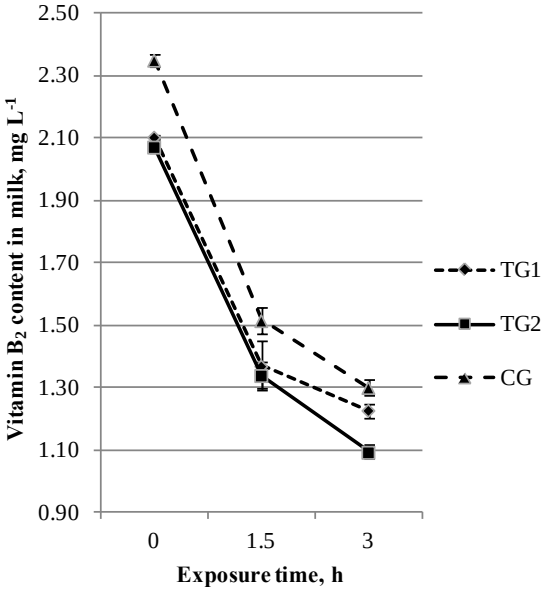

Figure 1. Decrease in vitamin B_2 content during exposure to direct sunlight in milk obtained after 25-day feed supplementation.

As the initial content of vitamin B_2 in milk obtained from 3 groups was significantly different ($p<0.05$), the oxidation intensity of vitamin B_2 was compared by its losses from the initial concentration (%). During the milk storage in direct sunlight for 1.5 and 3 h, the degradation of vitamin B_2 occurring in milk samples of all groups was high (see Table 2). After milk exposure to light during first 1.5 h, the losses of vitamin B_2 in all samples of milk were considerable – 34.9-35.5%, but did not differ significantly ($p>0.05$). During the remaining 1.5 h, the decrease in the content of vitamin B_2 was less intense, reaching 41.6-47.18%. A slightly and significantly higher ($p<0.05$) vitamin B_2 stability was seen in carrots-supplemented group (TG1) milk, where the losses of vitamin B_2 was by 3.01% less compared to CG. Regarding CAF-100-supplemented group (TG2) milk, there was not observed any superiority of vitamin B_2 stability over CG milk.

The results can be explained by the concentrations of such antioxidative compounds in milk as carotenoids and ascorbic acid that inhibit the degradation of vitamin B_2. The total carotenes content in TG1 (carrots-supplemented group) feed was considerably higher compared to CG, and also to TG2, and this can be the possible explanation of the slightly and significantly ($p<0.05$) higher stability of the vitamin B_2 in TG1 group milk samples as anticipated previously.

However, the carotenoids are not the only compounds giving protection to the riboflavin during photo-oxidation. It has been reported that ascorbic acid also has strong quenching ability against active-oxigen species and it effectively prevents the light-activated off-flavour formation and reduction of riboflavin in milk and aqueous solution (Trang et al., 2008; Lee et al., 1998). Lee et al. (1998) demonstrated that addition of 0.1% ascorbic acid resulted in 50% and 25.5% inhibition of reduction of riboflavin in whole milk and skim milk, respectively, after 10 h illumination at 3300 lux (Trang et al., 2008). Due to the significant role of ascorbic acid its average content in the raw milk that was obtained from differently fed cow groups was compared. It was 16.70±0.10, 16.99±0.10, and 17.20±0.09 mg L^{-1} in TG1, TG2, and CG milk, respectively, showing that both trial groups had a significantly ($p<0.05$) lower content of ascorbic acid that was by 0.5% less for TG1 and by 0.2% less for TG2, compared to CG. This can be one of the reasons why the riboflavin oxidative stability of carotenoids-supplemented group milks was only slightly higher (TG1-3h) or even lower (TG2-3h) compared to CG ($p<0.05$). The significantly lower content of ascorbic acid in TG2 possibly extinguished the positive effect of carotenoids giving a negative result as slight (2.57%) but significantly ($p<0.05$) higher losses of vitamin B_2 in this group's milk after 3-h-sunligt exposure compared to CG.

Table 2

Losses in vitamin B_2 content in milk during storage in direct sunlight (mean±SD)

Exposure time, h	1.5			3.0		
Groups	Experimental		Control	Experimental		Control
	TG1	TG2	CG	TG1	TG2	CG
Decrease in vitamin B_2, %	34.87 ± 2.54	35.31 ± 2.04	35.50 ± 0.87	41.60 ± 0.65	47.18 ± 0.81	44.61 ± 0.37

A more pronounced positive tendency that cow feed supplementation with carotenoids additives has potential to increase the stability of vitamin B_2 against photo-oxidative degradation in milk was seen in our previous investigation, showing that the vitamin B_2 photo-oxidative stability was higher in milk obtained from carotenoids-supplemented cows, saving 10-11% of vitamin B_2 during 1.5 h period, and 5-8% during 3 h period of milk exposure to sunlight (Antone et al., 2011).

However, the average losses of vitamin B_2 in our study were higher – 34.87-35.50% and 41.60-47.18% compared to the results from our previous investigations, namely, 10.03-21.22% during first 1.5 h and 15.66-23.48% during 3-h period of the milk exposure to sunlight (Antone et al., 2011). The differences in results can also be explained by the differences in light intensity (Bosset et al., 1994) and in the initial antioxidative capacity of milk as well (Walstra et al., 1999). The losses of vitamin B_2 in fluorescent light are also significant. Unpackaged pasteurized milk exposed to cool white fluorescent light for 7 h undergoes riboflavin losses of over 75% (Bosset et al., 1994). For the further research it would be valuable to take into consideration that the changes in vitamin B_2 content should be monitored earlier – during the first hour of sunlight exposure due to its extremely high sensitivity to photo-oxidation, thus possibly allowing detecting more pronounced effects from cow feed supplementation with different carotenoids supplements.

As mentioned previously, the photo-oxidative degradation is the main cause of riboflavin losses. This should be considered when choosing the packaging materials for food products containing vitamin B_2, among which milk and dairy products are rich and excellent natural dietary sources (Eitenmiller et al., 2008). Milk storage in packages made from glass or other transparent materials is quite often practiced in Latvia. Cardboard packaging is also frequently used, and while it is not transparent it is certainly not always impermeable to light (Walstra et al., 2006). The intensity of light and lengths of storage time of products containing riboflavin and other photo-sensitive vitamins should also be chosen cautiously to minimize the losses of nutritional value of food products.

The oxidative stability of milk fat measured as PV changes

The oxidative stability of milk fat was measured by changes in PV during storage at the temperature of 60 °C. PV is a sensitive indicator of oxidative and photooxidative changes in fats and oils. PV shows the concentration of primary oxidation products – hydroperoxides and peroxides – in the fat, however these are highly unstable substances producing secondary oxidation products – mainly carbonyl compounds (Bosset et al., 1994). In our test, the main cause of oxidation was the influence of temperature (60 °C) and the partial contact of fat with air, but for a half of samples – also the contact with sunlight at the beginning of the ageing process.

As seen from Figure 2, the oxidation process of milk fat that was not affected by light was very slow. During the 25-day period, only slow changes in the PV or induction period were observed showing the relatively high oxidative stability of butter oil. Significant ($p<0.05$) differences between samples of the three groups were not established, and PV did not exceed 0.012% of iodine at the end of the 25-day period. The induction period of the butter oil samples from all the three groups initially unaffected by light and stored at 60 °C temperature was > 25 days. Such a good stability of milk fat can be explained by the fact that it has a relatively low polyunsaturated fatty acid content and high proportion of saturated fatty acids compared to many other edible fats. Milk fat also contains natural fat-soluble antioxidants – carotenoids and vitamin E (tocopherols and tocotrienols) that increases the induction period (MacGibbon and Taylor, 2006). Vitamin E functions as the primary antioxidant and as the peroxy free radical scavenger. It is the primary, lipid-soluble, autooxidation chain-breaking action mechanism antioxidant that combines actions with other lipid- and water-soluble antioxidants to provide an efficient defense against free radical damage (Eitenmiller et al., 2008). The induction period usually is very slow until hydroperoxides are formed. Initially antioxidants are consumed; after

this has been achieved, peroxides are first liberated and subsequently broken down to form perceptible amounts of flavour products (Walstra et al., 1999).

Concerning the samples of milk fat exposed to light – the oxidative deterioration was much faster. At the beginning fat oxidation possessed with low intensity (induction period) followed by rapid increase in hydroperoxide concentration (Fig. 3) accordingly to the theory (Kamal-Eldin and Yanishlieva, 2005). For each type of fat, the end of the induction period was determined by the tangent method. The tangents as linear function lines of the phase of active hydroperoxide formation (B) of CG and TG1, respectively, were drawn, and the crossing points with corresponding linear function lines of induction period (A) were found.

The established induction periods are showed in Table 3. The induction periods were 10.97 and 12.03 days, for the CG and TG1 respectively. The induction period of TG2 was > 14 days, because the phase of active hydroperoxide formation (exponential phase) during the 14-day period was not observed yet. Induction periods of milk fat samples from both trial groups were longer than induction period of control group's milk fat, and a tendency of the impact of antioxidant protection on fat stability through the enrichment of cow's diet by its antioxidant content was evident.

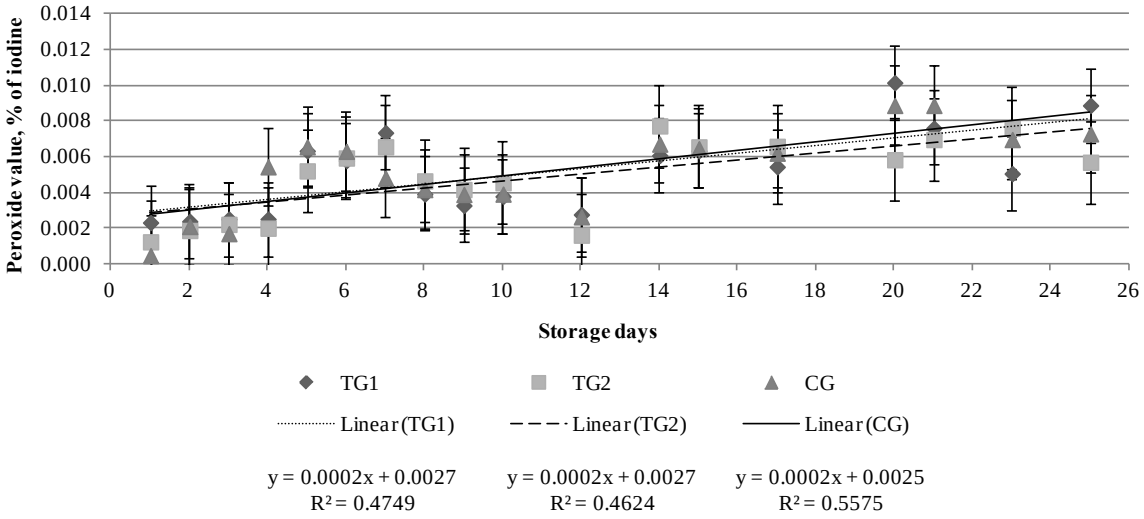

Figure 2. Peroxide value changes of milk fat stored in the dark.

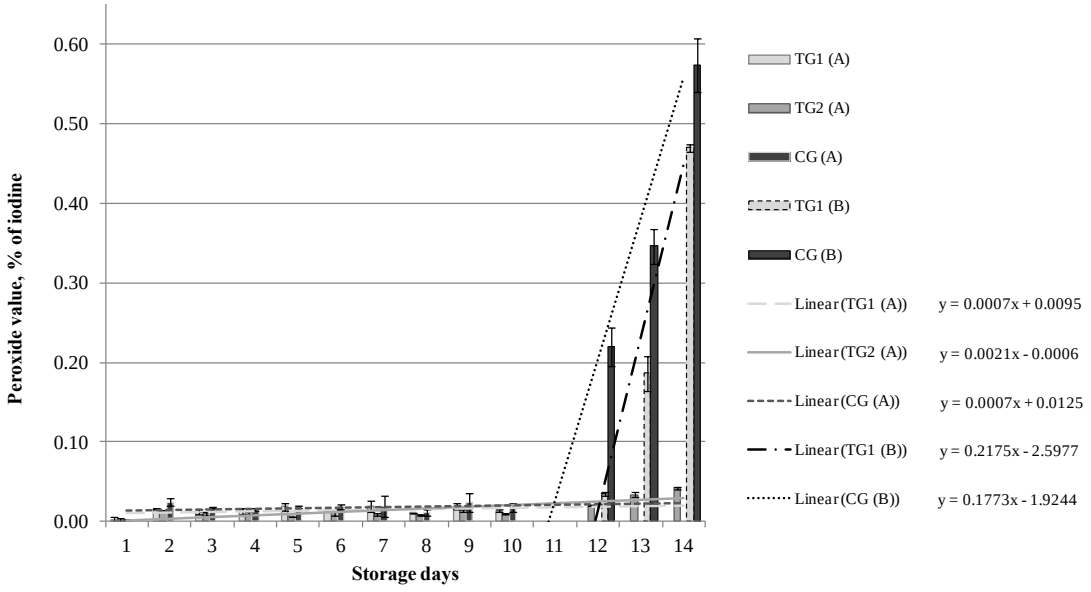

Figure 3. Peroxide value changes of milk fat exposed to light:
(A) – the induction period, (B) – the phase of active hydroperoxide formation.

Table 3

The established induction periods for the milk fat exposed to light

	Experimental groups		Control group
	TG1	TG2	CG
Induction period, days	12.03	> 14.00	10.97

The longest induction period and the highest ($p<0.05$) protection against lipid oxidation were observed in CAF-100-supplemented (TG2) cow milk fat, which can be related with increased tocopherol concentration in palm oil supplement and with the possible synergism between carotenoids and tocopherols in fat protection. The oxidative stability of the carrot-supplemented (TG1) group's milk fat was also higher compared to CG, showing the good potential of the enrichment of cow diet with carotenoids additives. The observed advantages of CAF 100 feed supplement were: longer shelf life, as well as easier storage and portioning compared to carrots. However, the benefits of carrots as feed supplements are that they are cheaper and locally grown vegetable products in Latvia.

Conclusions

1. A slightly and significantly higher ($p<0.05$) vitamin B_2 stability was seen in carrots-supplemented group (TG1) milk, where the losses of vitamin B_2 during 3-h sunlight exposure was by 3.01% less compared to CG.
2. The highest ($p<0.05$) protection against lipid oxidation was observed in CAF-100-supplemented cow milk butter oil.
3. The established induction periods for the fat samples initially affected by light and stored in 60 °C temperature for the CG and TG1 were 10.97 and 12.03 days, respectively. The induction period of TG2 was > 14 days.
4. The induction period of the butter oil samples from all the three differently fed cow groups initially unaffected by light and stored in 60 °C temperature was > 25 days and did not differ significantly.

Acknowledgments

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INFLUENCE OF PACKAGING CONDITIONS ON THE QUALITY OF PICKLED VENISON

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Abstract
Venison is well known as a traditional meat type in Europe, and it is lower in calories, cholesterol and fat content than common cuts of beef, pork or lamb. The aim of the current research was to determine physico-chemical parameters such protein, fat, pH, moisture content as well microbiological quality of pickled venison during storage. Beef as a control was analysed for comparison of obtained results. The meat (2×3×2 cm) pieces were pickled in vinegar marinade (composition: tomato sauce, mayonnaise, vinegar, lemon, onion, parsley, paprika, basil, black pepper, rosemary, salt) at 4±2 °C temperature for 48±1 h. The marinated meat was placed in polypropylene trays and hermetically sealed with high barrier polymer film under modified atmosphere ($C0_2$ 40%+N_2 60%) without and with iron-based oxygen scavenger sachets (Mitsubishi Gas Chemical Europe Ageless®). As a control, packaging in air ambiance packed pickled products was used. During storage time, the moisture and protein values significantly ($p<0.05$) decreased and pH, fat content and colony forming units significantly ($p<0.05$) increased in the pickled venison samples of all packages. Slower changes in pH of pickled venison and in the protein and moisture content of marinated beef were observed in modified atmosphere with oxygen scavenger during storage.
Key words: venison, marinating, modified atmospheres, oxygen scavenger, storage time.

Introduction

Venison is popular as a healthy food because of its low fat and high lean meat (Okabe et al., 2002; Stevenson et al., 1992). Venison is lower in calories, cholesterol and fat content than common cuts of pork, beef or lamb. All these attributes of venison are criteria demanded by today's discerning meat consumer (Hoffman and Wiklund, 2006). However, venison is a highly perishable product with a short shelf life. In scientific literature, investigation for venison shelf life extending using preservation of meat in modified atmosphere (Vergara et al., 2003), frozen packaging using vaccum- and nonvacuum-package (Farouk and Freke, 2008) is described.

Marination is an effective means of enhancing the quality and versatility of meats. Marination is the process of soaking or injecting of meat with a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids to flavour and tenderize meat products (Kargiotou et al., 2011; Pathania et al., 2010). This process could positively affect the shelf life of the meat due to the acidic or alkaline nature of the solution, and the antimicrobial and antioxidant activity of some marinade additives (Karigiotou et al., 2011). A good marinade will have a delicate balance of spices, acids and oil. Marinades are incorporated into meat by soaking texture and moisture retention, to enrich the meat flavor, to tenderize the fibers of muscle foods, and to preserve the products over a longer time (Alvarado and McKee, 2007).

Packaging makes food more convenient and gives the food greater safety assurance from microorganisms, biological and chemical changes so that the packaged foods may have a prolonged shelf life (Skandamis and Nychas, 2002). Packaging technology which modifies the atmospheric conditions of the package is popularly applied to extend the self-life of meat (Daszkiewicz et al., 2011). Modified atmosphere packaging (MAP) technology is one of the protection methods in which the surrounding atmosphere of the food is changed. Basic process in MAP is to remove the air inside the package and put in a gas or gas combination instead, and then seal hermetically (Gokoglu et al., 2011). Vergara et al. (2003) reported that for venison preservation gas composition ($C0_2$ 40%+N_2 60%) is the most appropriate. But modified atmosphere packaging technologies not always completely remove oxygen, and oxygen penetrates through the packaging film. Using of oxygen scavengers can reduce oxygen level in package. Oxygen scavengers are made from easily oxidisable substances. Almost all oxygen scavenger sachets are based on the principle of iron oxidation. The sachets are made up of finely divided powdered iron, ferrous compounds and various catalysts, which under appropriate humidity conditions initiate the reaction, using up any residual oxygen to form non-toxic iron oxide (Brandon, 2009).

The aim of the current research was to determine physico-chemical parameters such protein, fat, pH, moisture content as well microbiological quality of pickled venison during storage.

Materials and Methods

The experiments were carried out at the Department of Food Technology, Latvia University of Agriculture, in 2011. The meat of farmed red deer (*Cervus elaphus*) was obtained from a local farm 'Saulstari 1', located in Sigulda region, Latvia; the beef of farmed cattle *(Colloquially cows)* from Ltd. 'Kebeco' located in

Jekabpils region, Latvia, was used for control.

Initially, five types of marinades were used those main component was vinegar. After sensory evaluation, as appropriate for meat marinating was set marinade with following composition: tomato sauce, mayonnaise, vinegar (9 %), fresh lemon, onion, parsley, paprika, basil, black pepper, rosemary, salt. pH of marinade was 3.3.

Marinating process of the samples included the following steps:

1) *Longissimus dorsi* muscle from venison and beef saddle cuts were manually divided by knife in 0.250±0.020 kg pieces;

2) 0.250±0.020 kg pieces of *Longissimus dorsi* muscle were divided into smaller pieces of the size of 2×3×2 cm, and vinegar marinade was added;

3) prepared samples were marinated at 4±2 °C temperature in the refrigerator for 48±1 h.

Marinated meat samples were placed in polypropylene (PP) trays (210×148×35 mm) and hermetically sealed with high barrier polymer film Multibarrier 60 (composition: APA/TIE/PA/EVOH/PA/TIE/PE/PE; thickness 60±2 μm) under modified atmosphere ($C0_2$ 40%+N_2 60%) without and with iron-based oxygen scavenger sachets (Mitsubishi Gas Chemical Europe Ageless®), as a control packaging in air ambiance packed pickled products was used. Meat samples were analysed after 0, 4, 7 and 11 days of storage in a modified atmosphere (MA) packaging and in air ambiance. Samples were stored at 4±2 °C.

For physico-chemical analyses, meats were homogenised using a household blender according to ISO 17604:2003 standard procedure. Meat samples were prepared for microbiological analyses according to LVS EN ISO 6887-2:2004. Experiments were interrupted after 11 days of storage due to improper microbiological parameters of analyzed samples.

The following parameters were assessed:

- pH, measured using JENWAY 3520 (Barloworld Scientific Ltd., ESSEX, UK) pH-meter, according to LVS ISO 5542:2010;
- moisture content according to ISO 1442:1997;
- fat content according to LVS ISO 2446:1976;
- protein content according to ISO 937:1974 Kjeldahl nitrogen method.
- colony forming units according to LVS EN ISO 4833:2003.

The data was processed by analysis of variance (ANOVA) in order to determine the effect of packaging condition and storage time on each variable. Tukey's test was carried out to determine differences between groups. The level of statistical significance was $p<0.05$. Statistical analyses were performed using SPSS 15.0.software packages.

Results and Discussion

The conducted experiment did not indicate significant differences ($p>0.05$) in protein content among the in-air-packed and MA-packed meat with/without oxygen scavenger. Protein content tended to significantly decrease ($p<0.05$) with increasing storage period in all packages. Slow decline in protein content of venison package in air ambiance and beef package under MA with oxygen scavenger was observed due to solubility of protein fractions. The changes in protein content in pickled venison and beef samples during storage are shown in Figure 1.

The changes in proteins due to the storage of pickled meat samples we could explain with the protein composition and physical properties. Myosin is the most abundant muscle protein and exists as discrete thick filaments in the myofibril. Its solubility with respect to ionic strength and pH has been investigated widely, and it is well known that myosin is insoluble

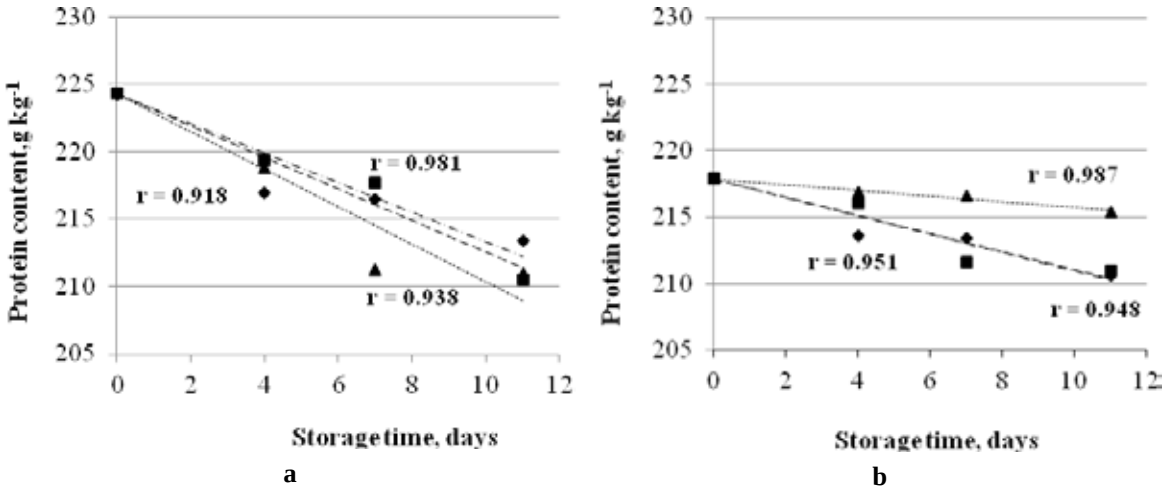

Figure 1. Changes in protein content of venison (a) and beef (b) pickled in vinegar marinade during storage: ♦- air ambiance; ■- $C0_2$ 40%+N_2 60% (without oxygen scavenger); ▲- $C0_2$ 40%+N_2 60% (with oxygen scavenger).

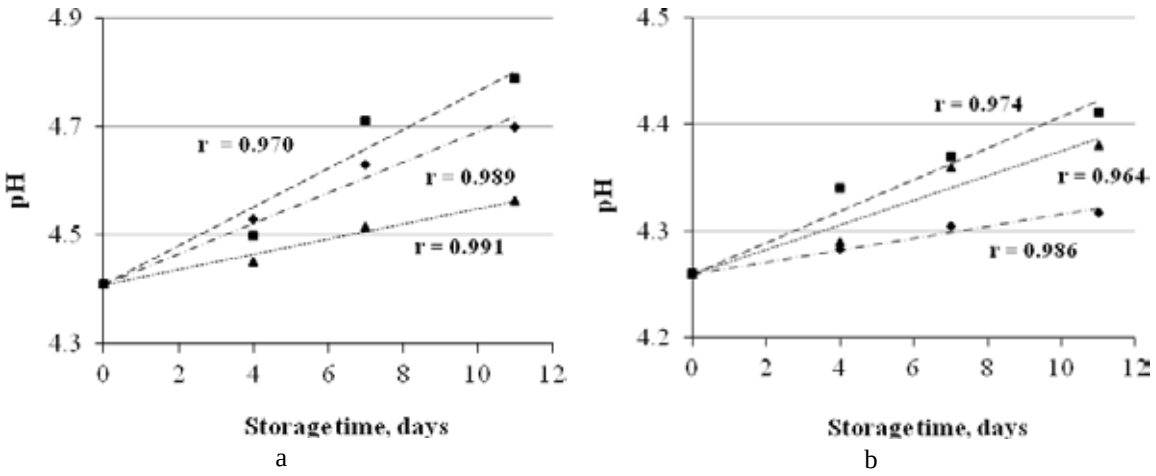

Figure 2. Changes in pH values of venison (a) and beef (b) pickled in vinegar marinade during storage: ♦- air ambiance; ■- $C0_2$ 40%+N_2 60% (without oxygen scavenger); ▲- $C0_2$ 40%+N_2 60% (with oxygen scavenger).

at physiological ionic strength (0.15-0.2 M) and high ionic strengths but soluble at intermediate ionic strengths 0.3-0.6 M on pH 5.5 (Kerry et al., 2002). Taking into account the above information, the protein loss during storage could be explained by the fact that the marinated meat pH values increase due to decrease in soluble proteins. The correlation coefficients showed close interconnection between protein content and storage time (Figure 1).

During the storage of pickled meat, significant differences ($p<0.05$) were observed in fat content among the in air-ambiance-packaged and in-MA-with/without-oxygen-scavenger-packed venison and beef samples packaged in air ambiance and in MA with/without oxygen scavenger. Fat content increased throughout the entire period of storage of pickled meat packaged in air and in MA. Significant ($p<0.05$) increase in fat content was determined after 4 days of storage in all samples irrespective of the packaging method. Initial fat content of venison was 16.8 g kg^{-1}, and of beef - 18.7 g kg^{-1}. After marinating of samples, the fat content increased until 36.5 g kg^{-1} (venison) and 25.2 g kg^{-1} (beef). Such results could be explained by the fact that fat from mayonnaise (one of the main ingredients of vinegar marinade) overpasses meat. This process continues the first four storage days. After that, no significant differences ($p>0.05$) were found in the fat content.

In the present research, no significant differences ($p>0.05$) were found among the pH of venison packaged in air ambiance and under MA with/without oxygen scavenger. Mean pH of beef packaged in air and MA without oxygen scavenger was significantly different ($p<0.05$). During storage of samples, pH increased. Results are similar to the finding of Pollard et al. (2002), Vergara et al. (2003) and Franco et al. (2012). Moore and Gill (1987) suggested that tissue breakdown during marinating and storage of marinating meat may be responsible for this increase. The average pH of meat was significantly ($p<0.05$) higher after 11 days than after 4 days storage irrespective of the packaging method. pH changes in venison and beef pickled in vinegar marinade during storage are shown in Figure 2.

A tight correlation was observed between storage time and pH for venison and beef in all packages (see Figure 2).

The results of the present study did not show significant variations ($p>0.05$) in the moisture content of beef packaged in air ambiance and in MA with/without oxygen scavenger. Significant differences were observed ($p<0.05$) in MA without oxygen scavenger and under MA with oxygen scavenger packaged beef. However, moisture content significantly ($p<0.05$) decreased in both investigated packaging techniques of meat samples. Less moisture loss during storage was observed in beef (727.0 – 690.8 g kg^{-1}) packed under MA with oxygen scavenger and in venison (729.8 – 695.7 g kg^{-1}) packed under MA without oxygen scavenger. It could be explained with water vapour permeation through the packaging materials. Moisture content of pickled venison and beef samples during storage is shown in Figure 3.

A tight correlation between the meat samples in storage time and moisture content was observed (Figure 3).

The Latvian current legislative act does not regulate the permissible level of mesophylic aerobic and facultative anaerobic microorganism count in marinated meat. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs requires that maximal threshold of mechanically

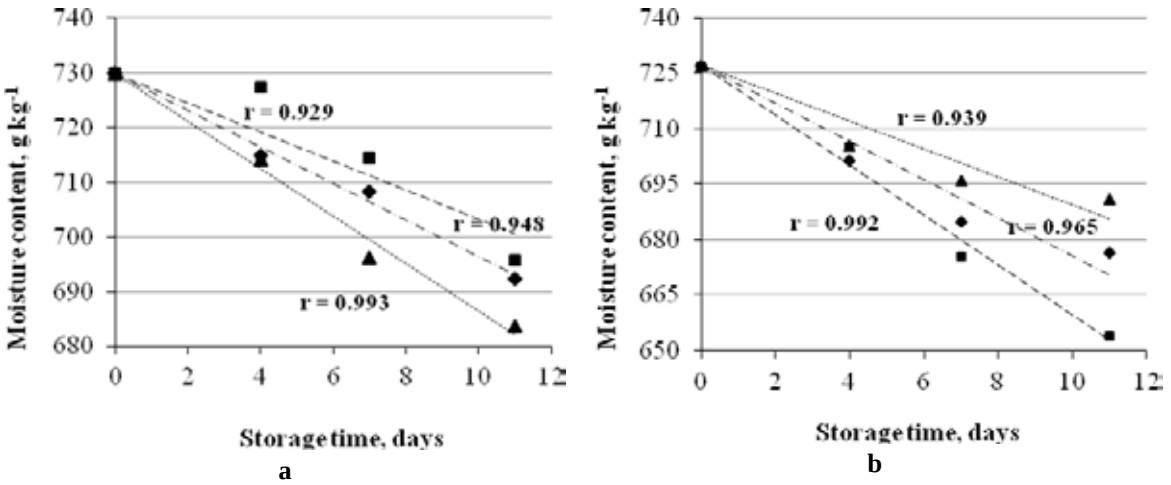

Figure 3. Changes in moisture content of venison (a) and beef (b) pickled in vinegar marinade during storage: ♦- air ambiance; ■- $C0_2$ 40%+N_2 60% (without oxygen scavenger); ▲- $C0_2$ 40%+N_2 60% (with oxygen scavenger).

separated meat is 5×10^6 cfu g^{-1} which also was seen as a critical threshold for microbiological analyses for this experiment.

During storage of meat samples, a significant ($p<0.05$) increase in colony forming units was observed (Figure 4). The applied packaging conditions (air ambiance and MA without/with oxygen scavenger) did not significantly ($p>0.05$) affect the microbiological quality of meat. Microbiological parameters of meat after 11 days exceeded the permissible level. The highest count was found in the meat samples packed in air ambiance (6.5×10^6 cfu g^{-1}).

The correlation coefficients showed a close interconnection between total microbial counts and storage time (Figure 4). During storage, a lower intensity of the increase in colony forming units in samples packed under MA with oxygen scavenger has been observed compared with the samples packed under MA without oxygen scavenger. At the beginning of storage, many liquid marinades have a pH of around 4.0, which makes them microbiologically stable but does not give the marinated meat a sour taste (Sheard, 2006). The obtained results showed that microbial counts increase when pH of marinated venison rises to 4.8, and of marinated beef – to 4.4.

Conclusion

1. Significant changes ($p<0.05$) in fat and moisture content were determined among the venison samples packaged in-air ambiance and under MA with/without oxygen scavenger, as well as in pH value and fat content among the packages of the analysed beef samples.

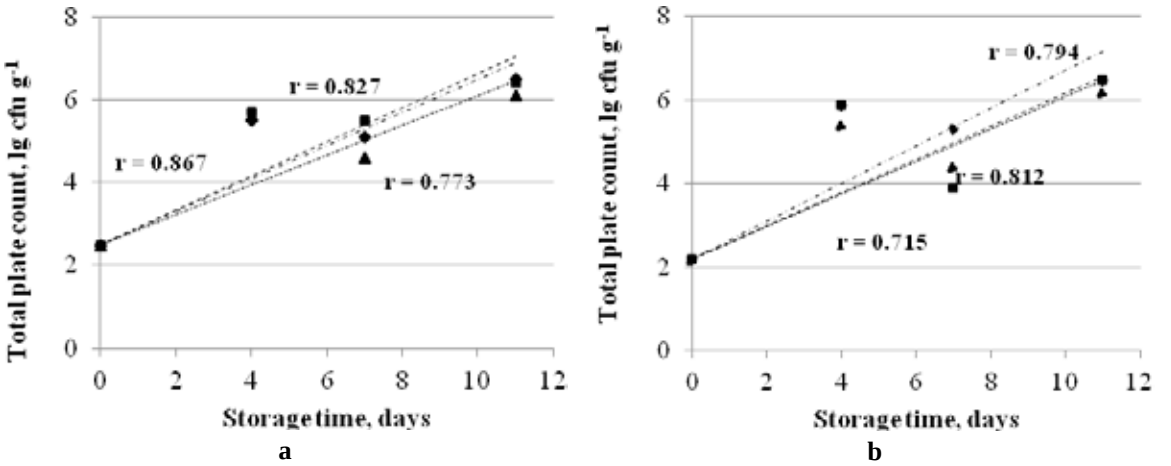

Figure 4. Microbial counts in venison (a) and beef (b) pickled in vinegar marinade during storage: —·— air ambiance; ——— $C0_2$ 40%+N_2 60% (without oxygen scavenger); --- $C0_2$ 40%+N_2 60% (with oxygen scavenger).

2. During storage, all quality parameters (protein, fat, pH, and moisture) significantly changed ($p<0.05$) irrespective of the packaging method.
3. In all packages during storage, colony forming units significantly ($p<0.05$) increased, but no significant ($p>0.05$) changes in colony forming units were observed between the venison and beef packages.
4. Changes in some quality parameters during storage tended to be slower in pickled venison and beef samples packaged in MA with oxygen scavenger.

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FATTY ACID COMPOSITION OF THE MEAT OF ELK, DEER, ROE DEER AND WILD BOAR HUNTED IN LATVIA

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Abstract
Every autumn and winter period, game animals - elk (*Alces alces*), deer *(Cervus elaphus*), roe deer (*Capreolus capreolus)* and wild boar *(Sus scrofa scrofa)* - provide an excellent input in the diversification of many consumer meals. In the recent years, consumption and assortment of game meat products has significantly increased. At the moment there are only few biochemical composition investigations of the game meat. The meat of wild animals is more favourable for human health, because it has a lower saturated fatty acid content, but a higher content of protein. Therefore the aim of our investigation was to compare biochemical composition of the game meats hunted in Latvia. Investigations were carried out in different regions of Latvia. In the studied samples, protein, intramuscular fat, fatty acids and cholesterol were determined. The biochemical analysis of 54 samples was carried out. The results of the analysis showed that protein content in all types of the meat samples was 22.36 - 22.92%, which is not statistically significant. The fat content, on the other hand, was significantly lower: 1.33 ± 0.88% in elk meat samples, and 1.59 ± 0.59% in roe deer meat samples. Content of cholesterol varied from 64.41% to 95.07% in the ruminant meat samples of different species. From the dietetic point of view, the meat samples of roe deer had the best composition of fatty acids.
Key words: game meat, intramuscular fat, dietetic product.

Introduction

The statistics show that there are around 33.000 hunters registered in Latvia, out of which 17.000 are active huntsmen. During hunting an average of 2624 tons of game meat is acquired in Latvia. Wild game meat is considered important and its share in the consumption has increased in the recent years. Currently, among consumers there is an increased interest in meat from animals kept in conditions as close as possible to the natural ones. Such a requirement is undoubtedly fulfilled in the game meat characterized by a high nutritional value and special sensory properties, desired by consumers (Soriano et al., 2006; Vergara et al., 2003).

Meat quality is a wide-ranging term, encompassing such diverse issues as technological, nutritional, hygienic and sensoric. Many factors have an impact on ruminant and wild boar meat quality; they can generally be divided into two categories: somatic factors (e.g. breed, age, sex) and environmental factors (e.g. diet, climate, hunting procedures) (Encyclopedia of meat sciences, 2004). Meat characteristics may be changed due to the dietary components, particularly fat content and composition (Petkov, 1986; Scolian et al., 2001). Any improvement of meat production by nutritional means should take into consideration the composition of meat as well as human health. Polyunsaturated fatty acids are not produced in human organism, therefore they must be consumed with products of animal origin, mostly fish, but wild animal meat is also a good source.

Composition of fatty acids, especially the ratio of polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) (PUFA/SFA), is more significant for human health than the total fat content. MacRae et al. (2005) noted that lowering the content of saturated faty acids, especially myristic acid (C14:0) and lauric acid (C12:0), improve the level of cholesterol in blood and lower the risk of heart diseases. Wood et al. (2003) reported that the recommended of ratio PUFA/SFA shold be higher than 0.4 and that in domestic animals it is too low - 0.1. On the other hand, too many polyunsaturated ω-6 fatty acids have undesirable impact on human health because the eicoasanoids (C20:3) produce inflammation, but inflammation is involved in the development of heart diseases and cancer. Enriching diet with polyunsaturated ω-3 fatty acids lowers the risk of atherosclerosis, hipertension and arthritis in human organism. Both ω-6 and ω-3 fatty acids are essential for human health, and our diet must contain balanced amounts (Gerster, 1998; Simopoulos, 2002). Therefore ratio ω-6/ω-3 is so significant. WHO (2003) suggests the above - mentioned ratio to be lower than 4. This ratio is lower if animals are grazed, because green forage has higher content of linoleic acid (Wood et al., 2003).

Typical for meat are saturated fatty acids (SFA). Such ruminants as cattle and sheep have higher content of SFA (44-46%), bat wild animals - lower content of SFA (41%) (Rule et al., 2002). Strategies that lead to an increase in the PUFA/SFA ratio in intramuscular fat would improve the health benefits of the meat from a consumer perspective (French et al., 2000).

Therefore the aim of this investigation was to compare biochemical composition of the meats of the game animals hunted in Latvia.

Materials and Methods

Meat samples (*m. logissimus lumborum*) were collected in the autumn-winter season. The research was conducted at the laboratory of Biochemistry and Microbiology of the Research institute of Biotechnology and Veterinary Medicine „Sigra". The chemical analyses of 54 samples were carried out, i.e. elk (8), deer (18), roe deer (16), wild boar (12) meat samples after hunting in whole regions in Latvia were collected. In the studied samples, protein, fat, cholesterol content and fatty acids composition were determined. Sample preparation was made in 48 hours after slaughtering or hunting of animals. Meat samples of about 300 g were homogenized with BŰCHI B-400 (ISO 3100-1).

Protein content was determined as total nitrogen content by Kjeldahl method and using coefficient 6.25 for calculation (ISO 937:1974).

Intramuscular fat content was determined by Soxlet method with hidrolysis procedure (boiling in the hydrochloric acid) using SoxCap 2047 and SOX TEH 2055 equipment (FOSS) (LVS ISO 1443:1973).

Cholesterol content was detected by Blur colorimetric method using spectrometer (Шманенков, 1973).

Fatty acids analysis of meat. The homogenized meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/Methanol followed by a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al. (2006). An ACME, model 6100, GLC (Young Lin Instrument Co.) equipped with a flame ionisation detector, an automatic sample injector, and an Alltech AT-FAME analytical column (fused silica 30 m×0.25 mm i.d.) were used. As the carrier gas, he was used with a flow rate of approximately 2 mL min^{-1}. Temperature conditions of the oven, injector and detector were the same as in the method of Aldai et al. (2006). Results were evaluated with the conventional integrator program (Autochro-2000, Young Lin Instrument Co.) The individual FAMEs (fatty acid methyl esters) were identified according to similar peak retention times using standard mixture Supelco 37 Component FAME Mix.

The statistical analysis was performed using SPSS 17. One-way ANOVA was used for comparison of mean values. Statistical significance was at $p<0.05$.

Results and Discussion

Biochemical composition of the meat samples was evaluated and results were summarized in Table 1. From the results of our investigation we can conclude that calculated content of protein in samples of game meet was 22.36 - 22.92%, of which the richest were the samples of wild boar meat. Statistical analysis showed that protein content of game meat samples did not differ significantly ($p = 0.297 > 0.05$).

The fat content in meat samples varied from 1.33 ± 0.88% to 2.82 ± 1.26%. Intramuscular fat content determined in meat samples of elk was the lowest, bat the highest - in meat samples of wild boar. Results of statistical analysis showed that content of intramuscular fat content in game meat samples differed significantly ($p = 0.021< 0.05$). The content of cholesterol range from 64.41 ± 4.99 mg 100 g^{-1} in meat samples of elk to 95.07 ± 7.88 mg 100 g^{-1} in the meat samples of wild boar ($p = 0.04 < 0.05$). Game meat possesses chemical composition as a raw material of high content of protein and low content of intramuscular fat in comparison with beef. The investigation of beef samples obtained in organic production in Latvia have showed that average protein content was 19.61%, but the content of intramuscular fat was 1.48% (Strazdina et al., 2010).

There are four inter-related factors that are important for human health: (1) the total fat content; (2) distribution of specific fatty acids; (3) the ratio of PUFA/SFA; and (4) the ratio of ω-6/ω-3 fatty acids. Each of these dietary lipid elements has been shown to influence the development of CHD (Cordain, 2002). Composition of dietary fat is more significant for consumers than the total fat content, therefore composition of fatty acids and sum of saturated, monounsaturated and polyunsaturated fatty acids were compared. Comparison is shown in Table 2.

Increase in the level of saturated fat, particularly 12:0, 14:0 and 16:0 (palmitic acid), has been identified as the major dietary factor responsible for raising total

Table 1

Biochemical composition of game meat

Group	n	Protein, %	Fat, %	Cholesterol, mg 100 g^{-1}
Elk	8	22.72 ± 0.60	1.33 ± 0.88	64.41 ± 4.99
Deer	18	22.36 ± 1.37	1.90 ± 1.29	70.57 ± 2.49
Reo deer	16	22.82 ± 1.76	1.59 ± 0.59	67.92 ± 4.46
Boar	12	22.92 ± 2.88	2.82 ± 1.26	95.07 ± 7.88

Table 2

Comparison of fatty acids composition of game meat

Fatty acids, % of total	Elk	Deer	Roe deer	Boar
Saturated fatty acids (SFA)				
C 12 : 0	0.19 ± 0.29	0.30 ± 0.22	0.01 ± 0.03	0.11 ± 0.15
C 14 : 0	2.44 ± 1.71	4.57 ± 2.33	1.32 ± 0.79	2.92 ± 1.37
C 15 : 0	0.35 ± 0.23	0.67 ± 0.13	0.44 ± 0.19	0.25 ± 0.28
C 16 : 0	18.08 ± 3.04	21.02 ± 6.67	18.72 ± 3.01	23.12 ± 1.19
C 17 : 0	0.90 ± 0.51	0.60 ± 0.38	1.07 ± 0.40	0.40 ± 0.20
C 18 : 0	13.56 ± 2.30	14.46 ± 4.76	15.63 ± 3.10	14.54 ± 2.89
C 20 : 0	0.08 ± 0.15	0.06 ± 0.01	0.02 ± 0.05	0.09 ± 0.07
C 22 : 0	0.00	0.04 ± 0.15	0.04 ± 0.08	0.00
C 24 : 0	0.15 ± 0.32	0.41 ± 0.47	0.29 ± 0.45	0.33 ± 0.66
Monosaturated fatty acids (MUFA)				
C 14 : 1	1.18 ±1.44	1.62 ± 1.25	0.35 ± 0.36	0.85 ± 0.92
C 15 : 1	0.23 ± 0.01	0.07 ± 0.03	0.02 ± 0.03	0.05 ± 0.06
C 16 : 1	4.35 ± 3.42	6.66 ± 4.61	1.95 ± 1.47	4.27 ± 1.62
C 17 : 1	0.27 ± 0.29	0.31 ± 0.20	0.31 ± 0.18	0.24 ± 0.21
C 18 : 1	27.89 ± 9.88	17.51 ± 3.38	26.15 ± 6.99	29.88 ± 9.27
C 20 : 1	0.04 ± 0.07	0.13 ± 0.12	0.04 ± 0.06	0.24 ± 0.21
C 22 : 1	0.00	0.03 ± 0.07	0.00	0.09 ± 0.17
C 24 : 1	0.13 ± 0.20	0.23 ± 0.36	0.14 ± 0.18	0.00
Polyunsaturated fatty acids (PUFA)				
C 18 : 2 ω -6	6.90 ± 1.99	12.34 ± 7.70	11.62 ± 3.34	11.70 ± 1.96
C 18 : 3 ω-3	4.77 ± 1.71	3.31 ± 1.84	3.94 ± 2.51	1.46 ± 0.92
C 18 : 3 ω-6	0.04 ± 0.07	0.14 ± 0.11	0.07 ± 0.08	0.03 ± 0.05
C 20 : 2	0.45 ± 0.66	0.10 ± 0.13	0.07 ± 0.06	0.26 ± 0.24
C 20 : 3 ω-6	0.20 ± 0.34	0.32 ± 0.33	0.35 ± 0.27	0.14 ± 0.20
C 20 : 3 ω-3	0.05 ±0.07	0.04 ± 0.05	0.00	0.12 ± 0.09
C 20 : 4 ω-6	4.59 ± 1.92	4.25 ± 2.70	5.00 ± 2.02	2.02 ± 1.53
C 20 : 5 ω-3	0.95 ± 0.62	1.36 ± 1.10	2.03 ± 1.29	0.39 ± 0.45
C 22 : 2	0.00	0.13 ± 0.20	0.05 ± 0.07	0.22 ± 0.13
C 22 : 5 ω-3	0.72 ± 0.24	1.29 ±0.85	1.87 ± 0.69	0.84 ± 0.64
C 22 : 6 ω-3	0.32 ± 0.54	0.20 ± 0.33	0.39 ± 0.40	0.08 ± 0.11
Sum of ω-3	6.81± 2.39	6.20 ± 3.01	8.23 ± 3.51	2.89 ± 1.03
Sum of ω-6	11.73 ± 4.48	17.05 ±6.61	17.04 ± 5.70	13.89 ± 5.83
ω-6/ω-3	1.72	2.75	2.07	4.81
P/S ratio	0.53	0.68	0.68	0.50

and LDL serum cholesterol concentrations (Howell et al., 1997). Elk meat samples had the lowest content of palmitic acid (18.08 ± 3.04%), but wild boar meat samples had the highest content - 23.12 ± 1.19% from all fatty acids. The results of statystical analysis that the sum of SFA contentof game meat samples did not differ significantly ($p = 0.283 > 0.05$).

WHO suggests the ratio ω-6/ω-3 to be lower than 4. Our investigation demonstrsted that in the samples of game meat this ratio varied from 1.72 in elk meat to 2.75 in deer meat, with the exception of the wild boar meat samples. Medeiros et al. (2002) have reported that ratio ω-6/ω -3 of deer meat was 3.45.

As mentioned above, the recommended ratio of polyunsaturated fatty acids to saturated fatty acids should be higher than 0.4. High relative percentages of PUFA are characteristic of the muscle tissue of all wild ruminants, whereas the relative percentage of PUFA in the muscle tissue of wild boar is lower than that found in wild ruminants. In our investigation, that the PUFA/SFA ratio was higher than 0.4 in all game meat samples and varied from 0.50 to 0.68. Medeiros et al. have found that ratio PUFA/SFA of beef samples was 0.38 (Medeiros et al., 2002).

The sum of saturated fatty acids, the same as the sum of monounsaturated and polyunsaturated fatty acids, was compared between the meat samples of elk, deer, roe deer and wild boar (Figure 1).

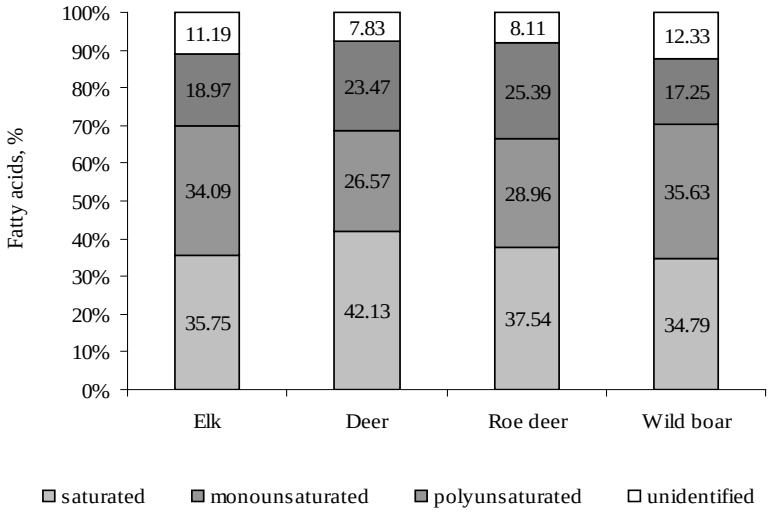

Figure 1. Comparison of fatty acids in game meat samples, %.

The lowest content of saturated fatty acids was determined in the meat samples of wild boar and elk meat - 34.79% and 35.75% respectively. The highest sum of saturated fatty acids was found in deer meat samples - 42.13% which is in agreement with the data obtained by Petkov (1986). The sum of PUFA in game meat samples varied from 17.25% to 25.39%. The highest content of PUFA was in meat samles of roe deer - 25.39%. Although ω-6 fatty acids are essential for health, people tend to consume too much of these substances, and arachidonic acid in particular is associated with inflammation. Inflammation can contribute to accumulation of plaque in arteries, which may increase the risk of coronary artery disease, heart attack and stroke (Calder, 2007).

Meat of wild animals is more favorable for human health because it has a lower SFA content but higher content of polyunsaturated fatty acids (Konjevic, 2008), for instance, wild boar meat. Meat and fat of wild animals have a significantly higher content of long chain fatty acids ω-3 than the meat of domestic animals (Cordan et al., 2002).

From the dietetic point of view, the „ideal" composition of fatty acids is when the amount of saturated, monounsaturated and polyunsaturated fatty acids is equal to 33.3% - 33.3% - 33.3% (Medeiros et al., 2002). Closest to this proportion were the meat samples of roe deer, where saturated fatty acids constituted 37.54%, monounsaturated fatty acids - 28.96%, and polyunsaturated fatty acids - 25.39%.

Conclusions

1. Game meat possesses chemical composition - high content of proteins (22.36 - 22.92%) and low content of intramuscular fat (1.33 - 3.23%).
2. The content of cholesterol was similar in the meat samples of different ruminant species - 64.41 - 70.57 mg 100 g^{-1}, but in the meat samples of wild boar it made 95.07 mg 100g^{-1}.
3. From the dietetic point of view, the „ideal" composition of fatty acids was found in the meat samples of roe deer, where saturated fatty acids constituted 37.54%, monounsaturated fatty 28.96% and polyunsaturated fatty acids 25.39%.

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ANTIMICROBIAL RESISTANCE OF ANIMAL PATHOGENS 2006-2009 IN ESTONIA

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Abstract
The present study describes situation of antimicrobial resistance of animal pathogens and resistance trends in Estonia in years 2006-2009. Bacterial strains isolated during period 2006-2009 were *Escherichia coli (E. coli)*, *Enterococcus faecium (E. faecium)*, *Enterococcus faecalis (E. faecalis)*, collected from healthy pigs faeces as well as from diagnostic submissions of pig samples. *Staphylococcus aureus (S. aureus)* isolates originated from cows with clinical mastitis and *Staphylococcus pseudointermedius (S. pseudointermedius)* isolates from dogs with pyoderma or otitis externa. Antimicrobial susceptibility was detected by microdilution method. Normal enteric microflora from health y pigs had resistance against streptomycin, tetracyclin, sulfametoxazol and trimethoprim. *E. faecalis* and *E. faecium* were resistant to erythromycin, tetracyclin, streptomycin and kanamycin. Multiresistance occured mainly against kanamycin, streptomycin and tetracyclin. *E. coli* strains isolated from pathological material showed high resistance to ampicillin, tetracycline, streptomycin, sulphonamides and trimethoprim. Multiresistance was detected between 60–73% during study years. In 2009, one ESBL (extended spectrum betalactamase) producing isolate was observed. *S. aureus* strains isolated from clinical mastitis samples were mainly penicillin resistant (58–86%). Meticillinresistant *S. aureus* was not found during the study. In 2009, resistance to lincomycin (30%) and fucidinic acid (22%) was detected. In *S. pseudointermedius* strains isolated from canine skin samples the prevalence of resistance to penicillin as high as 53–81% was found. Multidrug resistance was relatively stable being 38% in 2006, 29% in 2007 and 25% in 2009. In conclusion, antimicrobial resistance of animal pathogens in Estonia was high. Further improvement of prudent use of antimicrobials and infection control is needed.
Key words: antimicrobial resistance, staphylococci, enteric bacteria.

Introduction

Resistance to antimicrobial agents is an emerging problem worldwide. Antibiotic use selects for population of resistance bacteria target pathogens and normal bacterial flora, including food borne pathogens such as *Salmonella spp*., *Campylobacter spp*. and *E. coli*. Extensive antibiotic use accelerates the development of antibiotic resistance in the population. It includes increased morbidity and mortality from treatment failures and increased health care costs as newer, more expensive ingredients are needed to treat infections. Awareness of the undesirable consequences of its widespread occurrence has led to the initiation of antimicrobial agent resistance monitoring programs in several countries. The surveillance of emerging resistance and resistance trends are identified through the national antimicrobial resistance monitoring program with the purpose of facilitating timely and appropriate public health responses (Angulo et al., 2004). Separate program for human and veterinary medicine has been developed during the last ten years in the Nordic countries (SVARM, FINRES). In addition, integrated programs have been working in Denmark and Norway (DANMAP; NORM-NORMVET) for several years.

The monitoring of antimicrobial resistance is based on three categories of bacteria: human and animal pathogens, zoonotic bacteria and indicator bacteria. Indicator bacteria are included due to their ubiquitous nature in animals, foods and humans and their ability to readily develop antimicrobial resistance in response to selective pressure in both reservoirs. Human and animal pathogens are included because these cause infections that primarily reflect resistance caused by the use of antimicrobial agents in the respective reservoirs. Zoonotic bacteria are included because they can develop resistance in the animal reservoir, which may subsequently compromise treatment effect if resulting in a human infection.

Estonia University of Life Sciences in cooperation with Estonian Veterinary and Food Laboratory started the monitoring program for antimicrobial resistance of animal pathogens in the year 2000. Indicator bacteria (*E. coli*, *Enterococcus spp.*), zoonotic bacteria (*Salmonella spp.*) and different pathogenic bacteria isolated from clinical submissions (*Staphylococcus spp.*, *E. coli*) were hence investigated annually. The objective of the present study was to give an overview of the occurrence of antimicrobial resistance in bacteria isolated from healthy and diseased animals submitted between 2006 -2009.

Materials and Methods

Sampling strategy

The faecal samples representing normal intestinal microflora (*E. coli*, *Enterococcus spp.*) of healthy pigs were collected during National Salmonellosis surveillance programme. In the laboratory, systematic random sampling (every fifth sample) was used. Also, *E. coli* isolated from diseased pigs were collected during diagnostic submissions. *S. aureus* isolates originated from cases of clinical mastitis from cattle submitted to

the laboratory during routine microbiologial diagnosis. *S. pseudointermedius* isolates from dogs pyoderma or otitis externa were also included into the monitoring programme. Systematic random sampling was used among the *S. aureus* isolates, all *S. pseudointermedius* and *E. coli* isolates were included in the analysis.

Isolation and identification of bacteria

The material for identification of *E. coli* was inoculated directly to eosin methylene blue (EMB) agar (Sigma Ltd) based on the occurrence of a green-metallic sheen that appears on the surface of the bacterial colonies after incubation at 37 °C overnight. Phenotypic confirmatory test for production of extended spectrum beta-lactamases (ESBLs) in *E. coli* was performed by the double disc diffusion test according to Clinical and Laboratory Standards Institute (2008) (CLSI). Genotypic screening of ESBL and AmpC positive *E. coli* was performed by using Identibact Array Tube test according to the manufacturer (www.identibact.com). A polymerase chain reaction was complementary performed for identification of plasmid-mediated AmpC and CTX-M mediated ESBL according to J. F. Perez-Perez and N. D. Hanson (2002).

For isolation of enterococci one drop of faeces suspended in 2 mL sodium chloride (0.9 g kg^{-1}) was spread on Slanetz-Bartley agar and incubated for two days at 42 °C. Up to four colonies with morphology typical of *E. faecalis* / *E. faecium* were sub-cultivated on blood agar. Colonies were identified by the following criteria: colour, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose. All isolates of *E. faecium* and *E. faecalis* were stored (-80 °C) for the suspectibility testing.

Samples from the clinical submissions to identify staphylococci were inoculated onto ovine blood agar (Oxoid, Basingstoke, UK) and mannitol salt agar plates (Oxoid), and incubated aerobically at 37 °C for 18–24 h. Staphylococcal isolates were putatively identified by colony morphology, ability to grow on mannitol salt agar and Gram-stain characteristics. Representative isolates were then subcultured onto blood agar before being subjected to further confirmatory tests. Catalase activity was determined using 3 g kg^{-1} hydrogen peroxide (Sigma, Poole, UK). A slide test for bound coagulase was undertaken using rabbit plasma (Pro-Lab, Cheshire, UK). The absence of bacterial clumping indicated a negative result. All isolates negative for bound coagulase underwent a tube coagulase test where the formation of a clot following incubation of a bacterial suspension with an equal volume of rabbit plasma at 37 °C for 4–18 h indicated the presence of free coagulase. Coagulase-positive isolates were identified to the species level using a commercially available microbial identification

Table 1

Antimicrobial resistance of *Eschericia coli* isolated from faecal samples of healthy Estonian pigs 2006-2009

Antimicrobial agents	Breakpoint µg mL^{-1}*	2006 % (n=34)	2007 % (n=45)	2008 % (n=20)	2009 % (n=40)
Ampicillin	≥8	9	18	10	10
Ciprofloxacin	≥0.06	0	0	0	3
Nalidixic acid	≥16	3	0	0	3
Gentamycin	≥2	12	9	0	0
Ceftiofur	≥1	6	2	0	1
Streptomycin	≥16	26	31	20	15
Tetracyclin	≥8	6	27	15	13
Florfenicol	≥16	0	0	0	0
Kanamycin	≥8**	9	13	5	0
Sulfametoxazol	≥256	6	24	10	13
Trimethoprim	≥2	0	20	10	5
Chloramfenicol	≥16	3	9	0	3
Tsefotaxim	≥0.25	0	0	0	3

* Test: VetMIC™ GN-mo (version4).
Epidemiological cut-off values from EUCAST (European Committee on Antimicrobial Susceptibility Testing).
** Breakpoints from SVARM 2007 (Swedish Veterinary Antimicrobial Resistance Monitoring ISSN 1650-6332 Uppsala, http://www.sva.se).
SVARM 2007 (Swedish Veterinary Antimicrobial Resistance Monitoring ISSN 1650-6332 Uppsala, http://www.sva.se).

micro-tray kit according to the manufacturers' instructions (API ID32 Staph System; BioMerieux; Lyons, France). Oxford *S. aureus* NCTC 6571 was used as a quality control standard throughout the identification procedures. Screening for methicillin resistance in *S. aureus* from milk and S. *pseudointermedius* from dogs samples from cows was performed with microdilution according to CLSI (2008), testing oxacillin with 2 g kg^{-1} NaCl added to the broth, and oxacillin without added NaCl and cefoxitin. The *in vitro* antimicrobial susceptibility was determined by using microdilution method (VetMIC®, Sweden). Epidemiological cut-off values issued by the EUCAST (European Committee on Antimicrobial Susceptibility Testing) were used for interpretation of results of susceptibility testing of indicator bacteria and clinical breakpoints for *S. aureus*.

For interpretation of results for susceptibility testing of indicator bacteria (E. *coli* and enterococci) epidemiological cut off values (ECOFF) issued by the EUCAST (http://www.escmid.org) were used. When no ECOFFs were issued by EUCAST, the clinical breakpoints recommended for animal pathogens by CLSI were taken into consideration. The term 'multiresistance' is used with a meaning as proposed by S. Schwarz et al. (2010). Briefly, isolates with phenotypically identified acquired resistance to three or more antimicrobial classes are deemed multiresistant.

Propotion of resistance for each measured antimicrobial agent by dividing resistant isolates with all collected isolates was calculated.

Results and Discussion

Antimicrobial resistance of indicator bacteria isolated from faecal samples of healthy pigs is presented in Tables 1 and 2.

Normal gut microflora has developed resistance against several antibiotics detected. No resistance was detected to florfenicol. The highest resistance can be detected against streptomycin, tetracyclin, sulfametoxazol and trimethoprim. In 2008, resistance to streptomycin and tetracyclin decreased, no resistance to nalidixic acid and ciprofloxacin was observed.

For both, *E. faecalis* and *E. faecium*, resistance was most frequently detected against erythromycin, tetracyclin, streptomycin and kanamycin. Resistance to vancomycin was relatively high in 2008, but no *vanE* genes were found. Multiresistance occurs mainly against kanamycin, streptomycin and tetracyclin. Trends in resistance of *E. coli* strains isolated from pathological material (faeces or organs) are presented in Table 3.

Table 2

Antimicrobial resistance of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from faecal samples taken from healthy Estonian pigs submitted between 2006-2009

Antimicrobial agents	Breakpoint µg mL^{-1}*	2006 % (n=10)	2007 % (n=11)	2008 % (n=25)	2009 % (n=17)
Ampicillin*	≥4	0	0	0	0
Erythromycin*	≥4	40	18	44	41
Virginiamycin**	≥32	0	9	4	0
Gentamycin*	≥32	4	0	4	6
Streptomycin*	≥512	50	0	32	18
Kanamycin**	≥1024	40	18	28	6
Tetracyclin*	≥2	40	36	44	30
Chloramphenicol**	≥32	0	9	12	0
Vancomycin*	≥4	0	27	8	0
Narasin**	≥2	0	0	16	0
Bacitracin**	≥32	10	9	8	0
Linesolid*	≥4	0	0	4	0

Test: VetMIC™ E-cocci (version3).
* Epidemiological cut-off values from EUCAST (European Committee on Antimicrobial Susceptibility Testing).
** Breakpoints from SVARM 2007 (Swedish Veterinary Antimicrobial Resistance Monitoring ISSN 1650-6332 Uppsala, http://www.sva.se).

Table 3

Antimicrobial resistance of *Eschericia coli* isolated from pathological material from Estonian pigs in years 2006-2009

Antimicrobial agents	Breakpoint µg mL^{-1}*	2006 % (n=25)	2007 % (n=18)	2008 % (n=21)	2009 % (n=30)
Ampicillin	≥8	64	17	50	5
Ciprofloxacin	≥0.06	8	61	33	0
Nalidixic acid	≥16	16	22	33	50
Gentamycin	≥2	12	6	5	3
Ceftiofur	≥1	0	0	0	0
Streptomycin	≥16	56	56	62	37
Tetracycline	≥8	64	50	62	54
Florfenicol	≥16	4	0	5	0
Kanamycin	≥8	24	6	19.	7
Sulfametoxazol	≥256	72	83	62	70
Trimethoprim	≥2	72	22	57	67
Chloramfenicol	≥16	36	0	14	27
Cefotaxim	≥0.25	0	0	0	3

Test: VetMIC™ Gn-mo (version 4).
* Epidemiological cut-off values from EUCAST (European Committee on Antimicrobial Susceptibility Testing).

During the study years high resistance to ampicillin, tetracycline, streptomycin, sulphonamides and trimethoprim was observed. The resistance to gentamycin decreased till the year 2006, while resistance to ciprofloxacin increased from 8% in 2006 to 61% in 2007, also resistance to nalidixic acid increased from 16% in 2006 to 50% in 2009. Multiresistance has been detected between 60-73% during all study years. The contemporaneous resistance to ampicillin, streptomycin and trimethoprim-sulphonamides was the most common trait, occurring in 84% of the multiresistant isolates. In 2009, one ESBL producing isolate was observed.

Table 4

Resistance of *S. aureus* isolated from clinical mastitis milk samples in years 2006-2009 in Estonia

Antimicrobial agents	Breakpoint µg mL^{-1}*	2006 % (n=50)	2007 % (n=21)	2008 % (n=25)	2009 % (n=50)
Penicillin*	≥0.125	58	86	80	86
Cefalotin**	≥1	0	0	8.0	8
Oxacillin+2% NaCl	≥2	4	0	0	0
Erythromycin*	≥1	0	0	4	0
Chloramphenicol*	≥16	0	0	4	0
Clindamycin	≥0.25	0	0	0	30
Tetracyclin*	≥0.25	4	0	0	6
Fusidinic acid**	≥1	x	0	0	22
Gentamütsiin*	≥2	2	0	4	2
Kanamütsiin**	≥8	0	0	0	4
Ciprofloxacin*	≥1	0	0	0	0
Trimethoprim*	≥4	0	0	0	0

Test: VetMIC™ GP-mo (version 2).
* Epidemiological cut-off values from EUCAST (European Committee on Antimicrobial Susceptibility Testing).
** Breakpoints from SVARM 2007 (Swedish Veterinary Antimicrobial Resistance Monitoring ISSN 1650-6332 Uppsala, http://www.sva.se).

Antimicrobial resistance of *S. aureus* isolated from clinical mastitis milk samples is shown in Table 4.

The resistance levels of *S. aureus* strains were generally low, except against penicillin. Resistance to penicillin incerased over the four years from 58% to 85%. Also, 8% of cephalotin resistant isolates were detected in the years 2008-2009. Meticillinresistant *S. aureus* (MRSA) was found during monitoring program. In 2009, resistance to lincomycin (30%) and fucidinic acid (22%) was detected. Table 5 describes resistance of *S. pseudintermedius* and *S. aurues* isolated from ear and skin samples of Estonian dogs between 2006-2009.

The prevalence of resistance to penicillin due to production of betalactamases (penicillinase) in *S. pseudintermedius* is high, 52-81%. An oxacillin resistant *S. pseudointermedius* was isolated in 2007 and 2008 with presence of *meqA* gene. In 2008, resistance to ciprofloxacin was observed. Data from 2009 should be interpreted with caution due to very small amount of isolates that year. Multidrug resistance is relatively stable being 38% in 2006, 29% in 2007 and 25% in 2009. The present monitoring program describes the situation of antimicrobial resistance and trends in Estonia in the years 2006-2009.

The prevalence of acquired antimicrobial resistance in commensal bacteria of the enteric microflora of healthy animals indirectly indicates the magnitude of the selective pressure from the use of antimicrobials in animal population. The resistance level of enteric microflora in pigs is higher in Estonia in comaprison with Sweden and Norway (Bengtsson et al., 2010; Kruse and Skov, 2004), but is similar to reports from Denmark (Jensen et al., 2010) and Netherlands (Mevius et al., 2009). Both *E .coli* and enterococci showed highest resistance to tetracyclin which can be explained with wide use of doxycycline for oral treatment of pigs. Also, tylosin and sulfonamides

Table5

Resistance of *Staphylococcus spp*. isolated from dogs in case of otitis externa or from skin samples of Estonian dogs between 2006-2009

Antimicrobial agents	Breakpoint µg mL^{-1}*	2006 % (n=21)	2007 % (n=17)	2008 % (n=16)	2009 % (n=8)
Penicillin*	≥0.13 *S.intermedius* ≥ 0.13 *S.aureus*	53	71	81	63
Cefalotin	≥2 *S.intermedius* ≥1 *S.aureus*	0	0	31	0
Oxacillin+2% NaCl**	≥1 *S.intermedius* ≥2 *S.aureus*	0	18	31	0
Erythromycin*	≥1 *S.intermedius* ≥1 *S.aureus*	29	29	44	13
Chloramfenicol*	≥16 *S.intermedius* ≥16 *S.aureus*	24	12	19	13
Clindamycin**	≥4 *S.intermedius* ≥ 0.25 *S.aureus*	24	12	44	13
Tetracyclin*	≥8 *S.intermedius* ≥1 *S.aureus*	24	41	25	25
Fucidinic acid**	≥ 4 *S.intermedius* ≥ 0.5 *S.aureus*	14	12	6	13
Gentamycin*	≥4 *S.intermedius* ≥2 *S.aureus*	0	12	31	13
Kanamycin**	≥8 *S.intermedius* ≥8 *S.aureus*	0	24	44	25
Ciprofloxacin*	≥1 *S.intermedius* ≥1 *S.aureus*	0	0	38	13
Trimethoprim*	≥2 *S.intermedius* ≥2 *S.aureus*	67	76	63	38

Coagulase negative staphylococci (CNS) and *Staphylococcus aureus* (S.a.).
Test: VetMIC™ GP-mo (version 2).
* Epidemiological cut-off values from EUCAST (European Committee on Antimicrobial Susceptibility Testing)
** Breakpoints from SVARM 2007 (Swedish Veterinary Antimicrobial Resistance Monitoring ISSN 1650-6332 Uppsala, http://www.sva.se).

with trimethoprim are commonly used in Estonia where cross-resistance between macrolides and high level of sulfa/trimetoprim resistance was developed in normal microflora. As enterococci are intrinsically resistant to many antimicrobial agents, antimicrobial agents used for treatment of Enterococcus infection are limited. Although food-producing animals are not always a source of Enterococcus infection in humans, antimicrobial-resistant in animal origins may cause transmission of their resistance genes from animal to human bacteria. Therefore, prevalence of antimicrobial resistant enterococci, including vancomycin-resistant enterococci (VRE) in food-producing animals, has become a serious problem in several countries.

A higher resistance is expected in bacteria from diagnostic submissions compared to bacteria originating from healthy animals sampled on farm. For instance, *E. coli* from pathological material is more resistant than normal habitant of intestine. However, this data shows a high probability of bias towards animals with recurrent infections, previously treated with antimicrobials that could explain high levelof resistance. On the other hand, the number of isolates from animal pathogens is quite low. Veterinarians do not often send samples to the laboratory for isolation and identification of bacteria. Therefore, antibacterial treatment is initiated without bacterial diagnosis, which can lead to multidrug resistance.

In 2005, *S. pseudintermedius* in dogs, a novel staphylococcal species was described (Devriese et al., 2005). Further on T. Sasaki et al. (2007) and J. Bannoehr et al. (2009) reported that canine strains of *S. intermedius* should be classified as *S. pseudintermedius*. Therefore, it was proposed to report strains from dogs as if the strain belonging to a related species (Devriese et al., 2009). Pyoderma and ear infection are common causes for dog owners to seek veterinary consultation (Holm et al., 2002). These conditions are often treated with clindamycin or cephalosporins. Detected resistance against fluoroquinolones is probably related to frequent use of marbofloxacin and ciprofloxacin in treatment of several infections in dogs. To be able to control the resistance situation in *S. pseudintermedius*, a prudent use of antimicrobials together with an effective infection control programme is of highest priority.

The resistance of *S. aureus* strains isolated from clinical mastitis samples of cows is high against penicillins. Due to high prevalence of penicillin resistant *S. aureus*, veterinarians tend to choose lincosamides as the first choice of treatment. Statistical data from the Estonian State Agency of Medicine confirmed that all together 209880 single intramammary syringes for lactating cows and 205648 for dry cows were sold in the year 2009 for therapeutic purposes. Ampicillin and cloxacillin combinations, cephalosporins with aminoglycosides and lincomycin with neomycin were the most common choices for the treatment of mastitis in lactating cows. For example, 255 g of intramammary lincomycin (pure antimicrobial) and 44.2 g of intramammary cephalosporins per thousand dairy cows were sold for treatment of clinical mastitis in 2009. However, only 73.4 g of penicillin G was used per thousand dairy cows for intramammary treatment of clinical mastitis. The use of broad-spectrum antibiotics and antibiotic combinations may influence the resistance of mastitis pathogens.

Conclusions

Antimicrobial resistance of animal pathogens in Estonia is high. Further improvement of the implementation of prudent use of antimicrobials and infection control will be needed. In a long term perspective, the need for antimicrobials must be reduced by further improvement of animal health.

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THE SURVIVAL OF *LISTERIA MONOCYTOGENES* IN COLD-SMOKED SAUSAGES WITH AND WITHOUT STARTER CULTURE

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Abstract
The survival of inoculated in a cold-smoked sausages *Listeria monocytogenes* wild strains was studied. The sausages were prepared with and without starter cultures. The survival limits of *L. monocytogenes* and lactic acid bacteria (LAB) were determined as colony forming units per gram (cfu g^{-1}) depending on water activity (a_w) and pH on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation. The decreasing water activity conditioned by moisture (weight) loss during ripening and pH decrease ensured negative polynomial growth rate of inoculated *L. monocytogenes* - 0.27 lg (cfu g^{-1}) each day of ripening time, and - 0.65 lg (cfu g^{-1}) on the first 7 days of maturation. A significant Pearson's correlation ($p<0.01$) was established between decreased values of *L. monocytogenes* count, a_w, salt concentration and LAB growth in sausages during the ripening period of 21 days. The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in cold smoked sausages, are a_w value decrease and LAB (starter culture), which stopped *L. monocytogenes* growth at the beginning of cold-smoked sausage maturation. If fermentation process went technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice a low level contamination has been seen. The remaining count of *L. monocytogenes* in cold-smoked sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (cfu g^{-1}) for ready-to-eat products when contamination at first is more than lg 5.0.
Key words: *Listeria monocytogenes*, lactic acid bacteria, cold smoked sausages, water activity, pH.

Introduction

Listeria monocytogenes is an ubiquitous bacterial pathogen that can be found in a large number of food products and can survive and multiply at refrigeration temperature (Lunde´n et al., 2003). Processed meat products such as cold smoked sausages are a part of major products associated with listeriosis (Thevenot et al., 2005). *L. monocytogenes* infection has a high mortality rate - 20 - 30% (Farber and Peterkin, 199). In the United States, a zero tolerance of *L. monocytogenes* in ready-to-eat foods has been prescribed for several years (Shank et al., 1996), but the European Union regulation exceeded concentration of *L. monocytogenes* in ready-to-eat food to 100 colony forming units (cfu) per gram (Anonymous, 2005). K. Glass and M. Doyle (1989) found out that *L. monocytogenes* decreasing level in fermented sausages and ham would be 1-2 lg (sausages) in 14 days and 2-3 lg (ham) in 28 days. Because lactic acid bacteria (LAB) can grow under the same storage conditions as *Listeria* spp. (Bērziņš et al., 2007), many studies have been conducted to investigate if these gram-positive organisms can provide adequate competition against the pathogenic organisms that are also present.

The safety of cold smoked sausages depends on the presence of factors such as concentration of sodium nitrite and salinity, relatively low water activity (a_w), low pH value, and application of probiotics (Lahti et al., 2001), like LAB used in fermented meat products (Bredholt et al., 2001). D. Liu et al. (2005) investigated that acid, alkali, and/or salt treatments, commonly used in food product processing, may not be sufficient to eliminate *L. monocytogenes*. R.E. Petran and E.A. Zottolla (1989) observed the growth of *L. monocytogenes* at the minimum a_w of 0.92. Below these minimum a_w levels, cell death is proportionate to water activity (Miller, 1992). According to literature sources availabale, some recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about survival of *L. monocytogenes* found in different ripening stages of cold smoked sausages when main bacteria growth factors changed in time. Therefore, the aim of the study was to determine the survival limits of *L. monocytogenes* inoculated in manufactured cold smoked sausages depending on the LAB, water activity (a_w), and pH value changes in ripening time.

Materials and Methods

The experiments were held at the Institute of Food and Environmental Hygiene, Latvia University of Agriculture, and in the laboratory of a sausage manufacturer in 2010 - 2011.

Individual pieces of raw sausages before smoking, in initial weight mean value of 0.394 kg, were inoculated internally with a cocktail of local (wild) strains of *L. monocytogenes*. The inoculated samples were labelled and subjected to smoking and maturating processes. All manipulations with samples were done in laboratory conditions (20 °C, 75 - 80% RH). The measurements and tests were done on 0, 1st, 3rd, 5th, 7th, 14th and 21st day of maturation. Three batches of cold smoked sausages were investigated (a total of 60 samples) and the mean values of lg (cfu g^{-1}) were estimated between each other, and in addiction of pH, moisture content, and water activity (a_w) changes at ripening time.

Inoculation was prepared from persistent *L. monocytogenes* strains (serotypes 1/2a and 4b) originally isolated from surfaces and meat products of the mother factory (Bērziņš et al., 2007) in Latvia. *L. monocytogenes* strains were incubated in half-Fraser base medium for 18 h at 37 °C. The fresh concentrated culture of the selected strains was measured by optical densities (densitometer DEN-1B, UK) and prepared with sterile half Fraser broth (CM0895, SR0166E, Oxoid) to obtain approximately 8.0 lg (cfu mL^{-1}), and then samples of dry sausages inoculated portionally (1 mL of inoculate in 100 g of sample) randomly leading to beginning concentration of 6.0 lg (cfu g^{-1}) in the sausage.

Ingredients of a 100-kg-cold-smoked-sausage raw material were: pork - 30 kg, beef - 10 kg, bacon 35 - kg, structural emulsion - 25 kg. Salt and species summary was 3.25 kg and starter culture - 0.02 kg ('Optistart Plus', prepared by Raps GmbH and Co.KG, Germany). The fermentation and ripening process were carried out in climatic chambers (models HR-6000 and HR-9000 'Sorgo' Austria) at 28 °C with a relative humidity of 95% on first 3 days, down to 75% RH and 14-15 °C on 4^{th} to 21^{st} day.

The determination of *L. monocytogenes* count, cfu g^{-1}, was done according to Standard ISO 11290-2:1198 A:2005 'Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 2: Enumeration method'. Each experimental batch was free of *L. monocytogenes* before culture inoculation, detected with standard method. The samples were analyzed by numbering *L. monocytogenes* on 0, 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 14^{th} and 21^{st} days of maturation with the nine-tube most-probable-number (MPN) method. For analysis, 10 g of a carefully mixed cold smoked sausage sample were blended with 90 mL of sterile buffered peptone water in a laboratory blender (Stomacher 400, Interscience, France) for 1 min. Decimal dilutions were made to obtain samples of 1, 0.1, 0.01, 0.001, and 0.0001 g. To determine the MPN, three consecutive dilutions were used. Afterwards, 0.1 mL of each target dilution was spread on two LM-selective plates (PALCAM, Oxoid) and incubated for 48 h at 37 °C. For confirmation of *L. monocytogenes*, five typical colonies from two selective plates at each sampling time were streaked on sheep blood agar plates and incubated for 24 h at 37 °C. Catalase-positive, gram-positive rods, produced hemolysis on sheep blood agar (CAMP-test), were considered *L. monocytogenes* (McKellar, 1994). Total count (cfu g^{-1}) of *L. monocytogenes* in cold smoked sausage samples were calculated with classical formula given in Enumeration method standard.

The determination of LAB count, cfu g^{-1}, was done according to standard ISO 15214:1998 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C'.

pH was measured at the same time as other measurements. Three individual pieces of sausages were measured each time, and then mean pH value was calculated. The pH-meter Testo 205 (Testo AG Germany), with automatic temperature compensation, was applied. Meter calibration was done according to 2 point method with pH standard solutions 4.01 and 7.00.

Water activity was measured with PawKit (Decagon) water activity meter. Calibration of device was done with saturated NaCl (sodium chloride) 6.0 molal standard solution (0.760 a_w at 20 °C). Samples for water activity measurement were collected in original polyethylene vessels with caps and measured immediately after collecting.

Statistical analysis. All experiments were reiterated three times, and tests were triplicated. The results represent the mean ± standard deviations (SD). Means were compared by Student's t-test. Differences were considered statistically significant when $p<0.05$. Statistical analysis was conducted with SPSS 17.0 (SPSS, Chicago, Ill., USA). Tables and chart figures were done by means of MS Excel 2007 appliances. To show the parameter changes in time, regression curves have been made for LAB, *L. monocytogenes*, a_w, and pH. The main factors, affecting the bacterial growth, have been calculated by correlation (SPSS, Factor analysis).

Results and Discussion

The results of the physicochemical parameters and bacterial analysis of the cold-smoked sausages at the beginning and the end of the ripening time are reported in Table 1. The values of pH were about 4.6 in the final product - typical of medium acidity sausages, and this was the result of the classical trend of microbial growth in the fermented sausages, where LAB are increasing in numbers at the very beginning of the fermentations (Figure 1), producing acids and a decrease in the pH, followed in the phases of maturation by the activity of micrococci that are able to neutralize the acids produced (Comi et al., 2005). The value of water activity (a_w) showed a constant decrease during the maturation reaching final values of 0.80 … 0.82, and moisture of 251 … 258 g kg^{-1}. The final value of the salt content was around 40 g kg^{-1}, while the final nitrite about 9 mg kg^{-1} of NO_2^-. These parameter changes were due to the effect of dehydration (Comi et al., 2005). Mean value of weight losses over 21 days of ripening, when relatively constant weight reached 75-76% of relative humidity of air (RH) in the climatic chamber, was ~110 and 117 g of samples with and without starter

Table 1

The measured parameters values on start 0th day (S) and finishing 21st day (F)

Parameters	Time: start (S), finish (F)	Samples with starter culture	Samples without starter culture
L. monocytogenes, lg (cfu g^{-1})	S	6.57 ± 0.26	6.83 ± 0.12
	F	1.42 ± 0.43	2.86 ± 0.36
Total count of lactic bacteria, lg (cfu g^{-1})	S	5.72 ± 0.18	3.35 ± 0.15
	F	9.41 ± 0.32	6.95 ± 0.27
Mean sausage weight, g	S	396 ± 0.5	397 ± 0.5
	F	286 ± 0.5	280 ± 0.5
Moisture content, g kg^{-1}	S	347.8 ± 0.40	394.3 ± 0.38
	F	251.2 ± 0.38	258.8 ± 0.36
pH values	S	5.80 ± 0.018	5.76 ± 0.016
	F	4.67 ± 0.016	4.59 ± 0.015
a_w values	S	0.963 ± 0.002	0.956 ± 0.002
	F	0.817 ± 0.003	0.800 ± 0.003
Salt (NaCl) content, g kg^{-1}	S	29.4 ± 0.85	28.4 ± 0.64
	F	39.8 ± 0.75	40.2 ± 0.82
Nitrite (NO_2^-) concentration, mg kg^{-1}	S	14 ± 0.4	14 ± 0.4
	F	9 ± 0.3	9 ± 0.3

culture accordingly. Losses movement significantly ($p<0.001$) correlated with the mean value of moisture content.

Due to good adaptation of LAB to meat environment and their faster growth rates which were displayed during fermentation and ripening of sausages, they became the dominant microflora (Drosinos et al., 2005). The total count of LAB changes in cold-smoked sausages with and without starter culture is shown in Figure 1. Theoretically in sausage (A) by starter culture, calculated to 1 g sausage raw mass, lg 9.4 *Lactobacillus sakei* L110, lg 9.4 *Staphylococcus xylosus*, and lg 8.0 *Debaryomyces hansenii* were added.

The difference of detected LAB count between sausage A and B variants on 0th day was ~lg 2.37, that evident of artificially increasing count of LAB in sausage A by ~100 times in comparison to sausage B.

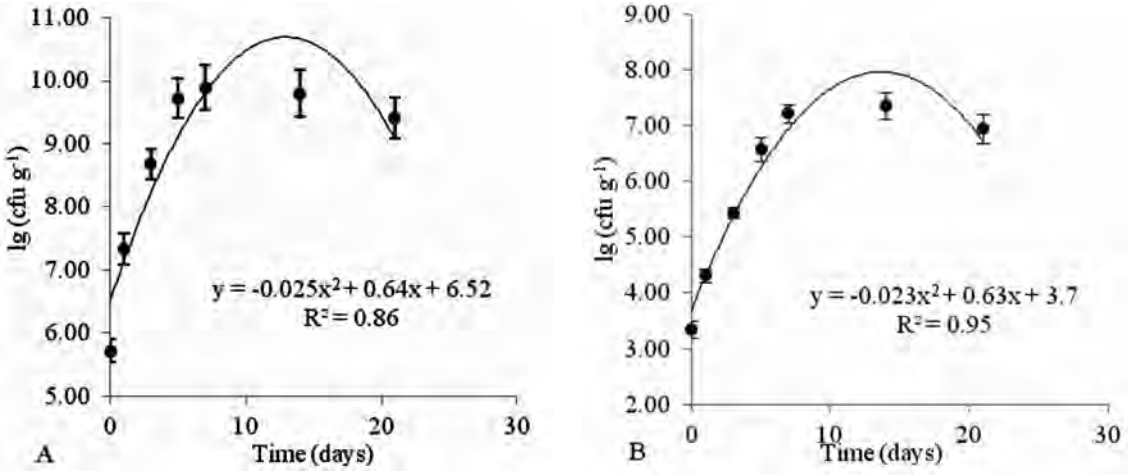

Figure 1. The polynomial changes of lactic acid bacteria count (lg values and SD values as ± bars) in cold-smoked sausages with (A) and without (B) starter culture during ripening ($p<0.01$).

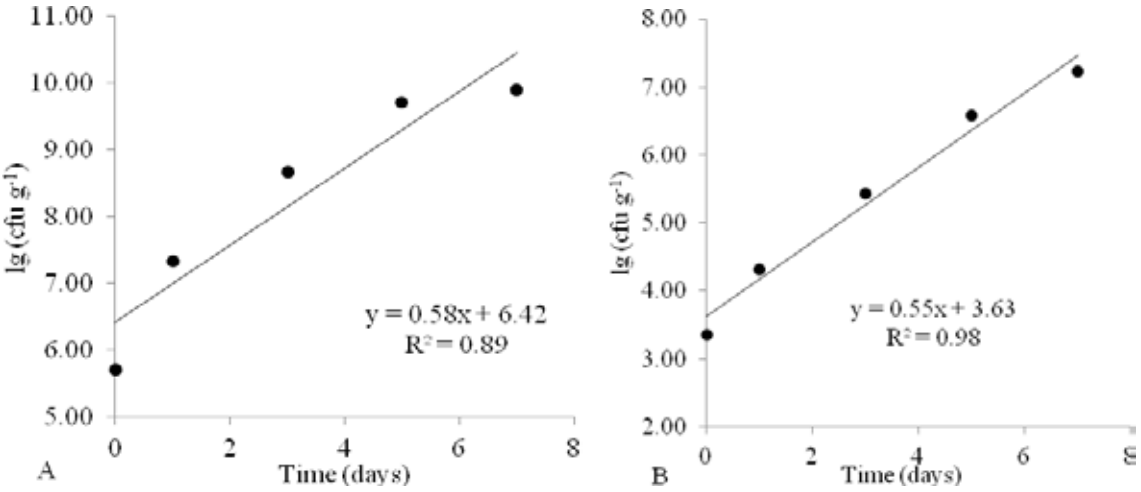

Figure 2. The linear regression trends of lactic acid bacteria count - lg (cfu g^{-1}) values in cold smoked sausages with (A) and without (B) starter culture in first 7 days of ripening ($p < 0.05$).

The most significant regression of bacteria count during all time investigated has been shown by polynomial equation (Figure 1). In the bacterial growth period on first 7 days the best conformity (R^2=0.98) to time, temperature, and interior factors are represented by linear regression in variant B (Figure 2).

It can be seen in Figure 1 and Figure 2 that exponential phase of lactic bacteria growth stops on the 7th day of ripening, when a_w decreases below 0.92 ... 0.90, and moves to stationary phase for next ~ 7 days. The main species of LAB, detected before in the meat products prepared at the mother factory, and its minimal a_w value by D. Vermeiren and A. Debevere (2004), were 0.94 for *Lactobacillus brevis* and 0.92 for *Lactobacillus plantarum*, which have been detected as the main LAB species in experimental sausage samples too.

The main bacterial growth factor a_w minimal values for starter culture components are: 0.91 *L. sakei* (Leroy and de Vuyst, 1999), 0.86 *S. xylosus* (Terra et al., 2007), and 0.81 *D. hansenii* (Aggarwal and Mondal, 2009). These different requests of minimal a_w guaranty a constant level of pH during necessary ripening time.

Due to its water binding and ionic characteristics, salt affects the metabolism of a starter culture. The growth of lactic acid bacteria is sometimes enhanced in the presence of low content of sodium chloride (1 to 2%, 10 ... 20 g kg^{-1}), but growth is clearly inhibited in the presence of NaCl content greater than 3% (30 g kg^{-1}) (Korkeala et al., 1992; Passos et al., 1993; Samapundo et al., 2010). Homofermentative LAB is more resistant to sodium chloride than heterofermentative LAB are, and strains resembling *L. sakei* have been shown to be more resistant than other strains.

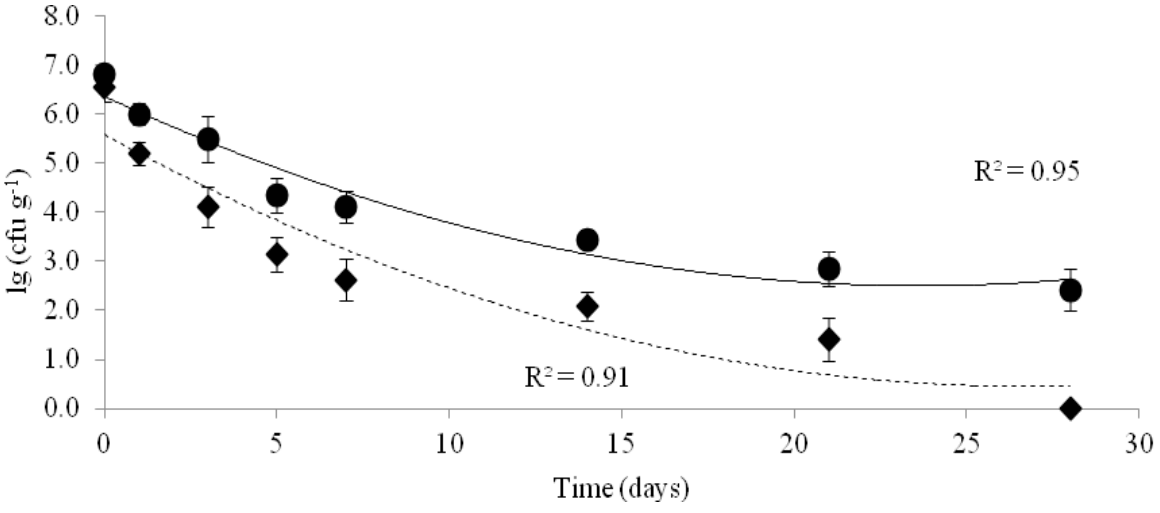

Figure 3. The polynomial regression trends of *L. monocytogenes* count - lg (cfu g^{-1})····· (A) with and —— (B) without starter culture in cold smoked sausages during ripening. Bars showed SD values ($p>0.05$).

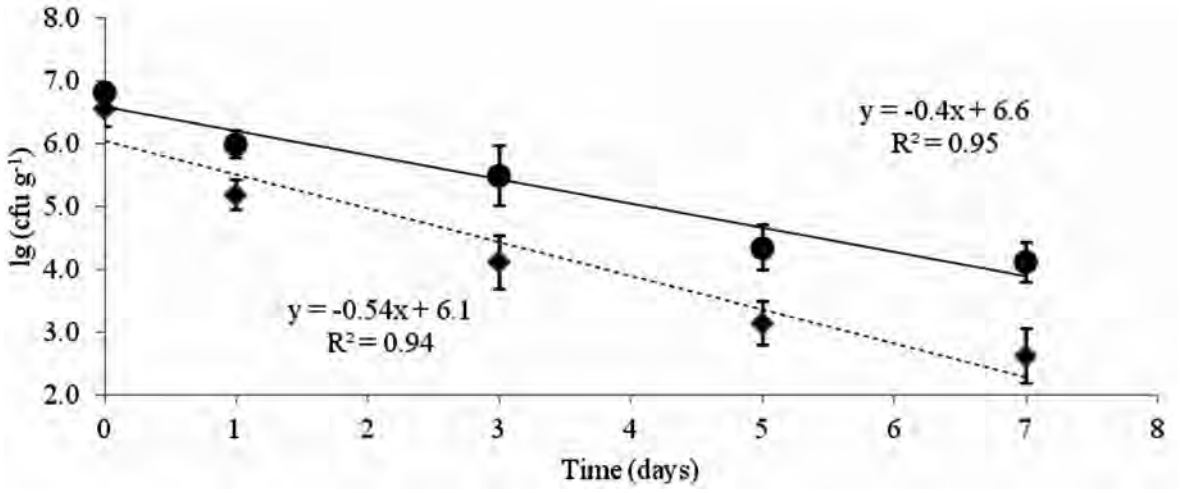

Figure 4. The linear regression trends of *L. monocytogenes* count - lg (cfu g^{-1})····· (A) with and —— (B) without starter culture in cold smoked sausages during the first 7 ripening days ($p>0.05$).

The initial *L. monocytogenes* inoculation concentration averaged 6.6 ... 6.8 lg (cfu g^{-1}) was significantly ($p<0.01$) reduced at any ripening stage in both (A and B) sample variants (Figure 3). The sausage samples from this study had finally pH < 4.7 and a_w < 0.82. Such values guarantee no growth of *L. monocytogenes* (Vermeulen et al., 2007) and the rest count possibly depends on initial count.

In both batches (A and B) the decrease of detected *L. monocytogenes* count showed a negative linear regression curve during the first 7 days (Figure 4) with lg (cfu g^{-1}) decreasing rate lg - 0.54 (A) and lg - 0.39 (B).

However, it can be said that *L. monocytogenes* were inhibited and did not exceed the growth in all observed ripening time (21 day). As it can be seen in Table 1, and Figure 4, the addition of starter culture hastened *L. monocytogenes* live cells, and detected count of *L. monocytogenes* decreased two times. All changes of the physicochemical parameters, except salt content, were decreased, but all of them did not support *L. monocytogenes* growth. All parameter changes more or less correlated (Table 2) between each other, but water activity is the parameter which summarizes these changes, and that is why it can be conferred as the main factor which limited pathogen growth in food products.

The latest papers described that the growth of *L. monocytogenes* ceased at a cell concentration of about 10^2 cfu mL^{-1} when natural microflora of foods, such as lactic acid bacteria, entered stationary phase (Al-Zeyara et al., 2011).

pH, a_w, NaCl (g kg^{-1}), and NO_2^- (mg kg^{-1}) values changes are shown in double graph in Figure 5.

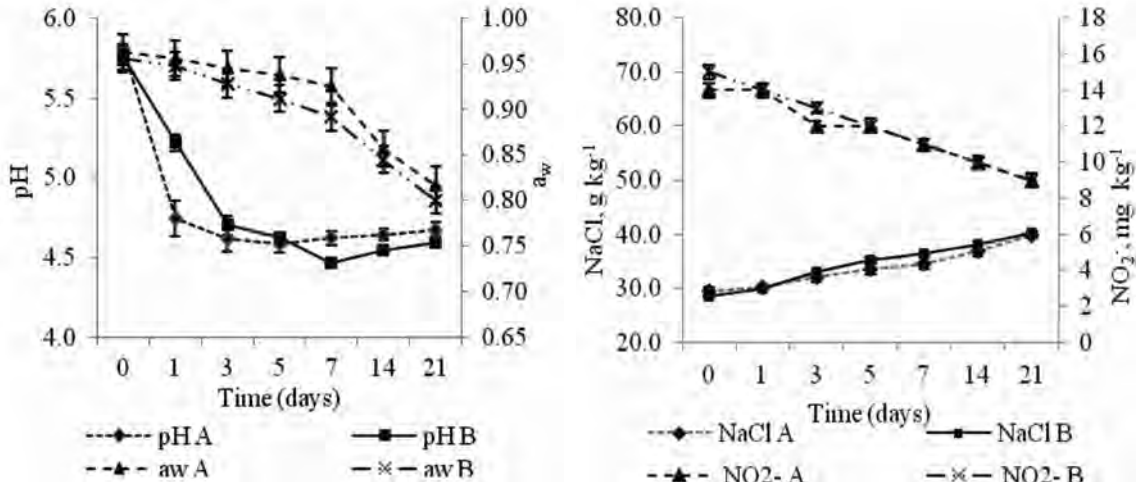

Figure 5. The changes of physicochemical parameters: pH and a_w (left), NaCl and NO_2^- (right) in cold-smoked sausages with (A) and without (B) starter culture during 21 ripening days.

Table 2

The correlation values and their significance levels between measured physicochemical parameters and changes of inoculated *L. monocytogenes* count

Calculation parameters		Time (days)	LAB, lg (cfu g^{-1})	pH	a_w	NaCl, g kg^{-1}
A	Correlation	-0.896	-0.865	0.609	0.867	-0.954
	p - value	0.003	0.006	0.073*	0.006	0.001
B	Correlation	-0.963	-0.873	0.765	0.980	-0.981
	p - value	0.001	0.005	0.023*	0.001	0.001

*Correlation is not significant.

The measurements of water activity show that *L. monocytogenes* growth would have been theoretically stopped on the 7th day of ripening when a_w decreased to 0.90 according to A. Vermeulen et al. (2007), but the observed results of bacterial count decrease made an idea of importance of a_w motion as the most significant factor against *L. monocytogenes* growing and survival in meat products.

The samples of cold smoked sausages had a mean initial pH value of 5.80 ± 0.02, which agrees with the results found by M. Paleari et al. (2003). A rapid decrease in pH was observed during the first three days of fermentation. The final pH of the fermented sausages had a mean value of 4.67 – 4.56 ± 0.02; this drop in pH was due to lactic acid production by the starter culture used for fermentation (Vermeiren and Debevere, 2004). Lactobacilli are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during fermentation (Schillinger et al., 1991). Lactic and acetic acids are often suggested to be major contributors to the acid aromas and tastes and the development of the texture of fermented sausage (Visessanguan et al., 2005).

Under the fermentation and maturation conditions in this work, the decrease of *L. monocytogenes* count in cold-smoked sausage was less intense than reported by other studies (Työppönen et al., 2003; Tolvanen et al., 2008) where an expressive decrease was observed at the beginning of ripening process. This is probably due to a lower pH and water activity in the first days of maturation noted in other studies, and higher initial *L. monocytogenes* concentration in our experiments.

The mean values of the decrease rate in *L. monocytogenes* count in batch B are bigger than those K. Glass and M. Doyle (1989) found in sausages without added lactobacilli cultures.

No significant correlation was calculated in both experimental batches with and without starter culture between *L. monocytogenes* count and pH value. That could be explained by a relatively short time when pH value decreased to constant level and long stationary phase of pH value.

Conclusions

The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in cold smoked sausages are a_w value decrease and lactic acid bacteria, which stopped *L. monocytogenes* growing at the beginning of cold-smoked sausage maturation. If fermentation process goes technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice we observed a low level of contamination.

The remaining count of *L. monocytogenes* in cold-smoked sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (cfu g^{-1}) for ready-to-eat products when contamination at first is more than lg 5.0.

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MICROBIOLOGICAL QUALITY OF COWS' MILK IN ORGANIC FARMING (PRELIMINARY REPORT)

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Abstract
The objective of the present study was to investigate the microbiological content of cows' milk in Latvia's organic farms with a purpose to detect potential microbiological threats in milk. Samples were collected in December 2011 at 12 biological dairy farms of Latvia. Raw milk samples (N=155) obtained from cow composite milk were studied. The total mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), the presence of coliforms and coagulase-positive staphylococci, count of yeasts and moulds were analysed using standard methods. Of the sampled cows 50% had a low somatic cell count (SCC) (<200,000 cells mL^{-1}), 23% - high, but 27% had a very high SCC (>500,000 cells mL^{-1}). The mean value of MAFAM in the samples with low, high and very high SCC was 4.7, 5.0 and 5.0 log10 colony forming units (cfu) mL^{-1}, respectively. The yeasts were present in 57% of milk samples with the mean concentration of 3.1 log10 cfu mL^{-1}. Moulds were found in 27% of all milk samples; their mean concentration was 4.4 log10 cfu mL^{-1}. Identified mould strains belonged to genera *Absidia*, *Aspergillus*, *Geotrichum*, *Mucor* and *Penicillium*. In cases of subclinical mastitis and latent mammary infection the most distributed mastitis pathogens were *Staphylococcus aureus*, *Micrococcus kristinae*, *Bacillus cereus* and coagulase negative staphylococci.
Key words: raw milk, microbiological quality, organic farming.

Introduction

Mastitis (an inflammation of the udder) is the most common disease affecting dairy cattle herds and the first cause of economic loss in milk production worldwide (Maréchal et al., 2011). Organic dairy farmers have identified mastitis as a major concern, mainly due to non-use of administration of long-acting intramammary antibiotics at dry-off. There is a study on mastitis that reveals that there are - more pathogenic and contagious species of mastitis causing bacteria obtained from cows on organic farms compared to milk samples collected from cows on conventional farms (Ruegg, 2009). This difference can be explained by inability of organic farmers to use effective mastitis control strategies sufficiently (Ruegg, 2009).

Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products determines that for organic herd prophylaxis and treatment of ill animals, phytotherapeutic and homeopathic products may be used. Only in cases, where the above treatments do not give desired results, it is allowed to use chemically synthesised allopathic veterinary products, including antibiotics. Antibiotics should not be used at drying off and are only allowed therapeutically during lactation in case of emergency. These measures could compromise the control and treatment of clinical disease and the herd health and welfare; therefore, it is necessary to establish an effective mastitis control strategy suitable for organic farming.

The objective of this study was to investigate the microbiological content of cows' milk in Latvia's organic farms with a purpose to detect potential microbial threats in milk. Further studies will be performed with the aim to develop effective mastitis control strategy including immunization of cows with mastitis vaccine in organic dairy herds in Latvia.

Materials and Methods

The collection of raw milk samples took place in December 2011 at 12 organic dairy farms of Latvia's regions – four farms from Zemgale, three – from Vidzeme, three – from Latgale and two – from Kurzeme. The organic farms were registered by the state control institutions. Herd size varied from 7 to 277 animals in a cow-shed including six herds with 7-50 cows, four - with 51-100 cows and two herds with more than 100 cows. Fifteen lactating cows from each herd were chosen for sampling. Milk samples in the herd less than 15 cows were collected of all lactating animals. The study included various breeds (Latvian Brown, Holstein and Danish Red) as well as different varieties of cross-breeds from the first to tenth lactation.

Sampling

Milk samples were collected by trained farm personnel from a cow level (cow composite milk) during sampling procedure of milk quality monitoring according to the standard LVS 175:1999 'Sampling of raw milk'. Samples for somatic cell count (SCC) evaluation were collected in 50 mL tubes with preservative, transported to the Laboratory of milk quality of the 'Siguldas Artificial insemination and Stock breeding station' (Sigulda, Latvia) and analyses were performed according to the standard LVS EN ISO 13366-2:2007. Samples for microbiological examination were collected in sterile vacutainers, 7 mL amount (Vacutest Kima, Italy) and transported to the Laboratory of Microbiology of the Research Institute of Biotechnology and Veterinary Medicine

'Sigra' (Sigulda, Latvia) in cold chain under temperature 10 °C and frozen at -20 °C for 2-6 weeks until an examination was done. A total 155 raw milk samples were analysed.

Microbiological examination

The samples were defrosted at room temperature and serially decimal diluted with Maximum recovery dilutent (Oxoid, England) according to the standard LVS EN ISO 6887-5:2011 'Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5: Specific rules for the preparation of milk and milk products (ISO 6887-5:2010)' and appropriate dilutions were plated on to agars.

For the enumeration of total mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), Milk agar (Oxoid, England) according to standard LVS EN ISO 7218:2007 'Milk and milk products - Enumeration of colony-forming units of microorganisms - colony count technique at 30 °C' was used. Acolyte Colony counter (Synbiosis, UK) for colonies enumeration was used. For the enumeration of yeasts and moulds Sabouraud Dextrose agar (Biolife, Italia) was used according to the standard LVS ISO 21527-1:2008 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0.95'. Plates were incubated at 22.5 °C for 10 days; subsequently, an enumeration was performed. Primary classification of moulds was based on colony characteristics (pigmentation, shape, background colour) and on microscopic examination according to Hungerford et al. (1998) and G. R. Carter and D. J. Wise (2004).

For the enumeration of bacteria and evaluation of haemolysis, we used blood agar medium containing 50 g L^{-1} sheep blood; plates were incubated for 24 h at 37 °C. If there were not present positive culture on following mediums, cultures from blood agar were identified using an identification system 'BBL Crystal Gram-positive and Enteric/Nonfermenter ID' (Becton, Dickinson and Company, USA). If on the blood agar Gram-positive bacillus was detected, colonies were transferred to a 'Bacillus cereus selective agar' (Oxoid, England). Incubation at 30 °C for 18 h and microscope examination for typical colonies of *Bacillus cereus* (*B. cereus*) was performed. Baird Parker agar with egg yolk supplement (Biolife, Italia) for the enumeration of staphylococci was used according to the standard LVS EN ISO 6888-1: 1999/A1:2003 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*S.aureus* and other species) - Part 1: Technique using Baird-Parker agar medium - Amendment 1: Inclusion of precision data'. Plates were incubated for 48 h at 37 °C. Presumptive coagulase-positive staphylococci colonies were transferred to a Brain heart infusion broth (Biolife, Italia), a Mannitol salt agar (Biolife, Italia) and tested for coagulase production using Rabbit Coagulase Plasma (Becton, Dickinson and Company, USA). *S. aureus* identification was confirmed with a diagnostic reagent 'Staphytect Plus' (Oxoid, England). Staphylococci other than *S. aureus* were identified using 'BBL Crystal Gram-positive ID' system. The isolation of Gram-negative bacteria including coliforms was carried out on Mac Conkey agar (Becton, Dickinson and Company, USA). Plates were incubated for 24 h at 37 °C. Isolated colonies were transferred to a 'Chromogenic E. coli/Coliform selective medium' (Biolife, Italia) for differentiation of *Escherichia coli* (*E. coli*) and other coliforms; tested by Reagent Stain dropper (Becton, Dickinson and Company, USA) for indole production and oxidase fermentation. For isolates not conformed to these methods, 'BBL Crystal Enteric/Nonfermenter ID' system was used.

Estimation of microbiological indices was done in accordance with the Council Regulation No 853/2004 of 29 April 2004 'Laying down specific hygiene rules for on the hygiene of foodstuffs', section IX 'Raw milk and dairy products'.

Categorisation of data

A milk sample was categorised as positive if at least one colony-forming unit of *S. aureus* or *Streptococcus agalactiae* (*S. agalactiae*) was isolated. For other microorganisms, the presence of at least three colony-forming units for positive categorisation was needed. If moderate to high growth of a major udder pathogen was found in combination with a few colony-forming units of several contaminating species, the sample would be diagnosed as positive for growth of the major udder pathogen. For the data analysis, milk secretion was categorised as Normal secretion, Disturbed secretion, Latent infection and Mastitis, according to International Dairy Federation (IDF), see Table 1. Threshold for somatic cell count estimation was 200,000 cells mL^{-1} in cow composite milk.

Table 1

Parameters for estimating milk secretion (adapted from IDF)

Bacterial culture	SCC is low	SCC is high
Negative	Normal secretion	Disturbed secretion
Positive	Latent infection	Mastitis

Using fix thresholds of 200,000 cells mL^{-1} and 500,000 cells mL^{-1}, three different somatic cell count categories were defined: low SCC<200,000, high SCC 200,000-500,000 and very high SCC>500,000.

Statistical analysis

The data were analysed using the SPSS 9.0.0 software package (SPSS Inc., Germany). Descriptive statistics including average, standard deviation and frequencies was done. To determine whether the effect of MAFAM, yeasts and moulds count was significant in explaining the variations in somatic cell count and secretion, the data were subjected to ANOVA followed by Univariate comparisons. Data are presented as mean ± standard deviation and a probability value $p<0.05$ was considered significant.

Results and Discussion

Milk quality can be estimated by count of somatic cells (SCC), mesophilic aerobic and facultative anaerobic microorganisms (MAFAM), coliforms and *S.aureus* (Nikolajeva, 2011). Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production, it is impossible to avoid contamination of milk with microorganisms; therefore, the microbiological content of milk is a major feature in determining its quality (Torkar and Teger, 2008).

Somatic cell count (SCC)

The SCC of milk is widely used to monitor udder health and milk quality (Sharif and Muhammad, 2008). SCC and bacteriological examination indicate the status of mammary gland as SCC in milk increases during intramammary infection (Harmon, 1994). Elevated SCC primarily consists of leucocytes, which include macrophages, lymphocytes and neutrophils. During inflammation, major increase in SCC is because of the influx of neutrophils into milk. Higher the SCC means greater the risk of raw milk contamination with pathogens (Sharif and Muhammad, 2008). Fifty percent of examined samples had a low SCC, 23% - high and 27% had a very high SCC. The mean value of SCC in the samples with low, high and very high SCC was 4.9, 5.5 and 6.3 log10 mL^{-1}, respectively. According to categorization of milk secretion, the mean SCC is the highest in cases of subclinical mastitis (1,293.333 or 6.1 log10 mL^{-1}), the smallest – in cases of the normal secretion (72,825 mL^{-1} or 4.9 log10 mL^{-1}), but the mean SCC of latent infection and disturbed secretion is not significantly different (Fig. 1). This means that the use of a single SCC analysis to classify quarters as uninfected or infected may not be a useful test, and bacteriological examination is strictly necessary.

C.B. Malek dos Reis et al. (2011) explain that the main factors responsible for SCC variation in mammary quarters are the occurrence of intramammar infections and the bacterial species. Milk samples with major pathogens isolation elicited higher SCC than those with minor pathogens.

Total mesophilic aerobic and facultative anaerobic microorganisms (MAFAM)

Milk may contain high bacterial numbers which form part of the product's natural microflora. The high MAFAM in some cases is related to mastitis, but not always. Mostly high MAFAM indicates milk contamination due to inadequate hygiene (Murphy, 2008). The mean value of MAFAM in the samples with low, high and very high SCC was 4.7, 5.0 and 5.0 log10 cfu mL^{-1}, respectively. As assessed by MAFAM, analyzed milk meets the Council Regulation 853/2004 requirements (maximum 5.0 log10 cfu mL^{-1}), however, it contains a large quantity of microorganisms; probably due to inadequate milking hygiene or insufficient cooling of milk and/or maintenance of cold chain thereafter.

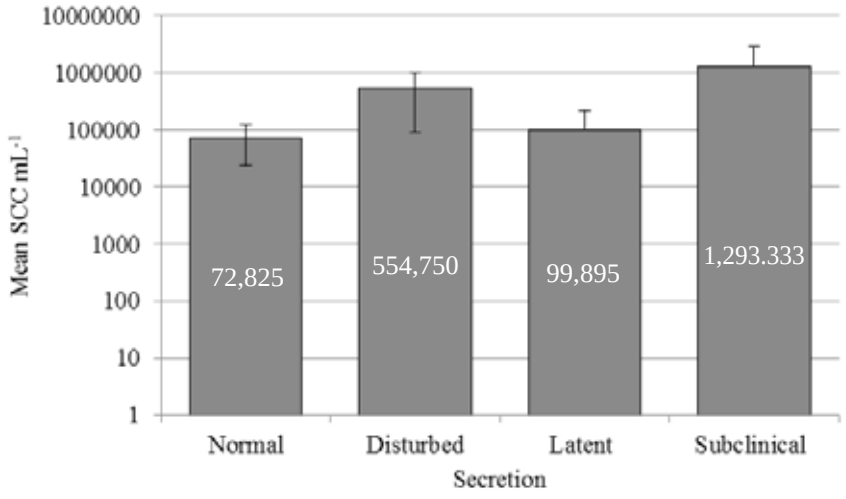

Figure 1. The mean SCC by categories of milk secretion.

Yeasts and moulds

Yeasts and moulds are normally regarded as spoilage organisms in milk. Moulds, mainly species of *Aspergillus*, *Fusarium*, and *Penicillium* can grow in milk and have ability to produce mycotoxins which can be a health hazard. Yeast spoilage is not a hazard to health (Douglas Goff, 1995). In rare cases moulds and yeasts can cause mastitis in cows, especially after the prolonged use of antibiotics (Britt, 1998). In our study the yeasts were present in 57% of milk samples with the mean concentration of 3.1 log10 cfu mL^{-1}. Moulds were found in 27% of samples and their mean concentration was 4.4 log10 cfu mL^{-1}. Correlation between SCC and count of yeast and moulds in milk was not established. For one half (n=21) of isolated moulds microscopic examination was performed and identified mould strains belonged to genera *Absidia* (n=4), *Aspergillus* (n=3), *Geotrichum* (n=1), *Mucor* (n=9) and *Penicillium* (n=4). There is the potential hazard from production of mycotoxins by moulds of genus *Aspergillus* and *Penicillium*, isolated of milk in this study.

Presence of *Enterobacteriaceae*

Enterobacteriaceae is a group of microorganisms which includes several that cause primary infections of the human gastrointestinal tract (Fox, 2010). In our study bacteria from genus *Enterobacteriaceae* were found in 11.6% of milk samples. tThis group includes *Serratia spp*., *Pseudomonae fluorescens* and coliform bacteria – *E. coli*, *Klebsiella oxytoca* and other undifferentiated. Researchers (Haguingan et al., 2010; Dadkhah et al., 2011) have found that udder infection with *Serratia spp.,* some strains of *E.coli* and *Klebsiella spp.,* less frequently *Pseudomonae spp.* may result in a severe clinical mastitis in cows. All of isolated bacteria can be causative agents for mastitis.

Isolated bacteria

1) In association with categories of somatic cell count

Bacterial growth occurred in 97% of samples. *S. aureus, Micrococcus kristinae* (*M. kristinae)* and coagulase negative staphylococci (CoNS) were the most prevalent agents with very high SCC and were isolated 29.6%, 11.1%, 11.1% milk samples, respectively. *S.aureus, M. kristinae* and microorganisms of genus *Enterobacteriaceae* were the most prevalent agents with high SCC and were isolated 20.5%, 13.6% and 13.6% milk samples, respectively. The most prevalent agents isolated from samples with low SCC were *M. kristinae, CoNS* and *S. aureus* with incidence 15.3%, 15.3% and 10.6% milk samples, respectively. All isolated bacteria groups are showed in Table 2.

Table 2

Incidence of bacteria in cows' composite milk samples

Isolated bacteria	Low SCC <200,000 mL^{-1}		High SCC 200,000-500,000 mL^{-1}		Very high SCC >500,000 mL^{-1}	
	n=85	%	n=44	%	n=54	%
S. aureus	9	10.6	9	20.5	16	29.6
M. kristinae	13	15.3	6	13.6	6	11.1
CoNS[1]	13	15.3	4	9.1	6	11.1
Bacillus cereus	2	2.4	3	6.8	5	9.3
C. aquatica	3	3.5	4	9.1	3	5.6
Enterobacteriaceae[2]	7	8.2	6	13.6	4	7.4
Lactic acid bacteria[3]	3	3.5	3	6.8	5	9.3
CoPS[4]	1	1.2	1	2.3	0	0.0
Other Gram-positive	15	17.6	1	2.3	2	3.7
Other Gram-negative	3	3.5	2	4.5	0	0.0
Culture negative	4	4.7	1	2.3	0	0.0
Other microorganisms[5]	12	14.1	4	9.1	7	13.0

CoNS[1] includes *S. saprophyticus*, *S. kloosi, S. equorum* and other undifferentiated; *Enterobacteriaceae*[2] include*Serratia spp*., *Pseudomonae fluorescens*, *E. coli*, *Klebsiella oxytoca* and other undifferentiated; Lactic acid bacteria[3] include *Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris* and *Pediococcus pentosaceus*; CoPS[4] (Coagulase positive staphylococci) includes *S. intermedius* and other undifferentiated except for *S. aureus*; Other microorganisms[5] include *Micrococcus spp*., *Bacillus spp., Enterococcus faecalis*, *Gemella haemolysans, Acinetobacter baumanii, Actinomyces pyogenes* and *S. agalactiae*.

Isolated microorganisms can be divided into several groups depending on their significance in milk quality and effect on udder health: Group of **lactic acid bacteria** *(Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris, Pediococcus pentosaceus, Enterococcus faecalis)* is able to ferment lactose to lactic acid. They are normally present in the milk and are also used as starter cultures in the production of cultured dairy products. **Spoilage bacteria** are involved in spoilage of milk, if they are psychrotrophic organisms *(P. fluorescens, Bacillus spp., Micrococcus spp., Corynebacterium spp.)*. Most psychrotrophs are destroyed by pasteurization temperatures; however, some like *P. fluorescens* can produce proteolytic and lipolytic extracellular enzymes which are heat resistant and capable of causing spoilage. Some species and strains of *Bacillus, Corynebacterium, Lactobacillus, Micrococcus*, and *Streptococcus* can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problems (Lin, 1997). **Coliform bacteria** *(E. coli, K. oxytoca)* are indicator organisms; they are closely associated with the presence of pathogens but not necessarily pathogenic themselves. They also can cause rapid spoilage of milk, because they are able to ferment lactose with the production of acid and gas, and are able to degrade milk proteins. The main bacterium of this group is *E. coli*. Some serotypes of *E. coli* can cause food poisonings and alimentary intoxications in human, the most dangerous among them are enterohemorrhagic *E. coli* strains, especially serotype O157:H7 (Usajewicz and Nalepa, 2006). **Pathogenic bacteria** can be divided into contagious *(S. aureus, S. agalactiae)* and environmental *(Staphylococcus spp., Bacillus cereus (B. cereus), M. kristinae, Acinetobacter baumanii, Actinomyces pyogenes)* pathogens. There have been a number of foodborne illnesses resulting from the ingestion of raw milk from a mastitic cow, or dairy products made from properly pasteurized milk (Nikolajeva, 2011; Douglas Goff, 1995; Guidelines, 2002). *S.aureus* is the bacterium with the largest interest in food toxicoinfections, because of some *S. aureus* strains are able to produce staphylococcal enterotoxins that cause gastroenteritis (Bennett and Hait, 2011).

2) In association with categories of milk secretion

According to categorization of milk secretion, the most distributed mastitis pathogens are *S. aureus, M. kristinae, B. cereus* and CoNS in cases of subclinical mastitis and latent mammary infection. (see Figure 2).

In this study ***S. aureus*** was the most frequent isolated pathogen - 35% of subclinical mastitis and 24% of latent udder infection cases. *S. aureus* is a common cause of bovine mastitis and its incidence is still high. A. Jemeljanovs et al. (2008) referred to 27%, J.M.B. Haguingan et al. (2010) - 31% and I. Klimiene et al. (2011) refered to 19% incidence of *S. aureus* in cases of subclinical mastitis. ***M. kristinae*** incidence in our study was 17% of subclinical mastitis and 34% of latent udder infection cases. Literature contains little information about *M. kristinae*. Most of these microorganisms are commensals to the human skin flora and cause infection in some cases. J.M.B. Haguingan et al. (2010) referred to 0.5% incidence of *M. kristinae* in cases of bovine subclinical mastitis. We isolated ***B. cereus*** with incidence of 11% of cases of subclinical mastitis. Since the *B. cereus* was isolated only from two herds, this incidence cannot be

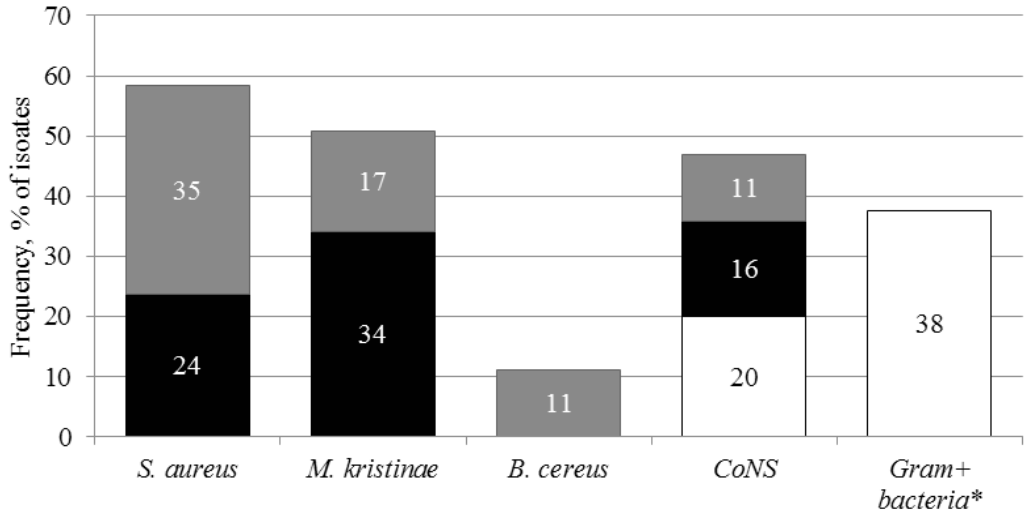

Figure 2. The incidence of the most distributed bacteria in cows' composite milk samples according to udder health categories: □ Normal secretion, ■ Latent infection, ▩ Subclinical infection, * other Gram-positive bacteria, including undifferentiated environmental non-pathogenic bacteria.

applied to all investigated herds and further data are required. *Bacillus* spp., including *B. cereus* are widely distributed in nature, and it is a frequent contaminant in raw milk and dairy products. In order to claim that *B. cereus* is a cause of intramammary infection, pure culture and association with high SCC or clinical signs of udder disease must be identified (Gonzales, 1996). There are authors that refer to fairly high incidence (30%) of contamination of raw milk by *B. cereus* (Hassan et al., 2010). In the context with subclinical mastitis, J.M.B. Haguingan et al. (2010) referred to presence of *B. cereus* in 7.6% of milk samples. Incidence of **coagulase negative staphylococci** in our study was 11%, 16% and 20% in cases of subclinical mastitis, latent udder infection and normal secretion, respectively. The most frequently diagnosed CoNS was *S. saprophyticus* - 5% of all cases of mastitis and latent udder infection. CoNS have traditionally been considered to be minor mastitis pathogens that can cause mastitis as opportunistic bacteria. The main reason for this is that mastitis caused by CoNS is mild, and usually remains subclinical (Taponen et al., 2006). The significance of CoNS, however, needs to be reconsidered as in many countries they have become the most common mastitis-causing agents (Pitkala et al., 2004). A. Jemeljanovs et al. (2008) admitted that CoNS incidence was 27% from subclinical mastitis secretion. Researchers have isolated more than ten different CoNS species from milk obtained from mastitis affected bovine udders. Most commonly reported species are *S. chromogenes, S. epidermidis, S. simulans* and *S. hyicus* (Thorberg et al., 2006; Pyörälä and Taponen, 2009; Klimiene et al., 2011). Some CoNS isolated from mastitis may be opportunists from the environment, but in S. Pyörälä and S. Taponen (2009) opinion, it is very likely that at least the main species infecting the bovine mammary gland are specifically adapted to the udder environment.

Further investigations are necessary to determine the source of microorganisms detected in milk and find out which ones are involved in mastitis aetiology.

Conclusions

1. The mean value of total mesophilic aerobic and facultative anaerobic microorganisms' count in the samples with low, high and very high SCC was 4.7, 5.0 and 5.0 log10 cfu mL^{-1}, respectively.
2. Bacteria from genus *Enterobacteriaceae* were found in 11.6% of milk samples, including coliforms of 2.6%.
3. The yeasts were present in 57% of milk samples with the mean concentration of 3.1 log10 cfu mL^{-1}.
4. Moulds were found in 27% of all milk samples and mean concentration was 4.4 log10 cfu mL^{-1}. Identified mould strains belonged to genera *Absidia*, *Aspergillus*, *Geotrichum*, *Mucor* and *Penicillium*.
5. In cases of subclinical mastitis and latent mammary infection, the most distributed pathogens were *Staphylcoccus aureus*, *Micrococcus kristinae*, *Bacillus cereus* and coagulase negative staphylococci. In cases of normal secretion the most isolated bacteria were gram-positive undifferentiated environmental bacteria and coagulase negative staphylococci.

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PORCINE CIRCOVIRUS-2 IMPACT ON THE MORPHOLOGICAL SIGHT OF PIG LYMPH NODES

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Abstract
The aim of study was to investigate porcine circovirus-2 (PCV2) impact on the morphological sight of pig lymph nodes. The research was carried out in 2010. Three Latvian farms were investigated (from Latgale and Kurzeme) and thirty 5-15 weeks old pigs with clinical signs of Postweanig Multisystemic Wasting Syndrome were selected and observed. Four lymph nodes from each animal for histological and immunohistochemical investigations were taken. Thirty structural parameters of a lymph node were detected on histological slides: visualization of follicle structure, lymphocyte depletion and amount of histiocytic cell infiltration. As a result, changes in lymph nodes with PCV2 antigen were detected in all these structural parameters: increasing amount of PCV2 antigen in pig lymph nodes, but the greatest changes were in the follicles structure in lymph node. The increased amount of PCV2 antigen in lymph node, significantly decreases the amount of lymphocytes and increases the amount of histiocytes in them. Porcine circoviruss-2 in lymph node basically distributes from the lymph node cortex into the medulla, as well the amount of PCV2 antigen in lymph node also increased.
Key words: lymph node, porcine circovirus-2, lymphocytes, histiocytes.

Introduction

It is considered that porcine circovirus-2 (PCV2) is the primary agent of Postweanig Multisystemic Wasting Syndrome (PMWS). This syndrome was first described in Canada in 1991. The first report about PCV2 infection in Lithuania and Latvia was published in 2007 (Stankevicius et al., 2007).

PMWS clinical signs and gross lesions are variable and non-specific (Harding, 2004). However, microscopic lesions in lymphoid tissues are almost unique and constitute the basis of PMWS diagnosis (Segales et al., 2004a). According to the data provided in many papers, PCV2 infected pigs have a systemic lymphadenopathy (Allan and Ellis, 2000; Sorden, 2000), which means different lymph node reactions – for example, an enlargement of the inguinal, mesenteric and bronchial lymph nodes (Segales et al., 1997; Harding, 2004). PCV2 specific lymphoid tissue reaction could be detected only by means of the histological test. It is known that lesions of lymph nodes in PCV2 infected pigs are as follows: lymphocyte depletion, hystiocytic cell proliferation, sometimes multinucleate giant cell infiltration, as well as multiple, sharply demarcated, spherical, basophilic cytoplasmic inclusions of bodies in histiocytic cells (Harding et al., 1998; Rosell et al., 1999; Segales, 2002, Segales et al., 2004b). Moreover, different reactions of lymphoid tissues between different pig species are possible (Opriessnig et al., 2009).

The effects of PCV2 on the pig immune system are not yet fully known, but it has been reported that the main target cells for PCV2 replication are the monocytes and macrophages (Rosell et al., 1999).

Summarizing the above stated, the aim of study was to investigate PCV2 impact on the morphological sight of pig lymph nodes. **The tasks of the study are as follow:**

1. to detect a PCV2 antigen by immunohistochemical analysis and it amount in *lnn. inguinales superficiales sinister et dexter, lnn. mesenterici craniale* un *lnn. tracheobronchales*;
2. to investigate the morphological changes in their lymph nodes;
3. to evaluate PCV2 amount in lymph nodes impacting the morphological changes.

Materials and Methods

The research was carried out in 2010. The investigations were done in three Latvian farms (from region of Latgale and Kurzeme). On their farms pigs with clinical signs of PMWS were observed. Before death, pigs had progressive weight loss or wasting, diarrhoea or respiratory disease, pallor or jaundice, which accord with PMWS (Harding et al., 1998; Rosell et al., 1999; Segales et al., 2004a). For research purposes thirty pigs of the age of 5-15 weeks were selected from all farms.

Pig necropsy was performed in 12 hours after pigs' death and samples of lymph nodes *lnn. inguinales superficiales sinister et dexter, lnn. mesenterici cranial* and *lnn. tracheobronchales* were taken for the histological and immunohistochemical investigation. In total, four lymph nodes were taken from each animal (n=30).

All lymph nodes (n=120) were fixed by immersion in 10% neutral buffered formalin. Fixed samples were dehydrated, embedded in paraffin wax, sectioned at 4 μm. Histological slides were stained with haematoxylin and eosin and investigated by Zeiss light microscope.

In order to detect PCV2 antigen, an immunohistochemical technique (IHC) was carried out on the lymph nodes tissue sections by using an avidine-biotin-peroxidase method described previously (Szczotka et al., 2011). Endogenous peroxidase activity was inhibited by immersing the tissue sections in a 3% solution of hydrogen peroxide in water for 30 min. Antigen retrieval was done by enzymatic treatment (protease K) in Tris-buffered saline (TBS; pH 7.55) for 3 min. After that, tissue sections covered with the monoclonal antibody Ingenasa 36A9 (dilution 1:250 in TBS with albumin), and the slides were incubated at +4 °C, 15 hours. Then, the secondary antibody Policlonal Goat–Anti Mouse Immunoglobulins Biotinylated (dilution in TBS 1:200) was applied for one hour at room temperature. Peroxidase-conjugated avidin kit was applied for two hours at room temperature. Sections were incubated in chromogen substrate (AEC) and stained in haematoxylin. Tissues sections were examined microscopically with Zeiss light microscope.

For each histological slide, three structural parameters of lymph nodes were detected. These parameters were evaluated by score ranges from 0 to 3 (Opriessing et al., 2004):

1. visualization of the follicle structure (0 – normal, no changes, 1 – visualization of the follicle structure a little deplete, 2 – the follicle structure poorly visualized, 3 – loss of the lymphoid follicle structure);
2. the amount of lymphocyte depletion for full lymph nodes (0 – normal, no changes, 1 – a mild amount of lymphocyte depletion, 2 – a moderate amount of lymphocyte depletion, 3 – a severe amount of lymphocyte depletion);
3. the amount of histiocytic cells (0 – histiocytic cells not detected in tissues, 1 – a mild amount of histiocytic cells, 2 – a moderate amount of histiocytic cells, 3 – a severe amount of histiocytic cells).

In each immunohistochemical slide was estimated amount of PCV2 antigen in different regions of lymph node – in follicle, paracortex near follicle area, paracortex near sinus area and medulla. This parameter was evaluated by generally accepted system (Opriessing et al., 2004), where PCV2 antigen presence in lymph node is mild (+), moderate (++) or severe (+++). In addition, PCV2 antigen was evaluated in percentages, where measured immunohistochemical positive and negative stained lymph nodes tissues in the field of microscope were observed. Measurements were done with the computer program Pannoramic DensitoQuant (3DHISTECH). Five fields (200 µm on 200 µm) were detected from each region of lymph node.

The results were statistically calculated by Microsoft Excel t-test: Two-Sample Assuming Unequal Variance for comparing the mean index of structural parameters in lymph node with different amount of PCV2 antigen in lymph nodes ($p<0.05$).

Results and Discussion

From 30 immunohistochemical tested piglets, the antigen of PCV2 was established in 16 animals lymph nodes, moreover, in different amount it was found in all 64 investigated lymph nodes (Table 1).

Table 1

The IHC results of PCV2 antigen in pig lymph nodes

PCV2 antigen in lymph nodes:	Number of animals (n=30):	Number of investigated lymph nodes (n=120):
is detected	16 (53.3%)	64 (53.3%)
not detected	14 (46.7%)	56 (46.7%)

As the PCV2 antigen could contain variable amount of antigen (from + to +++) in the investigated lymph nodes from one animal, animals were grouped into three groups – according to the amount of prevailing virus antigen in lymph nodes for future analysis.

In 28 lymph nodes from seven piglets mild amount (+) of PCV2 antigen prevailed, in 16 lymph nodes from four piglets the amount of PCV2 antigen was moderate (++), but in 20 lymph nodes from five piglets the amount of antigen was severe (+++).

First lymph nodes structural changes of piglets, whose lymph nodes contained mainly mild amount (+) of PCV2 antigen were analysed (Table 2).

Comparing the average score of each structural parameter, it was established that the tendency of the visualization changes of follicles were similar for all animals' lymph nodes in this group. On average, the tendency of the amount of lymphocytes decreasing and the amount of histiocytes increasing was also similar in all lymph nodes.

The visualization of the follicle structure in lymph nodes of animals No. 13, 26, 30 was only slightly depleted. Also, in these animals' lymph nodes the mild amount of lymphocytes decrease was detected and mild amount of histiocytes increase was observed. It is considered that slight morphologycal changes of lymph nodes could indicate an early stage of PCV2 infection. Other authors have reported it too (Ostanello et al., 2005; Opriessnig et al., 2007).

Structural parameters of lymph nodes in piglets No. 4, 5, 8, 11 had severe changes (Table 2). In addition, the most severe changes were found in *lnn. tracheobronchales*, where the follicles structure was

Table 2

The structural changes of lymph nodes (scores) mainly with mild amount of PCV2 in lymph nodes

The name of lymph node	Valued parameters	Number of the animal							Mean index of parameter
		No. 4	No. 5	No. 8	No. 11	No. 13	No. 26	No. 30	
lnn. inguinales superf. sinister	amount of PCV2 antigen	+	+	+	+	+	+	+	+
	visualization of the follicle structure	2	2	3	3	1	1	1	1.86
	decrease amount of lymphocytes	2	2	2	2	1	1	0	1.43
	increase amount of histiocytes	2	2	2	1	1	1	1	1.43
lnn. inguinales superf. dexter	amount of PCV2 antigen	+	+	+	+	+	+	+	+
	visualization of the follicle structure	2	2	3	3	1	1	1	1.86
	decrease amount of lymphocytes	2	1	2	2	1	1	1	1.43
	increase amount of histiocytes	2	2	2	1	1	1	1	1.43
lnn. mesenterici craniale	amount of PCV2 antigen	+	+	+	+	+	+	+	+
	visualization of the follicle structure	2	2	3	3	1	1	1	2.00
	decrease amount of lymphocytes	2	1	2	3	1	1	1	1.57
	increase amount of histiocytes	2	2	2	2	1	1	1	1.57
lnn. tracheobronchales	amount of PCV2 antigen	+	++	++	+	+	+	+	+
	visualization of the follicle structure	2	3	3	3	2	1	1	2.14
	decrease amount of lymphocytes	2	2	2	3	1	1	0	1.57
	increase amount of histiocytes	2	2	2	2	1	1	1	1.57

not visualized and two animals (No. 5 and 8) had moderate amount of PCV2 antigen in this lymph node. The mentioned structural changes of lymph nodes show that the PCV2 infection process had been more durable to these four animals. (Ostanello et al., 2005; Opriessnig et al., 2007).

Next estimated lymph nodes structural changes of piglets were in those, whose lymph nodes contained mainly moderate amount (++) of PCV2 antigen (Table 3).

It was noticeable that the visualization of follicles in all lymph nodes was changed mostly (average

Table 3

The structural changes of lymph nodes (scores) mainly with moderate amount of PCV2 in lymph nodes

The name of lymph node	Valued parameters	Number of the animal				Mean index of parameter
		No. 1	No. 2	No. 16	No. 28	
lnn. inguinales superf. sinister	amount of PCV2 antigen	++	++	++	++	++
	visualization of the follicle structure	3	2	3	3	2.75
	decrease amount of lymphocytes	3	2	2	2	2.25
	increase amount of histiocytes	2	1	2	1	1.50
lnn. inguinales superf. dexter	amount of PCV2 antigen	++	++	++	++	++
	visualization of the follicle structure	3	2	3	3	2.75
	decrease amount of lymphocytes	3	2	2	2	2.25
	increase amount of histiocytes	2	1	2	1	1.50
lnn. mesenterici craniale	amount of PCV2 antigen	+	++	++	+	++
	visualization of the follicle structure	2	2	3	2	2.25
	decrease amount of lymphocytes	2	2	2	1	1.75
	increase amount of histiocytes	2	1	2	1	1.50
lnn. tracheobronchales	amount of PCV2 antigen	+	+	++	+	+
	visualization of the follicle structure	2	2	3	1	2.00
	decrease amount of lymphocytes	2	3	2	1	2.00
	increase amount of histiocytes	2	2	2	1	1.75

Table 4

The structural changes of lymph nodes (scores) mainly with severe amount of PCV2 in lymph nodes

The name of lymph node	Valued parameters	Number of the animal					Mean index of parameter
		No. 3	No. 7	No. 9	No. 10	No. 15	
lnn. inguinales superf. sinister	amount of PCV2 antigen	+	+++	+++	+++	+++	+++
	visualization of the follicle structure	2	3	3	3	3	2.80
	decrease amount of lymphocytes	1	2	3	3	2	2.20
	increase amount of histiocytes	1	2	3	2	2	2.00
lnn. inguinales superf. dexter	amount of PCV2 antigen	+	+++	+++	+++	+++	+++
	visualization of the follicle structure	2	3	3	3	3	2.80
	decrease amount of lymphocytes	1	2	3	3	2	2.20
	increase amount of histiocytes	1	2	3	2	2	2.00
lnn. mesenterici craniale	amount of PCV2 antigen	+++	+++	+++	+++	+++	+++
	visualization of the follicle structure	3	3	3	3	3	3.00
	decrease amount of lymphocytes	3	3	3	3	2	2.80
	increase amount of histiocytes	3	3	2	2	2	2.40
lnn. tracheobronchales	amount of PCV2 antigen	++	++	+++	++	+++	+++
	visualization of the follicle structure	3	3	3	3	3	3.00
	decrease amount of lymphocytes	3	3	3	3	3	3.00
	increase amount of histiocytes	3	3	2	2	3	2.60

score 2.00 – 2.75) compared to other investigated parameters of lymph nodes.

Also, lymphocytes depletion in lymph nodes of these animals was relatively severe, score from 1.75 to 2.25; like the increase amount of histiocytes – from 1.50 to 1.75 (Table 3).

In the Table 4 are shown the lymph nodes structural changes of piglets in whose lymph nodes mainly severe amount (+++) of PCV2 antigen was observed.

It is observed, that generally in lymph nodes of animals in this group, all investigated structural parameters had greatly changed: visualization of the follicle structure was scored in the range from 2.80 to 3.00; the amount of lymphocytes was decreased and amount of histiocytes was increased – on the average from 2.00 to 3.00 scores (Table 4).

By analyzing lymph nodes group individually for each animal, it should be noted that one piglet (No. 3) had different morphological changes in *lnn. inguinales superficiales*. These changes were relatively smaller (scores 1-2), that could be associated with the mild amount of PCV2 antigen in them. Presumably PCV2 was not distributed throughout the whole organism of piglet No. 3 yet, as it was observed in other four animals of this group.

Summarizing data about all structural parameter changes in lymph nodes, it has been concluded that PCV2 was relatively more affected in the follicle of lymph nodes. Follicles lose their typical structure, and presumably also are affected by B lymphocyte population, because it is known that lymphocytes B concentrate directly in the follicles of lymph nodes (Willard-Mack, 2006).

The amount of lymphocytes decreased simultaneously with the PCV2 antigen increase in them. The question – which population of lymphocytes – T or B – decreased in lymph node, will be clarified in the further stage of research.

If the amount of PCV2 antigen in lymph nodes increases, then the amount of histiocytes increases accordingly. It is thought that main target cells for PCV2 replication are the monocytes and macrophages (Rosell et al., 1999). Then macrophages 'get old' and are overtaken by histiocytes (Willard-Mack, 2006). It is likely that 'fight' between macrophages and virus has been for a long time. It is possible that those pigs could have disorder of humoral immunity (Allan et al., 1999; Bolin et al., 2001; Krakowka et al., 2001).

To understand changes of individual structural parameters of lymph nodes with different amount of PCV2 antigen better, structural changes of lymph nodes were compared with prevailing amount of the virus antigen in lymph nodes (Table 5).

Comparing the histological structure of lymph nodes with mild (+) amount of PCV2 antigen and the structure of lymph nodes without presence of PCV2 antigen (0), it is observed that all investigated parameters have significant difference in structural changes in all lymph nodes. In all investigated parameters, there is a significant difference in structural changes in all lymph nodes with mild and severe amount of PCV2 antigen (Table 5).

Table 5

Significant difference in structural parameters of lymph node with mild (+), moderate (++) and severe (+++) amount of PCV2 antigen in lymph node

The name of lymph node	Valued parameters	The amount of PCV2 antigen and significant changes in structural parameters in lymph node			
		- (n=56)	+ (n=28)	++ (n=16)	+++ (n=20)
lnn. inguinales superf. sinister	visualization of the follicle structure	0	1.86*	2.75^	2.80¬
	decrease amount of lymphocytes	0	1.43*	2.25^	2.20¬
	increase amount of histiocytes	0	1.43*	1.50	2.00¬
lnn. inguinales superf. dexter	visualization of the follicle structure	0	1.86*	2.75^	2.80¬
	decrease amount of lymphocytes	0	1.43*	2.25^	2.20¬
	increase amount of histiocytes	0	1.43*	1.50	2.50°¬
lnn. mesenterici craniale	visualization of the follicle structure	0	2.00*	2.25	3.00°¬
	decrease amount of lymphocytes	0	1.57*	1.75	2.80°¬
	increase amount of histiocytes	0	1.57*	1.50	2.40°¬
lnn. tracheobronchales	visualization of the follicle structure	0	2.14*	2.00	3.00°¬
	decrease amount of lymphocytes	0	1.57*	2.00	3.00°¬
	increase amount of histiocytes	0	1.57*	1.75	2.60°¬

* – the significant difference between structural changes of lymph node with 0 amount of PCV2 antigen and mild amount (+)
^ – the significant difference between structural changes of lymph node with mild (+) and moderate (++) amount of PCV2
° – the significant difference between structural changes of lymph node with moderate (++) and severe (+++) amount of PCV2
¬ – the significant difference between structural changes of lymph nodes with mild (+) and severe (+++) amount of PCV2 in them

To view a PCV2 distribution in lymph nodes from follicle to other part of lymph node better, the amount of PCV2 antigen was reflected as a percentage in separate structures of lymph nodes (Fig. 1, 2, 3). Considering amount of PCV2 antigen (%) measured by five fields from each part of lymph nodes, graphical representations in the average amount of PCV2 antigen (%) were given.

First, the average amount of PCV2 antigen (%) for seven animals with mild (+) amount of PCV2 antigen was evaluated (Fig. 1). It appeared that PCV2 antigen is mainly located in the lymph nodes follicles.

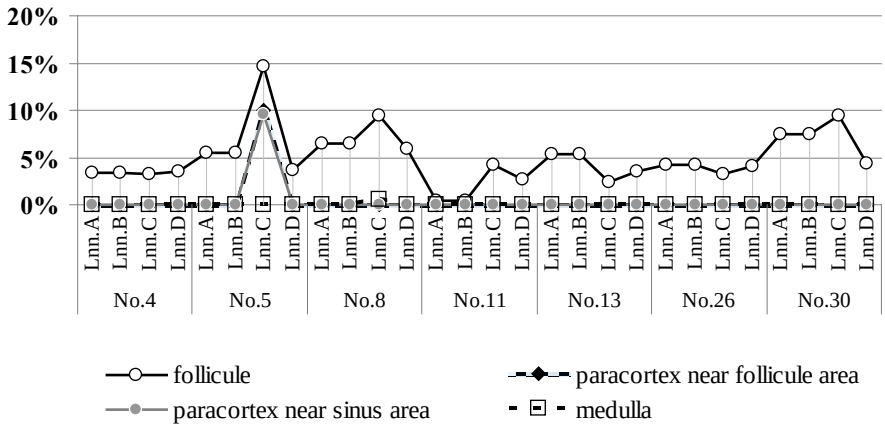

Figure 1. PCV2 antigen location in lymph nodes mainly with mild (+) amount of PCV2 in lymph nodes: Lnn. A – *lnn. inguinales superficiales sinister,* Lnn. B – *lnn. inguinales superficiales sinister dexter,* Lnn. C – *lnn. mesenterici cranial* and Lnn. D – *lnn. tracheobronchales*. No. 4, 5, 8, 11, 13, 26 and 30 – animal number.

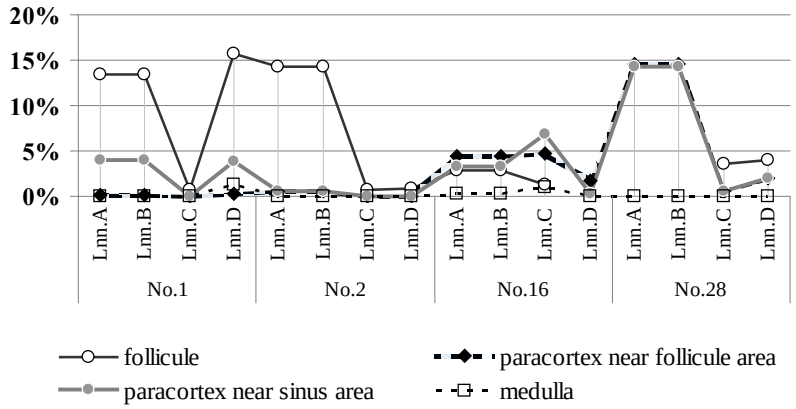

Figure 2. PCV2 antigen location in lymph nodes mainly with moderate (++) amount of PCV2 in lymph nodes. Lnn. A – *lnn. inguinales superficiales sinister,* Lnn. B – *lnn. inguinales superficiales sinister dexter,* Lnn. C – *lnn. mesenterici cranial* and Lnn. D – *lnn. tracheobronchales*. No. 1, 2, 16 and 28 – animal number.

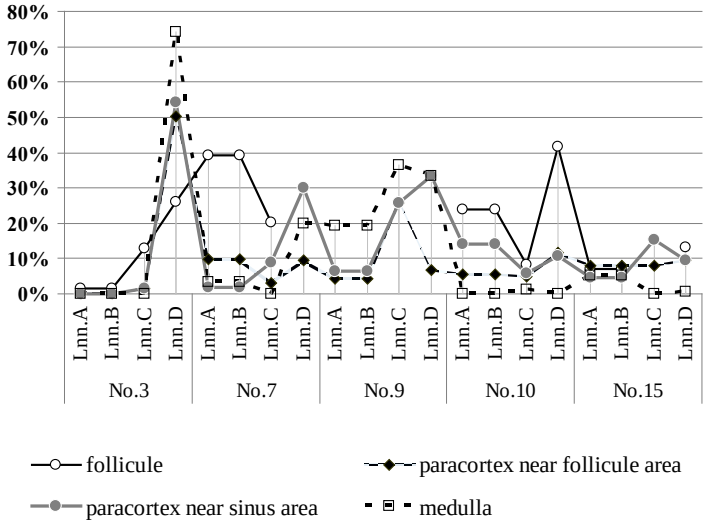

Figure 3. PCV2 antigen location in lymph nodes mainly with severe (+++) amount of PCV2 in lymph nodes. Lnn. A – *lnn. inguinales superficiales sinister,* Lnn. B – *lnn. inguinales superficiales sinister dexter,* Lnn. C – *lnn. mesenterici cranial* and Lnn. D – *lnn. tracheobronchales*. No. 3, 7, 9, 10 and 15 – animal number.

PCV2 antigen in follicle (14.5%), paracortex near follicle area (10%) and paracortex near sinus area (10%) was detected only in one animal (No. 5) *lnn. tracheobronchales.*

In lymph nodes mainly with moderate (++) amount of PCV2 antigen (Table 3), PCV2 antigen was located in follicle, paracortex near follicle area and paracortex near sinus area (Fig. 2).

Next, lymph nodes mainly with severe (+++) amount of PCV2 antigen were estimated (Table 4). In these lymph nodes PCV2 antigen mainly in medulla and paracortex near sinus area was detected (Fig. 3). PCV2 antigen percentage in medulla varied up to 75%, but in paracortex near sinus area – up to 55%.

Thus, it demonstrates that circovirus-2 at first was located in lymph nodes follicles, after that 'goes outside' the follicle into the paracortex near follicle area and near sinus area, and finally – into medulla. In lymph nodes PCV2 starts from cortical layer to medulla, from where it could enter into the organism lymphatic system.

Conclusions

1. Increasing amount of PCV2 antigen in pig lymph nodes, mostly changes the follicles structure in lymph node – visualization of the follicle structure becomes little deplete, and finally the lymph nodes completely lose the lymphoid follicle structure in the microscope.
2. The increase in amount of PCV2 antigen in lymph node significantly decreases the amount of lymphocytes and increases the amount of histiocytes in them.

3. Porcine circoviruss-2 in lymph node basically distributes from the lymph node cortex into the medulla, as well as the amount of PCV2 antigen also increases in lymph node.

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AORTIC LUMEN DIAMETER AND BLOOD PRESSURE CHANGES DYNAMICS AFTER REPLACING AORTA ABDOMINALIS WITH PROSTHESIS

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Abstract
The aim of this study was to find out the biointegration of innovative vascular prosthesis, made in Riga Technical University (RTU), in dog's abdominal aorta. The research is being performed in Veterinary Medicine Faculty of Latvia University of Agriculture since July 19. 2011. We used 9 female gender, 1-3 years old Beagle dogs in this study. The research is approved by the Latvian Republic Food and Veterinary Service. The study is carried out in the frame of European Social Fund co-financed project 'Establishment of interdisciplinary research groups for a new functional properties of smart textiles development and integrating in innovative products' (ESF No. 2009/0198/1DP/1.1.1.2.0./09/APIA/VIAA/148). 5-8 mm in diameter, 8 mm - 18 mm in length RTU produced aortic grafts were implanted retroperitoneal in dogs. Before the surgery the diameter of abdominal aorta was measured in cross sections, afterwards – cranial and caudal from the prosthesis, as well cross-section diameter of vascular graft was measured. Blood pressure was measured before the surgery and after the procedure on regular basis. Results are the following: there are no significant differences in aortic and graft diameter before and 1-2 months after the operation, there are no significant differences in systolic and diastolic blood pressure before and four months after the surgery at significance level $\alpha = 0.05$. The study shows that the innovative aortic prostheses don't change in diameter, and surgery like abdominal aorta transplantation doesn't cause significant variations in blood pressure. The study is being continued to find out later reactions to synthetic vascular graft.
Key words: vascular graft, synthetic prostheses.

Introduction

In medicine one of the major blood vessel diseases is atherosclerosis. As a result of atherosclerosis, the affected blood vessels become narrower and do not provide a complete tissue trophic of concrete area. Condition like this, decreases the patient's life quality. One of the options to restore normal blood circulation is to replace the pathologically affected blood vessels with synthetic prosthesis. The autologous blood vessels often are not enough to replace large blood vessels, so in such cases there is no other alternative as to replace the damaged blood vessel with an artificial prosthesis. During the last decades intensive development of prosthesis has taken place throughout the world. As A. Wesolow notes that there have been more than 450 different types of vascular prosthesis developed in the United States during the last 30 years (Wesolow, 2008). At the same time, using vascular prostheses in clinical practice, many failures have been associated not only with incorrect diagnoses, awkward operations, lack of patient compliance, infections, but also with the prosthesis design and inappropriate materials or incomplete work in the preclinical experimental verification.

Biomechanical and physiological properties of vascular prosthesis which are offered today do not conformwith human blood vessel properties. These wall structure characteristics do not allow the prosthesis to pulsate, resulting in failure to delete a high pulse wave fluctuations, and do not provide long-term hemodynamic processes in a live organism. Nowadays, one of the major medical problems is restoration of body functional unity after vascular reconstruction (Lukyanchikovs et al., 2010).

Successful introduction of new vascular prostheses with better rheological and immunological properties into clinical practice requires a comprehensive study of their properties in experimental animals *in vivo*. In such experiments there are no alternative methods *in vitro* (Podlaha et al., 2009).

New structure pulsating aortic prostheses are designed in Riga Technical University (RTU), under the supervision of the professor V. Kantsevicha. It is weaving technology, biocompatible with the surrounding tissue, inert, non-toxic polyurethane and polyester filament yarn used to make artificial blood vessels. In order to implement the aortic graft in human medicine, it is necessary to study their effects on the body and the possible resulting complications; therefore, the usage of laboratory animals is an integral part of this process (Lukyanchikovs and Kantsevicha, 2010)

One of the most serious postoperative complications is lumen narrowing of the implanted vascular prostheses caused by overgrowing with neointimal cells. The restricted artificial blood vessels phase also causes hemodynamic disturbances in the area around.

The aim of this study is to find out the biointegration of innovative vascular prosthesis, made in RTU, in dog's abdominal aorta. Biointegration in our study includes changes in vascular lumen cranial from the aortic graft, in graft place and caudal from it, and provides blood pressure and its changes

during postoperative period in dogs. In future we will investigate the animal body reaction to acceptance of the above mentioned innovative vascular prosthesis with histological and imunohistological examinations.

Materials and Methods

In this study, 9 female, 1 to 3 years old, Beagle dogs, purchased from experimental animal farms in France were used. The experiment is confirmed by the Food and Veterinary Service of Republic of Latvia, and has a permission to be carried out. Pulsating 5-8 mm in diameter and 8 mm to 18 mm long RTU produced aortic grafts were implanted retroperitoneal in experimental animals.

Dogs were weighed and one hour before the surgery 5% or 10% 'Enroxil' (the active ingredient enrofloxacin) 5 mg kg^{-1} was injected i.m. to prevent infection. Non-steroidal anti-inflammatory agent 'Loxicom' (the active ingredient meloxicam) was administered orally appropriate to the animal's weight to reduce inflammation and pain one hour before surgery. As premedication 0.1% Atropine sulfate 0.02 mg kg^{-1} and 1% Acepromazine 0.1 mg kg^{-1}were used i.m. Diazepam 0.5% 0.25 mg kg^{-1} and 10% Ketamine 10 mg kg^{-1} were used i. v. for induction anesthesia. During the operation inhalation of anesthetic Isoflurane was used.

Animals on the operating table were positioned in the right lateral recumbent position and surgery area -lumbar vertebral area was aseptically prepared. Incision was done parallel to the lumbar vertebrae below the longest dorsal muscle (*M. longissimus dorsi)*, caudal from the, last, left rib. The skin, abdominal external oblique muscle (*M. Obliquus externa abdominis*), the abdominal internal oblique muscle (*M. Obliquus interns abdominis*), abdominal transverse muscle (*M. transversus abdominis)* were split dissecting abdominal aorta without cutting peritoneum. Two specially designed clamps were placed on the aorta for blood vessel surgery, about 4 to 5 cm away from each other, and blood flow was stopped. Three minutes before this action heparin was administered intravenously. Next, a transverse cut or piece resection in abdominal aorta was made to divide it in two parts. Then the innovative aortic prosthesis made in RTU was implanted. Total ischemic time ranged from 30 – 60 minutes. For aortic graft sewing 7/0 'Premilene', for muscle and skin sewing 2/0 'Serafit' and skin - 3/0 'Supramide' threads were used.

In post-operative period all animals got 5% or 10% 'Enroxil' 5 mg kg^{-1} injections i.m. once a day and anti-inflammatory agent 'Loxicom' appropriate to the animal's weight was administered orally for seven days. For five days analgesic drug 'Tramadol' 4 mg kg^{-1} 2-3 times a day was used i.m. too. Twice a day care of the wound with 3% hydrogen peroxide, or sodium chloride 0.9% solution was managed. After 14 days the stitches were removed. Ultrasound abdominal aortic diameter control was performed prior the surgery and two weeks after surgery in all animals. Further investigation repeated once a month throughout the postoperative period. Postoperative period lasts 6 months for 5 animals and 1 year for 4 animals. Blood pressure measurements were managed prior to the surgery and every month after the surgery in six dogs.

Ultrasound measurements were performed using PHILIPS HD11 ultrasound device. Before manipulations all animals got intramuscular Acepromazine injections 0.1 mg per body weight i.m. to facilitate investigations. During ultrasound examination dogs were placed in the right lateral recumbent position and area for measurement taking was prepared by clipping the hair behind the ribs and ventral from the longest dorsal muscles in 10 × 20 cm square expansion. Before surgery abdominal aortic diameter was measured in cross sections in one place, but after the aortic prosthesis implantation diameter in cross section of abdominal aorta was measured cranial and caudal from the prosthesis, as well cross-section diameter of vascular graft was measured. Cursors in all cases were placed in the middle of prosthesis and aortic wall. Prosthetic length, was measured longitudinally.

Blood pressure was measured using High Definition Oscillometry (HDO) device. Experimental animals were placed in a quiet, room. After five minutes when dogs were relaxed the blood pressure measurements were taken with first size dog cuff, as it is stated in the instruction enclosed. The cuff was placed on metacarpal area and on the base of tail. During manipulation this part of body on which the cuff was placed, was located on cardiac level ± 10 cm. Five measurements were taken in each investigation and average measurement was calculated.

For statistical analysis average ± standard error was calculated and a T- test for paired samples in Microsoft Excel program was used.

Results and Discussion

By summarizing the results of blood pressure and calculating the average values ± standard deviation, we obtained data that are demonstrated in Table 1. The following table shows systolic blood pressure dynamics, where the average systolic blood pressure before aortic operation is 156.88 ± 5.01 mmHg and a minimal 140.8 mmHg to a maximal 166.80 mmHg. After the operation, it ranges from an average of 162.13 ± 10.12 to 139.65 ± 6.25 mmHg, and a minimal of 117.8 mmHg to a maximal 186.0 mmHg. Normal systolic blood pressure in dogs is 120 mmHg (Reece,

Table 1

Sistolic blood pressure (sys) dynamics measured on the base of tail

Animal name	sys (mmHg) before operation ± stand. error	sys (mmHg) 1-3 days after operation ± stand. error	sys (mmHg) 1 month after operation ± stand. error	sys (mmHg) 2 month after operation ± stand. error	sys (mmHg) 3 month after operation ± stand. error	sys (mmHg) 4 month after operation ± stand. error
Brille	166.8 ± 4.77	186.00 ± 4.92	174.40 ± 2.61	156.60 ± 4.11	153.80 ± 6.64	155.60 ± 5.14
Pienene	141.50 ± 7.81	160.80 ± 1.35	156.20 ± 3.24	122.40 ± 4.80	146.20 ± 3.18	144.40 ± 2.94
Minne	163.40 ± 3.38	165.80 ± 3.46	131.20 ± 3.87	149.40 ± 1.63	131.80 ± 4.31	133.80 ± 2.35
Knīpa	140.80 ± 4.05	179.40 ± 4.00	142.00 ± 5.29	117.80 ± 0.58	126.80 ± 4.55	129.80 ± 2.63
Smukā	166.20 ± 3.67	120.00 ± 2.34	122.60 ± 1.24	117.80 ± 2.13	-	-
Bumbulīte	162.60 ± 17.15	175.20 ± 6.65	163.00 ± 1.65	169.00 ± 2.03	-	-
average ± st.error	156.88 ± 5.01	162.13 ± 10.12	148.36 ± 8.11	142.50 ± 8.15	139.65 ± 6.25	149.90 ± 5.78
minimum	140.80	120.00	122.60	117.80	126.80	129.80
maximum	166.80	186.00	174.40	169.40	153.80	155.60

1997; Garančs, 2006). However, the blood pressure is a variable rate and there is a wide variation between dog breeds. For example, the Labrador Retriever dogs systolic blood pressure is 118 ± 17 mmHg, but Greyhaunds have 149 ± 20 mm Hg. In Beagle dogs systolic blood pressure ranges 140 ± 15 mmHg, but even between individuals normal blood pressure can vary, and each animal can have its own. To determine the exact blood pressure, the measuring should be done in healthy animals on a regular basis (Egner et al., 2007).

Based on the above stated information, the average data in this study complies with the reference values. Before the surgery the systolic blood pressure in most experimental animals is higher than it is mentioned in literature, but it can be explained with an additional stress, since this kind of manipulation was performed for the first time on these animals. It should be noted that animals with high blood pressure respond nervously to strangers such as the veterinarian (Marino et al., 2011). Increased systolic pressure 1-3 days after the surgery can be explained by the additional stress since during the post operative period the animals were in another room. Of course, the surgery remains the tissue injury and there is pain response (despite the use of analgesics) to which animals also respond with increased blood pressure (Egner et al., 2007; Reece, 1997). Subsequent blood pressure measurements as shown in Table 1. display the fact that the systolic blood pressure returns to normal and complies with the reference values mentioned above. In all tables there are some empty cells because the study continues and missed measurements are not done yet.

We wanted to find out if there are significant differences between the systolic blood pressure measurements before surgery and in the postoperative period. The result by statistical calculating shows that in all cases no significant difference at significance level $\alpha = 0.05$ was found.

Diastolic blood pressure results and the average values ± standard deviation are shown in the following data Table 2. The following table shows diastolic blood pressure dynamics, where the average diastolic blood pressure before aortic operation is 82.16 ± 4.50 mmHg and a minimal 69.8 mmHg to a maximal 98 mmHg. After the operation, it ranges from an average of 88.83 ± 8.13 to 69.56 ± 6.04 mmHg and minimal from 43 mmHg to a maximal 109.6 mmHg. Normal diastolic blood pressure in dogs is mentioned to be 70 mm Hg (Reece, 1997; Garančs, 2006). Similar to systolic blood pressure the diastolic blood pressure varies between animal species too. In Beagles it is 79 ± 13 mmHg, of course in this case the animal's individual characteristics and environmental conditions should be taken into account as in measuring systolic blood pressure (Egner et al., 2007).

In the data in Table 2. and Figure 2. we can see that the diastolic and systolic blood pressures are above normal reference range before surgery and 1-3 days after surgery, but later it returns to normal and corresponds to the values described in the literature. The reasons for this phenomenon is the same as mentioned in the systolic blood pressure, because both of these pressures and their changes has the same causes.

The diastolic blood pressure results are shown graphically in Figure 2. It shows the individual animal's response to external as well as internal conditions. Animals individual diastolic blood pressure curve is a marked decline and hikes. Comparing the average values before and after surgery we found no significant differences at $\alpha = 0.05$.

Ultrasound findings are reported in Tables 3., 4., 5. Diameter of the prosthesis before the surgery and 1 and

Table 2

Diastolic blood pressure (dia) dynamics measured on the base of tail

Animal name	dia (mmHg) before operation ± stand. error	dia (mmHg) 1-3 days after operation ± stand. error	dia (mmHg) 1 month after operation ± stand. error	dia (mmHg) 2 month after operation ± stand. error	dia (mmHg) 3 month after operation ± stand. error	dia (mmHg) 4 month after operation ± stand. error
Brille	90.60 ± 3.18	85.40 ± 4.96	91.00 ± 4.91	81.40 ± 3.48	81.20 ± 3.36	90.80 ± 2.08
Pienene	74.00 ± 6.77	91.80 ± 5.03	71.40 ± 5.60	73.00 ± 5.75	79.00 ± 2.70	66.00 ± 1.48
Minne	74.80± 2.85	99.20 ± 8.73	83.00 ± 3.76	75.20 ± 4.85	70.20 ± 2.20	74.80 ± 2.05
Knīpa	69.80 ± 1.46	109.60 ± 4.29	74.00 ± 3.25	44.00 ± 0.83	69.80 ± 4.77	64.80 ± 3.87
Smukā	98.00 ± 7.16	55.40 ± 2.03	62.40 ± 0.67	62.60 ± 2.27	-	-
Bumbulīte	85.80 ± 5.59	104.00 ± 5.94	76.20 ± 2.03	82.2 0± 1.65	-	-
average ±st.error	82.16 ± 4.50	88.83 ± 8.13	76.33 ± 4.01	69.56 ± 6.04	75.05 ± 2.95	74.10 ± 5.99
minimum	69.80	55.40	62.40	43.00	69.80	64.80
maximum	98.00	109.60	91.00	82.20	81.20	90.80

2 months after the surgery do not differ significantly at $\alpha = 0.05$. The statistical comparison for future months will be done when necessary measurements from all animals will be obtained, since the animals were not operated simultaneously.

In studies available histological and immunohistochemical evaluation of vascular prostheses are described. One of the major problems after explantation of the prosthesis is intimal hyperplasia found in vascular prostheses made from various material (Ao et al., 2000; Podlaha et al., 2009). Intimal hyperplasia is the cause for narrowing of the blood vessel lumen. In this study, narrowing of the lumen is not noticed during two months, but it is also important to determine lumen changes over time. Testing of commercial polyester prostheses three months after implantation revealed neointimal hyperplasia in varying degrees, and in individual animals prosthetic lumen was closed. Authors explained it by differences in experimental animal species (Uberrueck et al., 2005). In general, polyester, polytetrafluoroethylene, poliurethane prosthesis has been used for many years and found to be suitable for large vessel transplantation. The problems begin when replanted blood vessels are < 5 mm in diameter (Rashid et al., 2004; Alcantara et al., 2005).

After removing previously implanted polytetrafluoroethylene prosthesis in humans, prosthesis capsule thickening and the internal lumen narrowing of the prosthesis were found. In some cases formations of aneurysms (Formichi, 1988) were found. In this study, the aortic diameter was measured before and after the prosthesis, because we believe that there is a possibility that lumen diameter can change morphologically in post operative period. Our results show that the aortic lumen diameter before prosthesis implantation does not differ significantly with aortic lumen diameter cranially and caudally

Table 3

Aortic prosthesis diameter dynamics

Animal name	Prosthesis diameter prior operation/ cm	Prosthesis diameter 2 weeks after operation/ cm	Prosthesis diameter 1 month after operation/ cm	Prosthesis diameter 2 month after operation/ cm	Prosthesis diameter 3 month after operation/ cm	Prosthesis diameter 4 month after operation/ cm	Prosthesis diameter 5 month after operation/ cm
Fiksā	0.500	-	0.496	0.496	0.443	0.458	0.509
Poga	0.500	-	0.397	0.365	0.364	0.376	0.443
Melnīte	0.500	-	0.467	0.481	0.475	0.474	0.548
Brille	0.500	0.500	0.491	0.479	0.520	0.533	-
Pienene	0.500	0.477	0.463	0.467	0.467	0.522	-
Minne	0.500	0.467	0.467	0.436	0.424	-	-
Knīpa	0.800	0.672	0.684	0.670	-	-	-
Smukā	0.800	0.670	0.681	0.681	-	-	-
Bumbulīte	0.800	0.684	0.687	0.687	-	-	-

Table 4

Aortic diameter dynamics cranial from prosthesis

Animal name	Aortic diameter prior operation/ cm	Aortic diameter 2 weeks after operation/ cm	Aortic diameter 1 month after operation/ cm	Aortic diameter 2 month after operation/ cm	Aortic diameter 3 month after operation/ cm	Aortic diameter 4 month after operation/ cm	Aortic diameter 5 month after operation/ cm
Fiksā	0.780	-	0.790	0.795	0.788	0.760	0.775
Poga	0.688	-	0.720	0.740	0.763	0.784	0.776
Melnīte	0.811	-	0.837	0.833	0.849	0.833	0.892
Brille	0.826	0.769	0.760	0.812	0.820	0.785	-
Pienene	0.882	0.859	0.920	0.872	0.855	0.971	-
Minne	0.753	0.786	0.827	0.812	0.780	-	-
Knīpa	0.758	0.793	0.753	0.726	-	-	-
Smukā	0.721	0.701	0.731	0.731	-	-	-
Bumbulīte	0.727	0.714	0.784	0.784	-	-	-

Table 5

Aortic diameter dynamics caudal from prosthesis

Animal name	Aortic diameter prior operation/ cm	Aortic diameter 2 weeks after operation/ cm	Aortic diameter 1 month after operation/ cm	Aortic diameter 2 month after operation/ cm	Aortic diameter 3 month after operation/ cm	Aortic diameter 4 month after operation/ cm	Aortic diameter 5 month after operation/ cm
Fiksā	0.780	-	0.760	0.769	0.760	0.744	0.780
Poga	0.688	-	0.747	0.726	0.707	0.776	0.773
Melnīte	0.811	-	0.846	0.826	0.826	0.830	0.820
Brille	0.826	0.841	0.815	0.794	0.776	0.802	-
Pienene	0.882	0.811	0.763	0.828	0.795	0.846	-
Minne	0.753	0.820	0.814	0.814	0.859	-	-
Knīpa	0.758	0.694	0.688	0.678	-	-	-
Smukā	0.721	0.811	0.727	0.727	-	-	-
Bumbulīte	0.727	0.838	0.819	0.819	-	-	-

from prostheses one and two months after the operation at $\alpha = 0.05$. Mentioned aortic diameter changes are shown in Tables 4. and 5.

Conclusions

1. The study shows that the innovative aortic prostheses do not change in diameter after two month from the surgery.
2. Surgery like abdominal aorta transplantation does not cause significant variations in blood pressure during post operative period.
3. To find out later reactions to synthetic vascular graft, further observations are recommended.

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PHYSICAL MODEL OF TRACTOR IMPLEMENT

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Abstract
In order to perceive the pressure oscillation in the hydraulic hitch-system of the tractor and oscillation of the whole tractor aggregate at different loads on the hitch-system, a physical model is used. Changing the position of weight on physical implement boom, different moments of inertia were obtained and the appropriate load on the hydraulic hitch-system hydro cylinder. Results of driving experiments on the artificial roughness road present the maximum pressure peak of 220 bar in the tractor hydraulic system when weight was placed on further position, and the driving speed was 8 km h^{-1} and tyre pressure was 1.2 bar. At the driving speed of 11.2 km h^{-1} and with the same tyre pressure, the hydraulic system pressures reached up to 212 bar. On the weight on the middle position of boom, pressure in the hydraulic hitch-system reached 172 bar at the driving speed of 7.8 km h^{-1} and tyre pressure of 1.2 bar, but at the driving speed of 11.2 km h^{-1} and at the same tyre pressure, the hydraulic system pressures reached 165 bar. If the weight was placed on nearest position, pressure in the hydraulic hitch-system reached only 85 bar at the driving speed of 7.8 km h^{-1} and tyre pressure - 1.2 bar, but at the driving speed of 11.2 km h^{-1} and at the same tyre pressure, it was 98 bar. The investigation of the physical tractor-implement model allows the determination of the conformity of the physical model with real harrow implement.
Key words: physical model, pressure peak, oscillation, physical implement.

Introduction

During tractor movement, with the working equipment (plough, harrow) attached to the hitch-system, over rough road surfaces oscillation of machine takes place. These oscillations are a reason of pressure pulsations in hydraulic hitch-system. The tractor hydraulic system is characterized by a pressure pulse effect, which reduces service life of the hydraulic system components, especially the lifetime of hydraulic hoses.

The previous experiments of (Laceklis-Bertmanis et al., 2010) present results of pressure oscillation investigation in hydraulic hitch-system of tractor Claas Ares ATX 557 with a harrow implement during the motion around an artificial roughness road. During experiments pressure oscillation at the different driving speed, tyre pressure and hitch-system oscillation damping (turned on/off) were investigated.

The pressure pulses in hydraulic hitch-system mostly depend on the mounted soil cultivating aggregate weight and mass moment of inertia. Different implements attached to the tractor's hitch-system cause different degrees of pressure pulsations. Changing the position of weight on implement physical model, the different moment of inertia was obtained and the appropriate load on the hydraulic hitch-system hydro cylinder. Therefore, the physical implement model for evaluation of the simplified mathematical model would be used. The main purpose of our experiments is to determine the physical model conformity to the real harrow implement.

Materials and Methods

The Experiments were performed in the NP Jelgava Business Park. In order to study the dynamic oscillation of the tractor hydraulic hitch-system, the physical implement model was used. Before measurements, the tractor was fitted with physical implement boom (Fig. 1). The physical implement boom consists of two parallel 2.5-meter-long mounted rolled metal consoles (120x120x6.3), connected together with two screws and a triangle knot. Physical implement was connected to the tractor's three-point hitch-system with knuckles. For hitch-system physical implement loading tractor balance weight (1400 kg) was used. The weight consists of two parts – 900 kg, and 500 kg. The balance weight corresponds to the implement of the previous experiments LMKEN short disk harrow and LEMKEN rubber rings roller (LEMKEN GmbH & Co.KG, 2012). In the experiments, the weight was placed in three positions (A, B, and C (Fig. 1)) and fixed with lanyard.

For data acquisition and processing, software offered by company Pico Technology was used. This software is provided for devices manufactured by different companies (Kaķītis, 2008). Using a universal data collection and processing device PicoLog, the hydraulic pressure oscillation in the tractor's hydraulic hitch-system were measured. In addition, the current tractor hydraulic hitch-system was equipped with displacement transmitter DTCH1000C (Fig. 2).

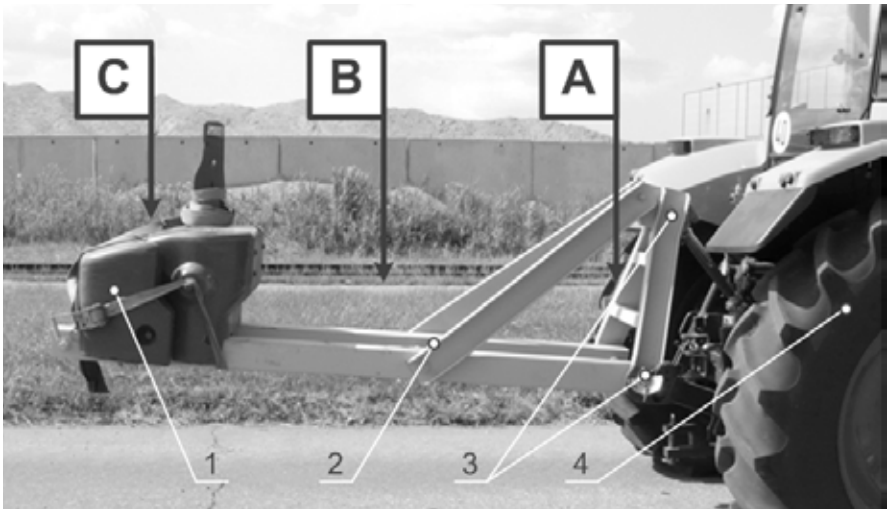

Figure 1. Physical Implement and Weight Position on Three Point Hitch-system of Tractor:
1 – weight; 2 – physical implement; 3 – three-point hydraulic hitch-system; 4 – traktor Claas Ares 557 ATX;
A, B and C – position of weight.

Pressure pulses and hydrocylinder displacement were measured with the pressure and displacement transmitters attached to the tractor's hydraulic hitch-system hydrocylinder. Displacement transmitter (RDP Group, 2012) was mounted on the hitch-system's hydrocylinder for measuring the displacement of hydrocylinder at different pressure peaks in the hydraulic system and the driving speed. One side of the displacement transmitter was attached to stationary position of the hydrocylinder, and another side - to the moving part. In order to protect the displacement transmitter from damage due to short range (±12.5 mm), one side was fitted with a magnetic clamp.

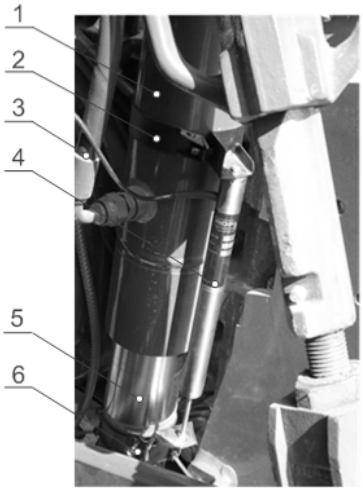

Figure 2. Position of the Diplacement Transmitter DTCH1000C on the Hidraulic Cylinder:
1 – hydrocilinder; 2 – mounting bracket; 3 – hose of oil supply; 4 – displacement transmitter;
5 – moving part of hydrocylinder; 6 –mounting bracket of magnet-type.

Using a universal data collection and processing device PicoLog, the hydraulic pressure hydrocylinder displacement oscillation in the tractor's hydraulic hitch-system was measured. The scheme of PicoLog gauge and transmitters connection is shown in Fig. 3. Artificial test road roughness, weight position, and the tractor's driving speed cause oscillation in the hydraulic hitch-system and the whole vehicle with different frequencies according to the driving speed. System oscillations were transferred to the hydrocylinder of the hydraulic system. Pressure pulses were immediate for a short time. A pressure and displacement converter receives and converts this process in a proportional electrical signal. The control unit was fed from the common network with voltage of 12 V. In the next step modified data were transmitted to the laptop. For data recording and processing, subprogram PLW Recorder is used. The acquired data values were saved in *.txt format for further processing. Data were processed by Microsoft Excel data analysis software.

Air pressures in tyres were selected from the tractor's manual, which provides different tyre pressures for different operating conditions. For example, for cultivation the 0.8 - 0.9 bar tyre pressure is provided, but for transportation it is 1.1 - 1.2 bar (Claas, 2007). Each measurement was repeated three times at certain gears and engine revolutions. From the acquired data, only the maximum values were taken into account, which characterized the greatest pressure peaks of the tractor's hydraulic hitch-system.

According to literature (Веденяпин, 1965), each experiment was repeated three times. Of each experiment, the nine maximum pressure value from resultant curves were used in further calculations, which is characterized by pressure changes in the

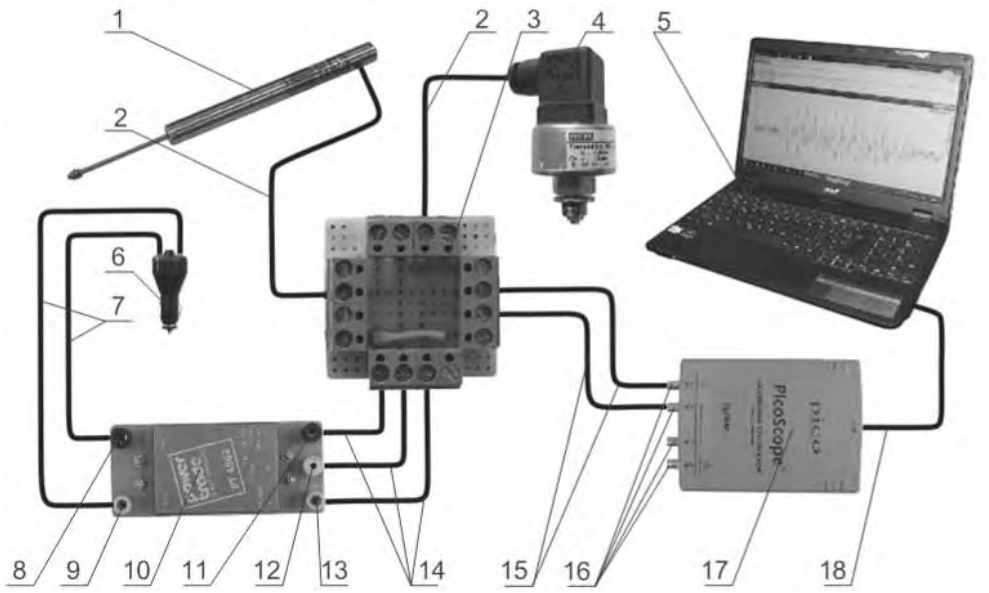

Figure 3. Installation Scheme of Transmitter and Measuring Device to PicoScope:
1 – diplacement transmitter *DTCH1000C*; 2 – data cable; 3 – alignement of transmitter and data; 4 – pressure transmtter ECO-1; 5 – computer with PicoTehnology software; 6 – plug box; 7 – cable of voltage; 8 – windrow „ – ”; 9 – windrow „ + ”; 10 – power trade (PT 4862); 11 – windrow of outlet with value „ – ”; 12 – ground bed; 13 windrow of outlet with value „ + ” ; 14 – cable of suppply voltage; 15 – data cable; 16 – inlet chanel BNC; 17 –PicoScope; 18 – data cable USB 2.0.

tractor's hydraulic hitch-system across the road roughness at different driving speeds. Average values were calculated from at least 27 values, and correlation between the series data points was at least 0.95, i.e., above 95%. After that, curves p=f (v) were constructed.

Results and Discussion

While changing the tractor hydraulic hitch-system oscillation damping position (switch on or off) that can be established on the tractor instrument panel, driving speed from 3 – 14 km h^{-1} and weight position on implement, various hydraulic hitch-system oscillation characteristics, which create the pressure pulse in hydraulic system were acquired.

Fig. 4 and Fig. 5 demonstrate how hydraulic pressure in the hydraulic hitch-system changes at all three weight positions and at tyre pressures 0.8 bar and 1.2 bar, and at driving speeds from 3 to 13.8 km h^{-1}. Initial operation pressures in the hydraulic hitch-system depend on weight position on the physical implement boom. In position A, initial pressure was 67 – 70 bar, but in position B it reached up to 120 – 125 bar and in position C up to 145 – 150 bar. The lower oscillation amplitude can be achieved if weight is placed near the hitch-system.

Fig. 4 describes changes in pressure at the tyre pressure of 1.2 bar and weight position A, B and C. If weight was placed in position C and the hydraulic hitch-system oscillation damping system was not used, then maximum average pressure of the hydraulic hitch-system reached 220 bar at the driving speed of 7.8 km h^{-1}, but when the hydraulic hitch-system oscillation damping system was used, the pressure pulse at the same driving speed reduced to 185 bar. If weight was placed on position B of boom and the hydraulic hitch-system oscillation damping system was not used, then maximum average pressure of the hydraulic hitch-system reached 170 bar at the driving speed of 7.8 km h^{-1} and tyre pressure 1.2 bar, but when the hydraulic hitch-system oscillation damping system was used, the pressure pulse at the driving speed of 11.2 km h^{-1} reduced to 165 bar. If weight was placed in position A of boom and the hydraulic hitch-system oscillation damping system was or was not used, the maximum average pressure of the hydraulic hitch-system reached 100 bar at the driving speed 11.2 km h^{-1} and tyre pressure 1.2 bar, but at the driving speed 7.8 km h^{-1} and at the same tyre pressure the hydraulic system pressures reached up to 88 bar.

Fig. 5 demonstrates changes in pressure at the tyre pressure of 0.8 bar and weight position C. If the hydraulic hitch-system oscillation damping system was not used, then maximum average pressure of the hydraulic hitch-system reached 210 bar at the driving speed of 6.4 km h^{-1}, but when the hydraulic hitch-system oscillation damping system was used, the pressure pulse at the same driving speed reduced to 185 bar. At the driving speed of 11.2 km h^{-1} and with the same tyre pressure, the hydraulic system pressures

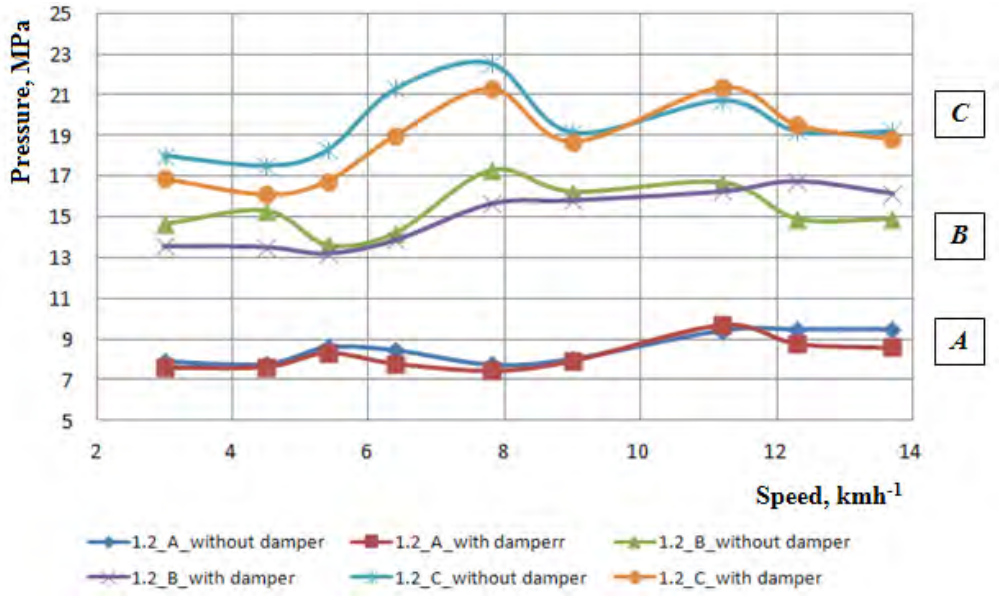

Figure 4. Pressure Peak in Tractor Hydraulic Hitch-System at Tyre Pressure 0.8 bar and Different Weight Positions A, B and C.

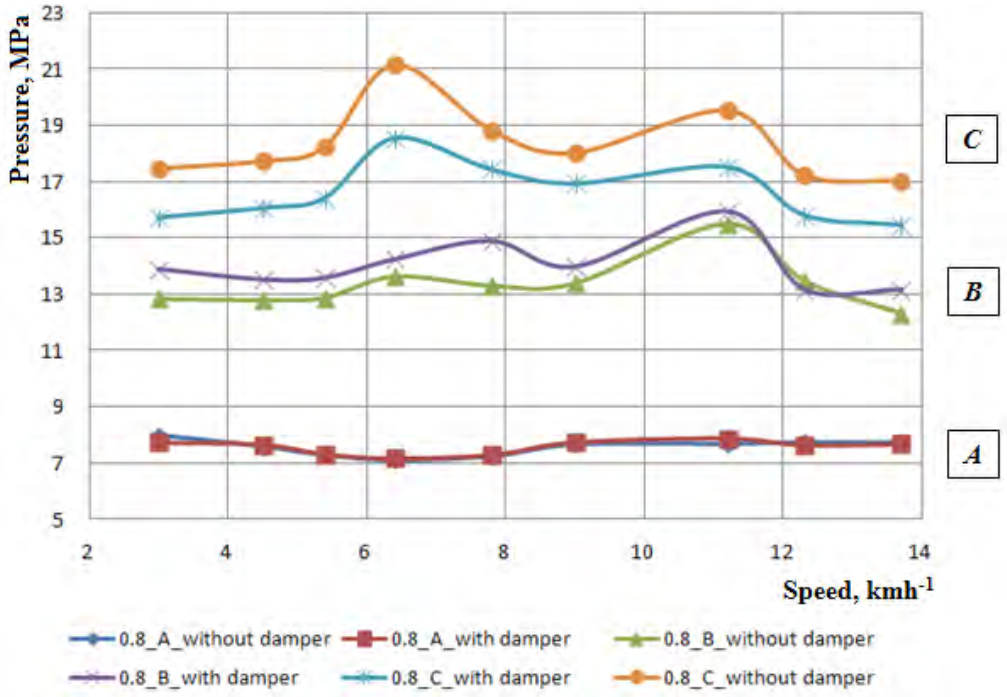

Figure 5. Pressure Peak in Tractor Hydraulic Hitch-System at Tyre Pressure 0.8 bar and Different Weight Positions A, B and C.

reached up to 195 bar. On the weight on position B of boom pressure in the hydraulic hitch-system reached 149 bar at the driving speed of 7.8 km h^{-1} and tyre pressure 0.8 bar, but at the driving speed of 11.2 km h^{-1} and at the same tyre pressure the hydraulic system pressures increased to 160 bar. If weight was placed in position A, the pressure in hydraulic hitch-system in the whole driving speed diapason reached approximately 70 – 80 bar.

Reducing the tractor tyre pressure from 1.2 to 0.8 bar, placed weight in position C and if the hydraulic hitch-system oscillation damping system was not used, then maximum average pressure of the hydraulic hitch-system decreased for 8.7% when the hydraulic hitch-system oscillation damping system was used, the pressure pulse at the same parameter decreased for 11.7%. Placed weight in position B and if the hydraulic hitch-system oscillation damping system

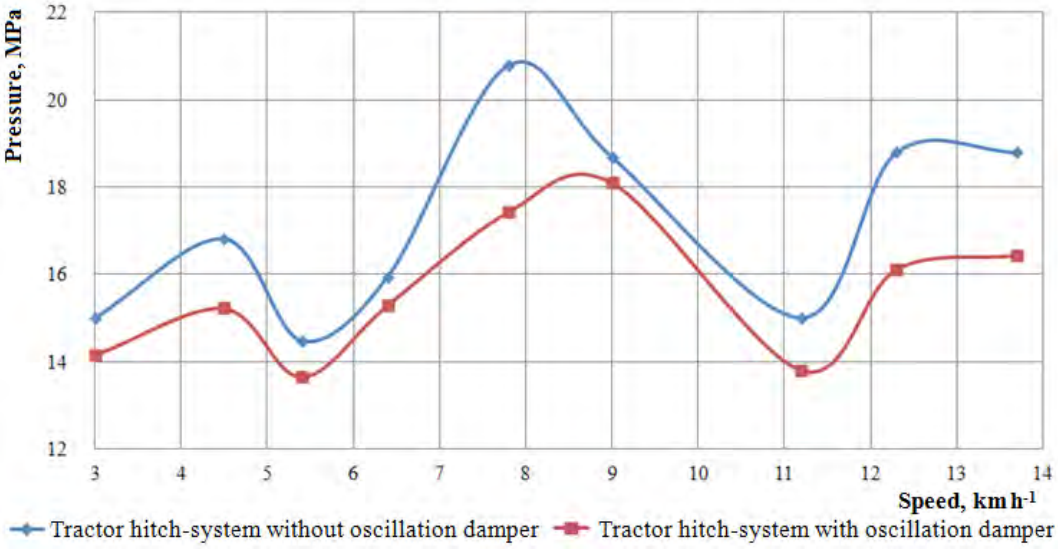

Figure 6. Tractor Hitch-System Oscillation at Different Speeds and tyre pressure of 1.2 bar.

was not used, then maximum average pressure of the hydraulic hitch-system decrease for 3% when the hydraulic hitch-system oscillation damping system is used, the pressure pulse at the same parameter decrease for 6.07%. Placed weight in position A of boom at the same parameters maximum average pressure of the hydraulic hitch-system decrease for 15.78%. The average displacement of the hydrocylinder in experiments amounted to 0.5 – 5 mm depending on the pressure in the hydraulic system hydrocylinder and weight position on the implement model.

Changing the position of weight on the physical implement boom, different moments of inertia and an appropriate load on the hydraulic hitch-system hydro cylinder was obtained. A lower oscillation amplitude can be achieved if weight is placed near the hitch-system.

Fig.6 shows maximal average values of the hitch-system pressure of the driving tractor with attached LMKEN short disk harrow and LEMKEN rubber rings roller (LEMKEN GmbH & Co.KG, 2012) at different motion speeds from previous investigations. Under way with tractor along artificial roughness test road, the air pressure in tyres of 1.2 bar was used.

Figures 5 and 6 suggest that the physical tractor implement model let it to arrange in correspondence with real - LMKEN short disk harrow and LEMKEN rubber rings roller, when tractor with implement oscillations on the artificial roughness test road is investigated.

For pressure peak values in graphs uncertainly was determined and for confidence level 0.95 it value do not exceed ± 0.194 MPa.

Conclusions

1. Decreasing the hydraulic hitch-system pressure impulse range is substantial for extending of service life of tractor metal constructions because a high leap of pressure in the hydraulic system corresponds to high forces and tension values in metal constructions.
2. The tractor implement physical model let it to arrange in correspondence with real - LEMKEN short disk harrow with rubber rings roller, when vertical oscillations on the artificial roughness test road is investigated.
3. For physical implement model, reducing the tractor tyre pressure from 1.2 to 0.8 bar, adjusting weight in position C and if the hydraulic hitch-system oscillation damping system was not used, then maximum average pressure of the hydraulic hitch-system decreased for 8.7%. When the hydraulic hitch-system oscillation damping system was used, the pressure pulse at the same parameter decreased for 11.7%.
4. Adjusting weight in position B and if the hydraulic hitch-system oscillation damping system was not used, then maximum average pressure of the hydraulic hitch-system decreased for 3%.W the hydraulic hitch-system oscillation damping system was used, the pressure pulse at the same parameters decreased for 6.07%.
5. Adjusting weight in position A of boom at the same parameters, the maximum average pressure of the hydraulic hitch-system decreased to 15.78%.

Acknowledgements

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SOLID FUEL BOILER AUTOMATION FOR BRIQUETTE USE

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Abstract
Nowadays all engineering technologies are based on the automation, but still there are some processes that are not easy to automate. One of them is the heating process using solid fuel boilers. The paper deals with automated double-link air flow control of the combustion process. The research target was to find more effective solutions of furnace air flow regulation to improve the combustion process efficiency. The research object was a 56 kW wood briquettes water heating boiler. The results of the experiment research show a higher efficiency of the combustion process with double-link air flow PI control using motor valves.
Key words: wood briquettes, furnace, double link air flow, motor-valves, temperature, PI control.

Introduction

Nowadays there is a tendency to use a renewable energy source for small, medium and large civil heating systems (Aršaņica et al., 2009). Today many private houses still mostly use the solid fuel boilers due to the reduced costs for family during the heating period. The problem lies in the fact that in many cases the non-automated boilers are used, what works with low efficiency. The most important issue for not using the new and automated or semi-automated solid fuel boilers is investments. At the beginning the investments are higher than a family can afford. At that point of view it is very important to find the main factors what can make the existing boilers more effective and user friendly.

The research target was to find more effective solutions of furnace air flow regulation to increase the combustion process efficiency. To achieve the target, very important is to research the burning process control methods and find a new way of furnace control. Make research with new control method. Improve the new control method efficiency on working process.

The first idea to consider is the automated double link control of the air flow in solid biofuel combustion process (Roderick et al., 2009) meaning the use of the motorized valves for the air flow regulation in furnace. That can give a chance to make more rapid control of flue gasses temperature to receive more effective burning process control. Boiler efficiency can be increased by using the furnace flue gasses temperature control. Every type of the furnace and the chimney has its own most effective flue gasses temperature Θ_F. It means that not every boiler can work as effective as other boiler with the same flue gasses temperature Θ_F. Flue gasses temperature above the needed working temperature causes additional direct heat losses (Blumberga et al., 2009).

New types of sensors and components should be implemented for automation of furnace air flow regulation. The thermocouples are the most popular devices to read flue gasses temperature in the air outlet pipe. Furnace air flow is regulated by using motor valves.

The results of the research show the efficiency of the double-link air flow control system. Efficient control of the burning process decreases emissions and heat losses (Aršaniča et al., 2008). To get correct results, all experiments were done according to the requirements of experimental research methods where every test should be done 3 times and the process start conditions for the tests should be the same during the whole experiment time.

Materials and Methods

The research object was a hot-water boiler for private house heating, using wood briquettes. The engineering solution was established in a private house. The solution was made taking into account all safety requirements for the heating systems.

The tests setup includes a controller unit for logic and data acquisition PM783F with analog input module AX722F and digital input/relay outputs module DX723F. The water and air temperature was measured by a PT100 standard probe. For flue gasses valve motorisation a Tyco produced actuator was used with added electrical DC (Direct current) potentiometer for feedback about real position on signal 4-20 mA types. For the air inlet valve, a standard bipolar stepper motor. For data logging standard trend displays were used with acquisition function blocks in PLC (Programming Logical Controller). This allowed the data not to be lost during the period of connection fault between the PLC and PC. Monitoring setup is shown in Figure 1.

Process monitoring data:

- Θ_F – flue gasses temperature, °C;
- Θ_S – supply water temperature, °C;
- C_{AI} – flue gasses motor-valve position, %;
- C_{AO} – inlet air motor-valve position, %.

The start conditions were as follows: the flue gasses temperature – 90 °C, supply water temperature – 75 °C, return water temperature – 67 °C, boiler room

temperature – 28 °C, ambient temperature – -3 °C, biomass weight – 3630 g.

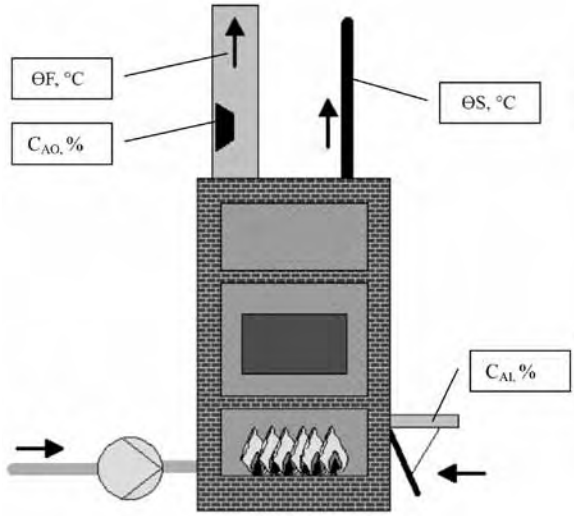

Figure 1. Tests monitoring setup.

To get correct result of each experiment, the same start conditions were established for each test. The start conditions were as follows:

- the boiler was cleaned after each experiment;
- the inlet air temperature difference was +/- 5% of average temperature in the boiler room;
- the briquette temperature difference before putting in the furnace was +/- 5% around average temperature in the boiler room;
- ambient temperature was +/- 5% of average temperature of the previous experiments;
- in each experiment the same amount of briquettes is added and each portion was weighed, the weight did not exceed +/- 2% around average weight of the experimented portions.

The experiments consisted of 6 separate series. To receive more accurate results, all series was repeated 3 times. Variables established for all experiments was motor-valves positions (C_{AO} – for outlet air flow valve, C_{AI} – for inlet air flow valve) as a percentage of full open state:

1. C_{AO} = 100%, C_{AI} = 100%, (Full opened inlet air and flue gasses valves);
2. C_{AO} = 50%, C_{AI} = 50%;
3. C_{AO} = 50%, C_{AI} = 5%;
4. C_{AO} = automatically regulated (from 15% to 100%), C_{AI} = 5% (const.). The setpoint of Θ_F is 140 °C;
5. C_{AO} = 50% (const.), C_{AI} = regulated automatically (from 0% to 100%). The setpoint of Θ_C is 140 °C;
6. C_{AO} = regulated automatically (from 15% to 100%), C_{AI} = regulated automatically (from 0% to 100%). The setpoint of Θ_C for this task was 140 °C.

The logged data from each experiment were entered into MS Excel software and analysed in a graphs form. Data statistical analysis was performed to receive information about the processes. To receive more accurate information, each test was done 3 times.

Control algorithms and block diagram

Automatic control block diagram (Fig. 2) for motor-valve positions using setpoint temperature and actual temperature Θ_F gives information about the structure of the process. Regarding the components in the diagram, the process controller unit was Proportion–Integral controller (Šnīders, 2008). Other components are motorized actuator motor valves with a linear work line controlling the air flow through the boiler. Both motor valves work with the same setpoint and control the air flow simultaneously. The hot water boiler and temperature transmitter are first-order inertial objects.

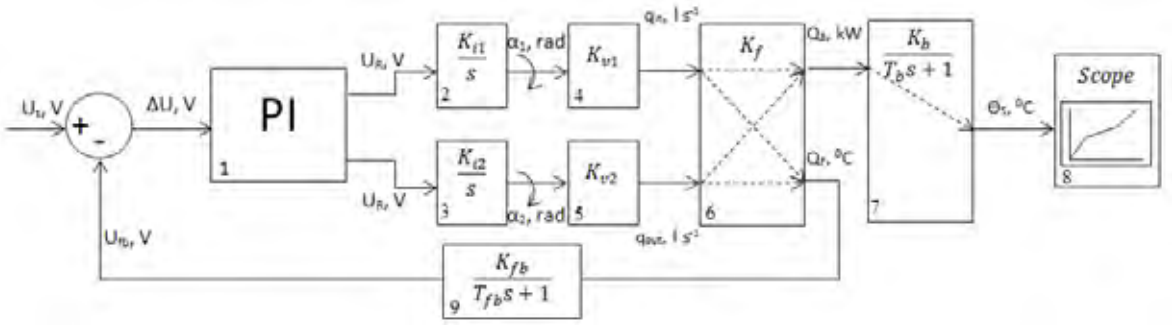

Figure 2. Block diagram of an automatic control system: 1) PI controller; 2) electric motor for furnace flue gasses valve; 3) electric motor for air inlet valve; 4) furnace flue gasses control valve; 5) air inlet control valve; 6) furnace; 7) boiler; 8) monitoring unit; 9) flue gasses measuring unit.

Transfer function of the PI (Proportion-Integral) controller:

$$W_{PI}(s) = \frac{U_R(s)}{\Delta U(s)} = K_P + \frac{1}{T_i s}, \quad (1)$$

where T_i – integral time constant, min;
K_p – transfer coefficient (gain) of the proportional link;
$U_R(s)$ – Laplace transform of PI output variable, V;
$\Delta U(s)$ – Laplace transform of PI input variable, V;
s – Laplace variable, min^{-1}.

Transfer functions of motor-valves:

$$W_{in}(s) = \frac{k_{v1}k_{i1}}{s}, W_{out}(s) = \frac{k_{v2}k_{i2}}{s}, \quad (2)$$

where k_{v1} – transfer coefficient of inlet valve, l s^{-1};
k_{i1} – coefficient of inlet valve angular speed, V^{-1} s^{-1};
k_{v2} – transfer coefficient of inlet valve, l s^{-1};
k_{i2} – coefficient of inlet valve angular speed, V^{-1} s^{-1}.

Transfer function of the boiler for transient process simulation:

$$W_h(s) = \frac{\Theta_S(s)}{Q_B(s)} = \frac{K_b}{T_b s + 1}, \quad (3)$$

where $\Theta_S(s)$ – Laplace transform of boiler output variable, °C;
$Q_B(s)$ – Laplace transform of boiler input variable, kW;
T_b – time constant of the boiler, min;
K_b – transfer coefficient of the boiler, °C kW^{-1}.

Transfer function of the temperature transmitter for transient process simulation:

$$W_{fb}(s) = \frac{U_{fb}(s)}{\Theta_F(s)} = \frac{K_{fb}}{T_{fb} s + 1}, \quad (4)$$

where $U_{fb}(s)$ – Laplace transform of temperature transmitter output voltage , V;
$\Theta_F(s)$ – Laplace transform of furnace output air temperature, °C;
T_{fb} – time constant of temperature transmitter, min;
K_{fb} – transfer coefficient of temperature transmitter, V $°C^{-1}$.

The transfer coefficient of the furnace:

$$k_f = \frac{Q_B}{q_{in}}, \quad (5)$$

where Q_B – boiler input heat power, kW;
q_{in} – furnace input air flow, l s^{-1}.

Results and Discussion

The humidity of the wood briquette is 10.6%. The optimal briquette humidity for burning is 10 – 18% (Križan et al., 2009).

First experiment

In the first experiment, the main target was to determine the burning process time in the stationary position where the position of both motor valves were 100% – they were fully opened. Burning process time starts from the briquette load in the furnace till the end condition achieved. Period end conditions were the same process data values as they were in the start conditions. In the process graph, temperatures Θ_S and Θ_F (Fig. 3) were obtained as an average values of three retries of one experiment. The whole process time was 1 hour 8 minutes (01:08:46).

The Θ_{F1} temperature rose up to 169.0 °C and within 30 minutes reached the process end temperature 85.0 °C. The supply water temperature Θ_{S1} remained in values of 72.2 ± 3.0 °C. Motor valve positions during the whole process time were opened for 100%. The furnace flue gasses temperature was high and losses from the flue gasses were considerable. The flue gasses average temperature Θ_{F1} = 128.2 ± 35.0 °C (Fig. 3).

Second experiment

In the second experiment, the main target was to determine the time of the burning process at the stationary position where the position of both motor valves were 50%. The whole process time was measured 1 hour 18 minutes (01:18:04). The flue gasses temperature Θ_{F2} rose up to 196.0 °C in 21 minutes, remained the temperature for 18 minutes, and within 40 minutes reached end temperature of the process - 85.0 °C. The supply water temperature Θ_{S2} remained of the value of 72.6 ± 4.0 °C. The motor valve during the whole process time was opened for 50%. The average temperature of flue gasses Θ_F = 152.7 ± 44.0 °C.

Third experiment

The third experiment showed the burning process with motor valve stationary position where inlet motor-valve was closed (5%) and outlet motor-valve was opened for 50%. The whole process time was 1 hour 16 minutes (01:16:51). The flue gasses temperature Θ_{F3} rose up to 183.0 °C in 41 minute, within 35 minutes reached end temperature of the process - 85.0 °C. The supply water temperature Θ_{S3} varied within71.3 ± 4.0 °C. The average temperature of flue gasses Θ_{F3} = 139.87 ± 37.0 °C.

Fourth experiment

In the fourth experiment, the main target was to observe the burning process time in the automatic

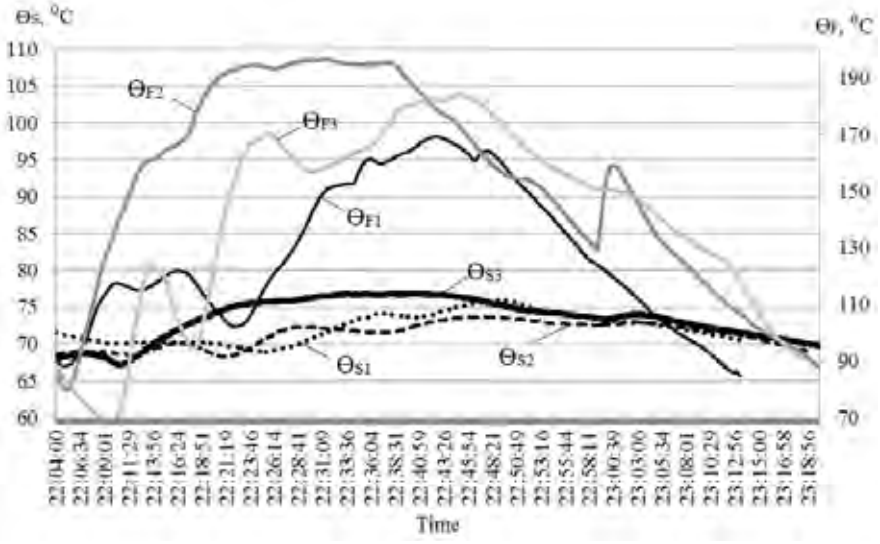

Figure 3. Results of experiments No. 1, No. 2, and No. 3: Θ_S – supply water temperature; Θ_F – outgoing air temperature.

control process where the furnace flue gasses motor valve was controlled by the controller with analog signal by the setpoint of the flue gasses temperature, but air inlet motor-valve was closed (5%). The controlled temperature value was 140.0 °C. The whole process time was 1 hour 38 minutes (01:38:12). The flue gasses temperature Θ_{F4} max rose up to 168.0 °C, and the process controller held the temperature at the value of 140.8 °C. The supply water temperature Θ_{S4} remained at the values of 72.3 ± 2 °C. The flue gasses average temperature Θ_{F4} was 140.8 ± 12 °C. The furnace flue gasses temperature is controlled by the process controller, and heat losses of the outgoing air are considerably less (Figure 4).

Fifth experiment

In the fifth experiment, the target was to explore the burning process time in the automatic control process where the air inlet motor valve was controlled by the controller with an analog signal by the setpoint of the outgoing air temperature, but furnace flue gasses motor-valve was closed (50%). The controlled temperature value was 140.0 °C. The whole process time was 1hour 33 minutes (01:33:58). The maximum flue gasses temperature increased up to 194.0 °C, and held the outgoing air temperature in average value - 141.2 °C. The supply water temperature Θ_{S5} remained in values - 73.3 ± 2.5 °C. The furnace flue gasses temperature was controlled by the process controller

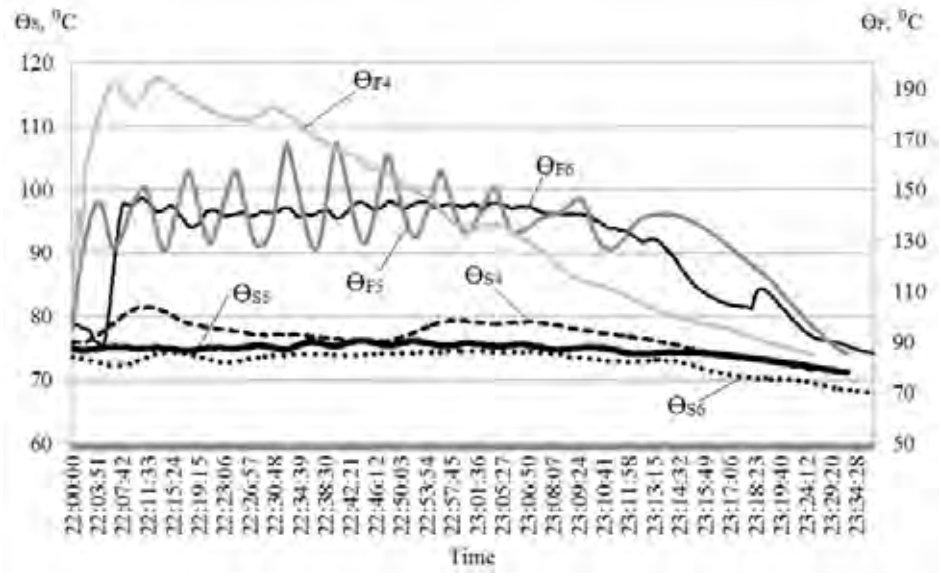

Figure 4. Results of experiments No. 4, No. 5, and No. 6: Θ_S – supply water temperature; Θ_F – outgoing air temperature.

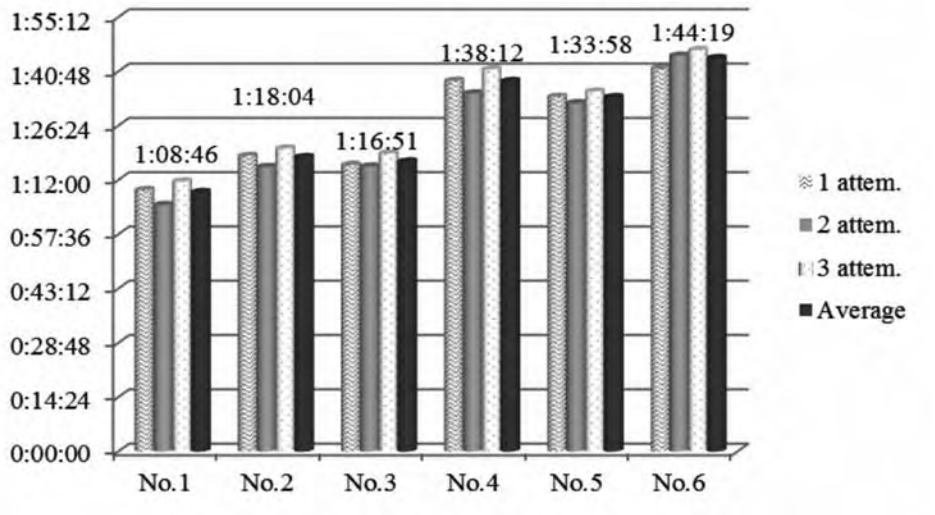

Figure 5. Process table with each test time and average test time.

controlling the air inlet motor valve. The flue gasses average temperature Θ_{F5} was 143.8 ± 26.0 °C.

Sixth experiment

In the sixth experiment, the target was to determine the burning process time in the automatic control process where the air inlet and flue gasses motor-valves are controlled by the controller with an analog signal by the setpoint of the flue gasses temperature. The controlled temperature value was 140.0 °C. The burning process time was 1 hour 44 minutes (01:44:19). The flue gasses temperature Θ_{F6} rose up to 146.0 °C, holding the temperature in average value of 141.9 °C. The supply water temperature Θ_{S6} remained in values of 71.3±3.0 °C. The flue gasses average temperature Θ_{F6} was 141.9± 5.0 °C. The furnace flue gasses temperature was controlled by the process controller and the losses of the outgoing air were considerably less. Result of sixth experiment was the best of others ones.

Time comparison

When examining the final results regarding individual burning process times, it was very clear that the processes equipped with an automatic control indicated better results (Fig. 5) and the time is even 50% longer in comparison with the results of the first experiment and is 35% longer than second and third experiment process time. Process time showed how long the combustion process is active. The longer the combustion time, the more efficient the process. Three things are important for the combustion process: 1) decreasing of heat losses; 2) elimination of gas emission; 3) holding the supply water temperature at the needed values. The longer the burning time, the slower the burning of wood briquettes and the lower level of flue gasses emission (Barmina et al., 2007). If the combustion process is longer the flue gasses temperature is lower and the heat loses are lower regarding the flue gasses temperature. It is important to hold also the flue gasses temperature at the needed level to keep the necessary supply water temperature (Barmina et al., 2011).

Control of air flow in double link motor valves control mode gave 6% better results than only outlet air motor valve automatic control and 11% better results than only inlet air motor-valve automatic control. The graph with automatic mode times shows only data with temperature setpoint 140 °C. The most effective outgoing air temperature was not fully explored, but even if it was not the most effective for that type of boiler, the results assure the efficiency of the double link control of the outgoing air temperature to be higher than using other burning methods.

Conclusions

1. The results show that using double link air flow control system with simultaneous control of the inlet air and outlet air valves, the burning process time increases by 35% in comparison with static motor-valves positions. Controlling of air flow in double link motor-valves control mode gave 6% better results than only outlet air motor-valve automatic control and 11% better results than only inlet air motor-valve automatic control. A longer burning process gives higher efficiency.
2. The automatic control process PI controller settings cannot be used the same for all control modes. The transfer coefficient of the proportion link and the time constant setting of the integral link need auto tuning for every control mode change.

Acknowledgement

The author is very grateful for support to thesis supervisor Andris Šnīders.

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INVESTMENT COSTS OPTIMIZATION OF MULTI-ROBOT SYSTEM USING GENETIC ALGORITHM

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Abstract
Forethought deployment of an industrial production system is a significant step towards improving economic benefit of an industrial company. The author proposes the procedure for finding an optimal specification of multirobot system, which considers it on the level of components of the robotic system. Components are grouped into mobile or stationary units of the system. A set of agents is considered as a solution for particular mission, it defines a specification of a heterogeneous multi-robot system. The paper presents the concept of the optimization procedure and describes the implementation of investment costs optimization step, which uses genetic algorithm.
Key words: multi-robot system, optimization, genetic algorithm.

Introduction

Last decade is peculiar by a growth of applications of robotic system in various fields. Robotic systems are being for such purposes as production process automation (Jammes and Smit, 2005) and intelligent manufacturing (Almeida, 2011). An increasing number of applications is reported in such domains as medicine (Davies, 2010), elderly care (Hansen et al., 2010) or daily life as household companions (Parlitz et al., 2007). An increasing interest in the research of heterogeneous multi-robot systems is caused by variety of their advances in comparison with homogeneous robotic systems (Shen and Norrie, 1999; Bi et al., 2008). Because of expected increase of the demand for heterogeneous robotic systems, the task of design and development of an optimal system becomes important (Kiener and Stryk, 2010). The economic benefit of a company depends on the effectiveness of robotic production system.

Active researches within robotics field primarily focus on novel methods for such aspects as intelligent control (Nouyan et al., 2009), world modeling (Coltin et al., 2010), communication (Mathews et al., 2011), etc., but the configuration of the system is not considered among the improvable parameters and is usually predefined or selected intuitively. However, the performance of the whole system is strongly influenced by characteristics and functionalities of the individual robots (Levi and Kernbach, 2010). The author proposes a specification optimization procedure, which purpose is to overcome aforementioned drawbacks.

The paper describes a concept of proposed optimization procedure and presents investment costs estimation model for heterogeneous robotic system which is used for evaluation of solution candidates. The implementation of evaluation step of specification optimization procedure is described in details. It is based on genetic algorithm (Holland, 1975) and uses costs estimation model as an objective function.

Materials and Methods

The specification of a multi-robot system defines types of agents (classes), their functions as well as a number of instances of each class of agents in the system. Optimal specification of a multi-robot system is such a configuration of the system that maximizes the objective function. The author uses investment costs as a primary evaluation criterion for current research. Investment costs define all expenses required to design, implement and deploy a multi-robot system from the scratch into production environment and do not include expenses related to the operation of the system. A usual business requirement is to reduce investment costs, therefore an inversed optimization objective function is used.

According to the developed specification optimization procedure, several concepts have been defined (see Figure 1). *Component* stands for a definition of function of the robotic system. Components are grouped together in order to form an *agent* (rather, mobile robot or a stationary unit). *Solution* is a specification of a heterogeneous robotic system, it defines types of agents and a number of their instances used to carry out a mission. A number of rules is applied before considering any combination of agents as a solution.

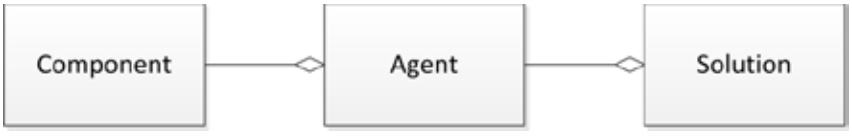

Figure 1. Conceptual model of the solution.

Figure 2. Specification optimization procedure.

Specification optimization procedure specifies three consecutive steps (see Figure 2). First of all, business requirements are defined by an industrialist, and then the optimization objective function is developed. Finally, heuristic algorithms are used to find the fittest solution. Detailed description of steps of the procedure is provided in (Komasilovs and Stalidzans, 2012).

An investment costs estimation model is developed with the aim to perform fast evaluation of a large number of solution candidates. According to the concept of specification optimization procedure, the mission for a multi-robot system is defined using a list of components. Investment costs model assumes that components have additional properties, which are related to the costs of a particular component.

Investment costs could be divided into several positions described below. The proposed model imply that investment costs of the whole system (Q_{inv}) equal to the sum of investment expenses for agents (Q_{inv_agent}). Expenses for system design are applied as an additional fraction (c_{sys_design} coefficient):

$$Q_{inv} = (1 + c_{sys\_design}) \times \sum Q_{inv\_agent} \quad (1)$$

Investment costs of agent (Q_{inv_agent}) consist of design costs of a particular type of agents (Q_{design}) and production expenses (Q_{prod}) of all instances of a particular class of agents:

$$Q_{inv\_agent} = Q_{design} + Q_{prod} \times N_{inst} \quad (2)$$

Design costs (Q_{design}) depend on the number of components (N_{comp}) involved into design of a particular class of agents, and the author assume that it grows exponentially. Coefficients (c_{lin} and c_{exp}) are used to tune the growth dynamics according to real prices of the design:

$$Q_{design} = c_{lin} \times exp\,(c_{exp} \times N_{comp}) \quad (3)$$

Production costs of an agent (Q_{prod}) equal to the sum of expenses, required to purchase the components (Q_{comp}) as well as agent assembly expenses (Q_{assy}):

$$Q_{prod} = \sum Q_{comp} + Q_{assy} \quad (4)$$

Assembly costs of an agent (Q_{assy}) grow exponentially depending on the number of components used in the agent (N_{comp}):

$$Q_{assy} = c_{lin} \times exp\,(c_{exp} \times N_{comp}) \quad (5)$$

Values of Q_{comp} for each component are defined by the user of optimization procedure; coefficients c_{lin} and c_{exp} both for design and assembly costs are defined on the level of an optimization problem.

Results and Discussion

The paper describes implementation of the third step of a multi-robot system specification optimization procedure which uses investment costs estimation model presented in the previous chapter. The author uses genetic algorithm as a heuristic optimization method. In general, genetic algorithm mimics the processes of natural evolution such as inheritance, mutation, selection and crossover. The population of solution candidates evolves towards better solutions through generations. The fitness of every solution candidate is evaluated in each iteration, and the fittest candidates are used to form the next generation (Eiben and Smith, 2003).

One of the most challenging aspects in any application of a genetic algorithm is the development of a fitness function which is used to evaluate solution candidates in each iteration. The author uses costs estimation model as a fitness function for the genetic algorithm described in the previous section.

Another challenging aspect is the implementation of genetic representation of a solution domain. It should cover complete solution space and be stable against local extremes. According to the concept, a solution is a set of agents. Thus, every solution candidate should encode such set of agents. There are two attributes related to a particular agent: class of agent, and the number of its instances used in the solution. Because of the requirement to cover a full space of solutions, all types of agents should be encoded within a chromosome.

Classic application of a genetic algorithm implies the use of bit-value genes. In case of specification optimization problem, such genes could define types of agents, but not the number of instances. Also it is possible to add additional bit genes for encoding the number of instances, but in such case the number of

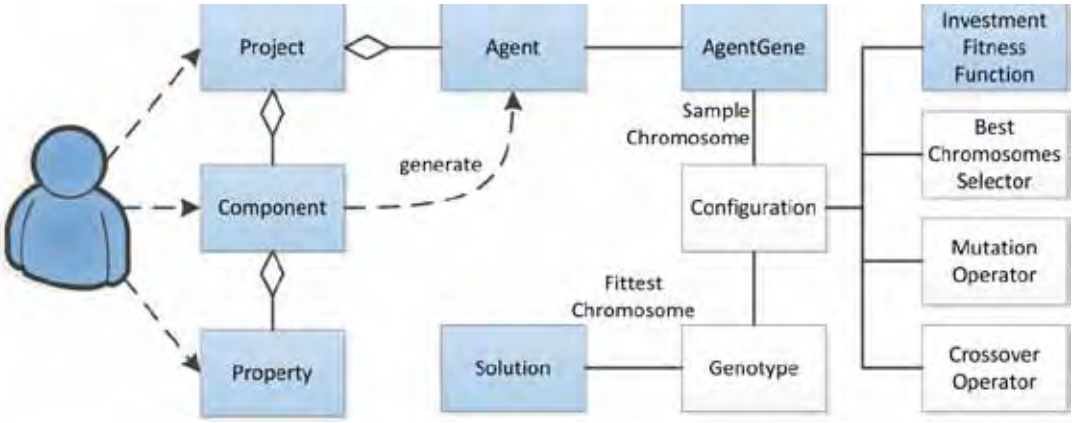

Figure 3. Conceptual object diagram.

genes within a chromosome (its length) would grow dramatically.

Because of that the author has selected integer genes to encode the set of selected agents within the chromosome. The range of values of agent genes is limited to non-negative integers. Gene with a value of zero means that particular type of agents is not used in the solution. Genetic operators for such genes are implemented randomly changing the value of the gene by the defined threshold.

The multi-robot system specification optimization software was developed using Java programming language and JGAP genetic algorithms package (Meffert et al., 2012). A number of special objects were involved into implementation of specification optimization procedure (see Figure 3): objects from JGAP package (white), as well as custom objects (gray).

Every specification optimization case (the mission) is defined as a project. The user defines a list of components required for performing the mission, as well as properties of these components. Then the list of components is used to automatically generate the list of possible agent classes. The optimization problem for a genetic algorithm is defined trough a special configuration object. It has a lot of specific properties; most important of them is the sample chromosome, which is used to breed the population of solution candidates. Agent genes are used in the sample chromosome. They are the extension of integer genes with the only added field, which refers to the agent instance they represent.

Another important object related to configuration of the genetic processor is the custom fitness function, which in this case implements the investment costs estimation model described above. Configuration also allows specifying a number of genetic operators involved into processing. Natural selection is implemented by selecting best 90% of solution candidates into breeding of the next generation. Mutation operator is applied to genes with probability of 0.08. Crossover is applied to 35% of candidates selected for the next generation.

Configuration object specifies also general properties for the genetic algorithm, such as maximum size of population (value of 200 was used in implementation) or allowance of identical individuals. After setting all required properties, the configuration object is used to create a genotype – the population of solution candidates. Then genotype evolves according to the specified fitness function and genetic operators. The number of generations is limited to 500. After finishing evolution, a solution of the optimization problem (the best specification of the multi-robot system in terms of investment costs) can be retrieved from the fittest chromosome of the genotype.

Conclusions

1. The paper presents investment costs estimation model for a heterogeneous multi-robot system. Investment costs for a particular system are estimated using user specified properties of the components (e.g. price). Also an assumption is used within the model, according to which the design and assembly costs of an agent (a robot, in particular) grow exponentially from the number of components used in it.
2. The implementation of heuristic evaluation is based on a genetic algorithm. The optimization task setup is described in the paper, as well as the software implementation model is presented. Adjusted parameters of the genetic algorithm allowed a fast and reliable evaluation of the solution candidates. The fitness function of the genetic algorithm corresponds to investment costs estimation model.
3. Specification optimization procedure was developed as a formal analysis method for heterogeneous multi-robot systems and it allows elimination of non-optimal solution branches with minimal effort.

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DYNAMIC MODEL OF BIOCHEMICAL NETWORK OF *ZYMOMONAS MOBILIS* ADAPTATION FOR GLYCEROL CONVERSION INTO BIOETHANOL

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Abstract
One of the biodiesel production problems is occurrence of a significant quantity (about 10%) of the by-product – glycerol. This problem is offered to solve by adaptation of bacteria *Zymomonas mobilis*, which is notable for ethanol production facilities. To be able to process glycerine into ethanol using *Z.mobilis* bacteria, the bacteria must be modified. At the same time, computer modelling analysis is required to assess specific modification affectivity in interconnection with other processes in bacteria. Computer model results of simulated experiment to understand and predict that the cells and biological processes are essential to reduce the number of experiments. This in turn reduces the necessary financial resources and time, bio-medical biotechnology, pharmaceutical and environmental problems. The model describes conversion of glycerol into bioethanol in *Z.mobilis* bacterial cell. First phase of model creation is creation of a structure model based on biochemical reactions using computer software CellDesigner. On the second phase of model creation, kinetic parameters which are available in literature were identified. Using the databases KEGG, SABIO-RK, BRENDA, reactants, kinetic parameters and reaction equation types were defined. Dynamic model of *Z. mobilis* biochemical network was created using computer software COPASI. The dynamic model describes conversion of glycerol into bioethanol in *Z.mobilis* bacterial cell. In this time simulation data of the computer model of natural organisms are not to confirm laboratory experimental data. Simulation data of the computer model are not correct, to prevent this problem is required parameter estimation in computer software COPASI.
Key words: Computer modelling, *Zymomonas mobilis*, ethanol.

Introduction

Z mobilis is undoubtedly one of the unique micro-bacteria in the world. It is known since 1912 as *Termobacterium mobilis*, *Pseudomonas Linder*, and finally as *Z.mobilis*. The first reviewing of their uniqueness was published in 1977 and 1988. *Z.mobilis* features manifest not only in biochemistry but also in growth, energy production, and response to the growing conditions. These features have caused great interest in science, biotechnology, and industrial areas. *Z.mobilis* is a bacterium which is notable for ethanol production facilities.

In the biodiesel production, washing process removes all water-soluble contaminants (methyl alcohol, glycerol, phosphates, etc.). One of the biodiesel production problems is generation of a significant amount (10%) of the by-product – glycerol. In order to process the glycerine into bioethanol using *Z.mobilis* bacteria, it must be modified. Biological experiments have revealed that by expressing bacterium *E. coli GlpF* and *GlpK* genes bacteria *Z.mobilis* is capable for processing glycerol into ethanol.

Biochemical reactions and process regulation network (Odzina et al., 2010) are too complicated to be able to predict the system's response without extensive computer modelling after changing any of its components. The aim of our research was to create a computer model of biological process by making simulation and analysing the results.

First phase of model creation was creation of the structure model based on biochemical reactions using computer software CellDesigner (Odziņa et al., 2010). On the second phase of model creation is stoichiometric analyses using computer software MatLab COBRA toolbox (Odzina et al., 2011), and then on the third step creating the dynamic model where kinetic parameters available in the literature are identified. Using the databases KEGG, SABIO-RK, BRENDA, reactants, kinetic parameters and reaction equation types were defined.

The dynamic model has characterized the conversion of glycerol into bioethanol inside the cell of bacteria *Z.mobilis*. The dynamic model has 22 reactions with 26 metabolites. The reactions are irreversible and reversible, and they work according to adapted Michaelis-Menten mechanism. The equation contains rate of reaction (*Vmax*), affinity constant (*Km*), and concentrations of metabolites. The problem is that databases do not feature all kinetic parameters suitable for bacteria *Z. Mobilis*. Therefore many kinetic parameters were taken from other organisms (as *E.Coli, S.cerevisiae*) whose structure resembles *Z. Mobilis*. Parameter estimation is performed to fit the model behavior with the one of laboratory experiments.

Computer model of *Z.mobilis* biochemical network was created using computer software COPASI (4.8.35.).

Materials and Methods

COPASI is a software application for simulation and analysis of biochemical networks and their dynamics. COPASI is a stand-alone program that supports models in the SBML standard and can simulate their behavior using ODEs or Gillespie's stochastic simulation algorithm; arbitrary discrete

events can be included in such simulations. COPASI carries out several analyses of the network and its dynamics and has extensive support for parameter estimation and optimization. COPASI provides means to visualize data in customizable plots, histograms and animations of network diagrams (http://www.copasi.org/tiki-index.php?page=OD.Events&structure=OD).

Systems Biology Markup Language (SBML) is a modular language, with a core comprising a complete format that can be used alone. SBML is oriented towards representing biochemical networks common in research on a number of topics, including cell signaling pathways, metabolic pathways, biochemical reactions, gene regulation, and many others. Broken down into its constituents, this model contains a number of components: reactant species, product species, reactions, rate laws, and parameters in the rate laws. To analyze or simulate this network, additional components must be made explicit, including compartments for the species, and units on the various quantities. The top level of an SBML model definition simply consists of lists of these components: beginning of model definition: list of unit definitions, list of compartments, list of species, list of reactions, list of parameters, list of rules; end of model definition (Hucka, 2003).

The dynamic model of modified organism describes second parameters: unit definition, compartment, species, reaction, parameters and events. The meaning of each component:

Unit definition: A name for a unit used in the expression of quantities in the model. The units are: time unit is second (s), volume unit is liter (l), quantity unit is micromole (µmol).

Compartment: A container of the finite volume for substances. In SBML Level 1, a compartment is primarily a topological structure with a volume but no geometric qualities. The dynamic model has two compartments: cellular and extracellular.

Species: A substance or entity that takes part in a reaction. The dynamic model has 26 species, of which 23 species are into cellular compartment and 3 species are into extracellular compartment.

Reaction: A statement describing some transformation, transport or binding process that can change the amount of one or more species. For example, a reaction may describe how certain entities (reactants) are transformed into certain other entities (products). Reactions have associated rate laws describing how quickly they take place. The dynamic model has 22 reactions, of which 13 reactions are irreversible and 9 are reversible. In the dynamic model, part of the reactions are taken from publication Mehmet M. Altintas 'Kinetic Modeling to optimize pentose Fermentation in *Zymomonas mobilis'* (Altintas et al., 2006). From the publication take reactions which characterized Entner Doudoroff patway. Reactions which are not included in the E. Doudoroff pathway were taken from database - SABIO-RK (http://sabio.villa-bosch.de/index2.jsp), and kinetic parameter from other databases – KEGG (http://www.genome.jp/kegg/) and BRENDA (http://www.brenda-enzymes.org/).

Each reaction is described by an equation. An equation types are in Table 1.

Table 1

Equations of the dynamic model

Reactions type	Reactions	Equation
Irreversible – one substrate, one product Irreversible – one substrate, two products	G2P -> PEP KDPG -> GAP + PYR	$\frac{Vmax \cdot \frac{KDPG}{KmKDPG}}{1+\frac{KDPG}{KmKDPG}}$
Irreversible – two substrates, two products	PEP + ADP -> PYR + ATP	$\frac{Vmax \cdot \frac{PEP}{KmPEP} \cdot \frac{ADP}{KmADP}}{1+\frac{PEP}{KmPEP}+\frac{ADP}{KmADP}+\frac{PEP \cdot ADP}{KmPEP \cdot KmADP}}$
Reversible – one substrate, one product	DOAP = GAP	$\frac{\frac{Vmaxf \cdot DOAP}{KmDOAP}}{1+\frac{DOAP}{KmDOAP}+\frac{GAP}{KmGAP}} - \frac{\frac{Vmaxr \cdot GAP}{KmGAP}}{1+\frac{DOAP}{KmDOAP}+\frac{GAP}{KmGAP}}$
Reversible – two substrates, two products	BPG + ADP = G3P + ATP	$\frac{Vmaxf \cdot \frac{ADP \cdot BPG}{KmADP \cdot KmBPG} - Vmaxr \cdot \frac{ATP \cdot G3P}{KmATP \cdot KmG3P}}{1+\frac{ADP}{KmADP}+\frac{BPG}{KmBPG}+\frac{ADP \cdot BPG}{KmADP \cdot KmBPG}+\frac{ATP}{KmATP}+\frac{G3P}{KmG3P}+\frac{ATP \cdot G3P}{KmATP \cdot KmG3P}}$

Parameters: A quantity that has a symbolic name. The dynamic model has 26 initial concentrations and 85 kinetic parameters: the equation contains rate of reaction (Vmax, Vmaxf, Vmaxr), affinity constant (KmS, KmP) and concentrations of metabolites (for example, ATP, ADP).

Events: which can be viewed as a discrete conditional state transition of the model, consist of two required parts: a trigger, which causes the event, and at least one assignment, which modifies the model (http://www.copasi.org/tiki-index.php?page=OD.Events&structure=OD). The dynamic model has one event: glucose impulse after 200 seconds.

Results and Discussion

In Table 2 describes reactions of the dynamic model:

One of the principles of the dynamic model transactions: the system must be a steady state. A system in a steady state has numerous properties that are unchanging in time. The concept of steady state has relevance in many fields, in particular thermodynamics and economics. Steady state is a more general situation than dynamic equilibrium. If a system is in steady state, then the recently observed behavior of the system will continue into the future. In stochastic systems, the probabilities that various states will be repeated will remain constant. In many systems, steady state is not achieved until some time has elapsed after the system is started or initiated. This initial situation is often identified as a transient state, start-up or warm-up period. While a dynamic equilibrium occurs when two or more reversible processes occur at the same rate, and such a system can be said to be in steady state, a system that is in steady state may not necessarily be in a state of dynamic equilibrium, because some of the processes involved are not reversible.

The dynamic model has a steady state. If the dynamic model has a steady state, the fallowing steps can be performed: model analyses, time course and parameter estimation and optimization.

Table 2

Reactions list of the dynamic model

No	Reactions Name	Equation
1	GAPD Glyceraldehyde-3-P dehydrogenase 1.2.1.12 (7)	GAP + NAD + Pi = BPG + NADH
2	G3PK 3-phosphoglycerate kinase 2.7.2.3 (8)	BPG + ADP = G3P + ATP
3	GPM Phospoglycerate mutase 5.4.2.1 (9)	G3P -> G2P
4	ENO Enolase 4.2.1.11 (10)	G2P -> PEP
5	PYRK Pyruvate kinase 2.7.1.40 (11)	PEP + ADP -> PYR + ATP
6	PYRD Pyruvate decarboxylase 4.1.1.1 (12)	PYR -> ACET + CO2
7	ADH Alcohol dehydrogenase 1.1.1.1 (13_1)	ACET + NADH = ETOH + NAD
8	Triose Phosphate isomerase 5.3.1.1 (17)	DOAP = GAP
9	Glycerol-3-P dehydrogenase 1.1.1.94 (16)	GP + NAD = DOAP + NADH
10	Glycerol kinase 2.7.1.30 (15)	GL + ATP = GP + ADP
11	Oxygen consumption	NADH -> NAD
12	ATP -dissipiation	ATP -> ADP + Pi
13	GK Glucokinase 2.7.1.2 (2)	GLUC + ATP -> GLUC6P + ADP
14	GPD Glucose-6-P dehydrogenase 1.1.1.49 (3)	GLUC6P + NAD -> PGL + NADH
15	PGLS 6 phospogluconolactonase 3.1.1.31 (4)	PGL -> PG
16	PGD 6-phosphogluconate dehydratase 4.2.1.12 (5)	PG -> KDPG
17	KDPGA 2-keto-3-deoxy-6-phosphogluconate aldolase 4.1.2.14 (6)	KDPG -> GAP + PYR
18	ADH II Alcohol dehydrogenase 1.1.1.1 (13_2)	ACET + NADH = ETOH + NAD
19	GF Glucose Fascilitator (1)	GLUCout = GLUC
20	Ethanol export (14)	ETOH = ETOHout
21	Glycerin transport	GLout -> GL
22	EthanolOut evaporate	ETOHout ->

Results from time course plots:

Plot 1 'Concentrations, Volumes, and Global Quantity Values' (Fig. 1); (x axis – s (second), y axis - µmol s^{-1}). In this model accumulate product GAP and GLUC6P. This problem in model is currently being addressed with parameter estimation methods, but in this moment are not good results. Other product achieves stability after two thousand seconds. Other species has low flow. The model aims to produce ethanol from glycerol and here you can see that the ethanol is produced, but not sufficiently and model still needs to improve.

Plot 2 'Reactions fluxes' (Fig. 2) (x axis – s (second), y axis - µmol s^{-1}). All reaction fluxes achieve stability flux after two thousand seconds.

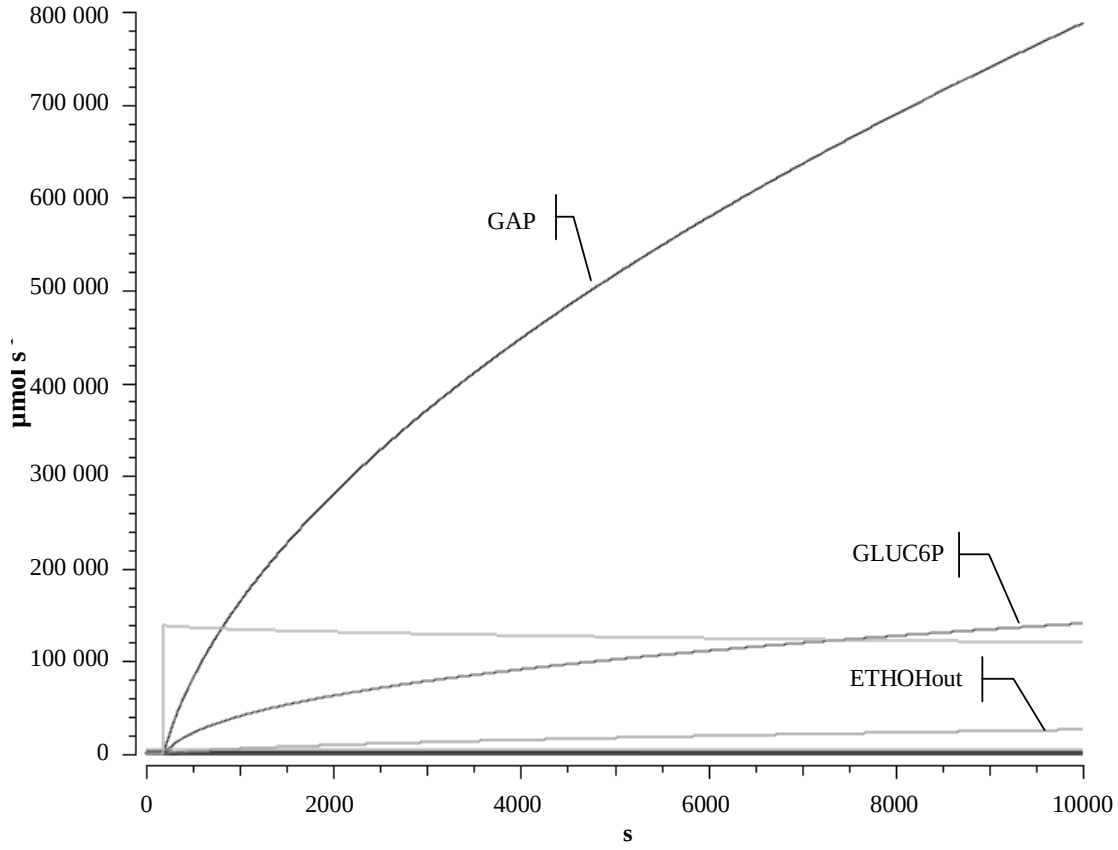

Figure 1. Concentrations, Volumes, and Global Quantity Values.

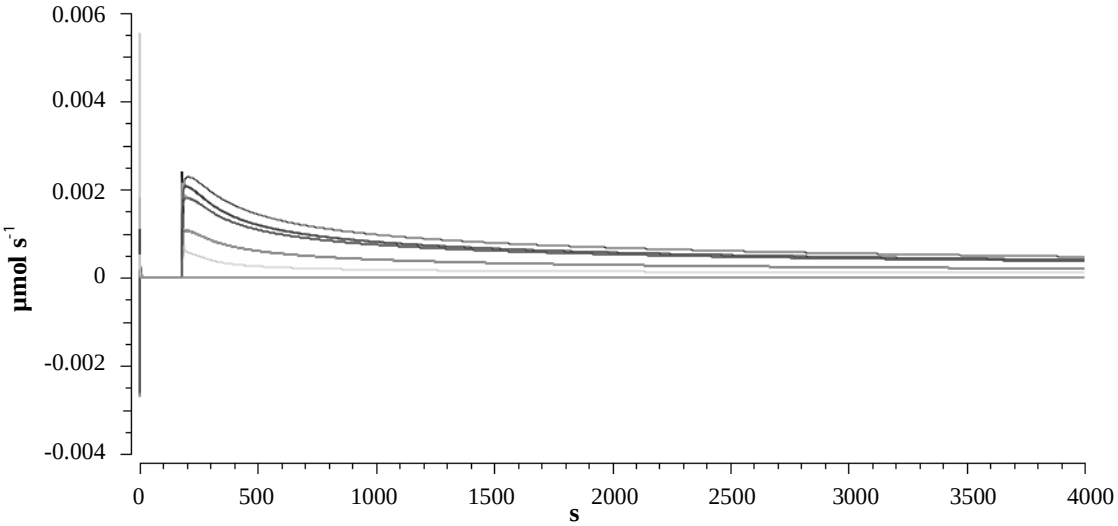

Figure 2. Reaction fluxes.

Conclusions

1. Results of simulated experiment to understand and predict that the cells and biological processes essential to reduce the number of laboratory experiments. Better results of the computer model simulation results can pruf in laboratory experiments. This in turn reduces the necessary financial resources and time, bio-medical biotechnology, pharmaceutical and environmental problems to be solved.
2. Programm Copasi is user friendly interface to create the dynamic model. The dynamic model has 22 reactions and 26 species.
3. The dynamic model describes conversion of glycerol into bioethanol in *Z.mobilis* bacterial cell. In this time simulation data of the computer model are not to confirm laboratory experimental data. Simulation data of the computer model are not correct, to prevent this problem is required parameter estimation in computer software COPASI.

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CHILDREN WITH SPECIAL NEEDS FAMILY EDUCATION AS A PARTNERSHIP COMPREHENSION IN RURAL AREA

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Abstract
Family education as a support to families where children are with special needs is mentioned in family politics statements. Unfortunately, family education problems in rural areas characterise present day situation in Latvia. Professionals with less specific knowledge essential to deal with special needs satisfaction is a reality in rural areas in Latvia. It is vital to advance family autonomy, develop an opportunity to deal with consequences that disabilities cause and, improve environmental conditions for children with special needs. The aim of this article is to fortify necessity of family education as a partnership comprehension development to children with special needs in rural area. The investigation was carried out in National Rehabilitation centre "Vaivari" in April 2012, by involving families with children with special needs. Analysis of scientific resources has been done; survey and statistical analysis of data were carried out, by using non-parametric method - Mann Whitney U test. During the research, the main family education comprehension aspects to families living in rural or city area and having children with special needs are identified. The advantages in special needs satisfaction context in rural areas and city environment are analysed. Measurements of respondent attitude to innovative family education e-solutions are made. Objectively and subjectively determined social isolation risk of families having children with special needs in rural area show the necessity to use family resources related to consequences that disabilities create. It is necessary to develop ways to use modern technological opportunities, provide appropriate family education and partnership with professionals.
Key words: children with special needs, family education, partnership, rural area.

Introduction

The education in a globalised knowledge society has become a process with comprehension not limited to analysis of formal education. Today education is related to fields of human life that are viewed as an economical and national existence base in the industrial societies, for example, work and family. Work meaning reproduction of available resources that was observed in previous centuries, today puts forward a demand of excellence that declares a necessity for educated employees and organisations that are ready to change. The family whose historical input over the centuries was to provide patriarchal hereditary rights, in the 21st century from unitary concept is transformed in alternative and even ambivalent existence justifications that points to crisis and development (Darling and Turkki, 2009). Searching the developmental opportunities as an instrument, education today is related to family existence for two significant aspects: collaboration necessary in order to merge work with family life and liberal statements based on the family value that is revaluated from which present families as a self-organised system idea results. This assumption denies the vertical hierarchies axis in family and professional relationships and introduces a mutually responsible partnership principle. The family becomes a research object and subject. The family that is able to learn is a future paradigm and also today's paradigm – learning ability paradigm.

The family functionality that is the base of wider society development differs in various culture environments (Beveridge, 2005). The city and rural areas (the countryside) are different environments due to recourse differences and capacity, but both of them are necessary components in a common society developmental structure.

Also, the family functionality establishes a satisfaction with family internal needs – standardised or special benefits. The children with special needs have an influence on family functionality if ways to compensate or creatively overtake the objective physical and mental limitations are not found.

Those families having children with special needs that live in rural areas are subjected to longer adaptation process than families living in cities. Care, medicine, rehabilitation, social benefits, education and environmental accessibility barriers are one part of difficulties to these families. Although in 1999 in State Family Principles support for families with children with special needs, an action platform for development of institutional family support mechanism was formulated, there are still uncertainties between state and local authorities support administration (Koncepcija valsts atbalsts..., 1999). The family politics that is reflected in State Family Principles reflects a discrepancy for local authorities to support families with children with functional disabilities, training and consulting and lack of accessible benefits for those living outside cities (Ģimenes valsts politikas pamatnostādnes, 2011).

In Latvia, support for families having children with special needs is treated as a social help or environmental availability improvements, ignoring the family educational necessity aspect. There is lack of political platform for debates on family education.

At the same time, academic discussion on family education definition and constructive development of it has not been started. As a result, the empirically taken measures are considered to be a self-initiative of researchers, and for its realisation it was not possible to avoid limitations that created theoretical uncertainties.

In present researches on families having children with special needs, their psychological, medical, care and rehabilitation questions, orienteering to disability or treating a child as an individual but not family and part of wider environmental system are discussed. In researches on children with special needs' family role is insufficiently analysed (Bērziņa, 2010). The characteristic influences of rural environment are discussed as minor aspects in separate local researches, underlining that necessary benefits are not available in local area of residence (Bērnu un jauniešu..., 2011). Family inability to receive education and information about opportunities of disability overcoming, using e-technologies (Vientuļo vecāku..., 2007) is mentioned.

The aim of article is to justify children with special needs family education as a partnership comprehension developmental necessity in rural environment, gathering information about children with special needs and their comprehension of family education.

In this research the author has described the difference between comprehension of family education in rural environment and city environment, underlining characteristic family necessities and interaction with environment aspects. The author paid attention to political and institutional platform necessity of innovative family education comprehension, defining families that have children with special needs and professional groups as partners.

Materials and Methods

Theoretical Framework of the Study

The article is based on a multifunctional approach for family education concept as well as theoretical analysis of J. Thomas, M. Arcus (1992) and R. McPherson (1998) etc. works where this approach is outlined. The family and multi-professional resource interaction, cycle of family life and sustainable statements of continuous family development were compiled in a family education comprehension. The research of family education is insufficient in author works from Latvia and other countries. The newer researches point to support searching for families with children with special needs as an existence of social efficient functional structure, considering professional and family connection basis. Family information and education determine opportunities of disability's overcoming, it helps to prognosticate the further development (Ray et al., 2009; Stewart et al., 2006).

Since beginning of partnership idea in work with families having children with special needs in 90ties when N. Dale (1996) offered a conceptual negotiating model for partnership (Negotiating Model for Partnership), asking to revise responsibility's distribution of families and specialists and underlining a division with knowledge meaning, the wider view in partnership comprehension is established. For theoretical partnership relationship comprehension of research in family education, the main statements of human ecological developmental theory, defining a partnership from other interaction forms of environment structures and underlining activity's necessity and essential modifications of involved parties (Beveridge, 2005) are used. An innovative approach in family (where children are with special needs) education as a partnership comprehension is pointed at political and institutional resource involvement, research development of school and family, family and treatment, care and rehabilitation specialists interaction. Family oriented care manifests a future aim whose significant parts are both family education and professionalization of family educators that includes necessity of certification. Latest researches show that this approach provides an optimal help for children with special needs (Gonzaless et al., 2004; Knapp and Madden, 2009).

In order to solve a unique problem spectrum families having children with special needs face with, one cannot ignore an environmental background. Satisfaction of special needs can take different family resources in cities and rural areas because benefits offered in cities are of wider choice than those in rural areas, availability of them is preferential, minimising the possible barriers. The research analysis on necessity of family education in the rural area where children are with special needs shows that there are few researches that focus on specific barriers of rural area environment. Differences of socio-economical segment and health-care infrastructures in rural areas and cities are observed. In rural area that is defined as a populated area with less than 2,500 inhabitants (Darling and Gallagher, 2004), children often encounter benefit availability difficulties such as -, transport availability aspects, mental health-care, paediatric, family doctor and dentist availability and general poverty tendency both in Latvia and foreign countries (Darling and Gallagher, 2004; Skinner and Slifkin, 2007). A recent specific factor interpreted as a barrier is difficulty in development context of children with special needs in Latvia that manifests both in unsolved discussion between country and local authorities about responsibility division in family politics realisation, and closure of small rural schools, creating significant difficulties and isolation risk exactly to children with special needs and their

families. In this situation a partial solution could be the e-technologies that might help to educate and provide necessary information to families. It could help to adopt families the definite inner psychological support and children care and education function as a self-organised capable systems that traditionally are comprehended as specialist competence. Today there are few researches about family education comprehension in rural area and family attitude to partnership introduction in relationship with specialists, e-technologies as an informative, educational and communication instrument.

The family perspective where children are with special needs today is connected not just with adaptation of environment conditions but capability to change environment themselves. Family education that is based on partnership offers new development opportunities to families, professionals, institutions and politicians.

Methods

The investigation was carried out in National Rehabilitation centre ”Vaivari” in April 2012. The service in this rehabilitation centre is avaible for every Latvian inhabitant, with respect of regional belonging.

In order to realise a family education research of children with special needs as a partnership comprehension in rural environment, the quantitative and qualitative research methods were used. The author has summed up and analysed 40 respondent answers about family education comprehension as a partnership, 20 from them were rural inhabitants who have a child with special needs and 20 citizens. Family members taking part in this research were 5-15 years old; questions are connected with children with special needs. Elder children with wide physical and mental disability spectrum are represented in this research that is connected with special needs concept.

14 men and 26 women in the age group of 22 to 47 participated in this research. The average age of respondents was 32.36 years (Mean (M) = 35.83; Standart Deviation (SD) = 8.506). 22 respondents have secondary special education and secondary education, but 18 have higher education or not completed higher education (SD=0.504).

15 respondents with secondary special or secondary education lived in the rural area, but 7 in the city (SD=0.477); 12 citizens and 6 inhabitants from the rural area (SD=0.485) have obtained higher education. Inhabitants who are living in the city have a higher education level. Case of relatively similar education level does not support a discriminating myth about correlation of children with special needs and education level with uneducated and uninformed parent social strata.

The participants answered to 16 open and closed type questions, in order to specify family education comprehension, gather information about family education actuality and possible comprehension differences in families having children with special needs in cities and rural areas. The open-type questions provoked respondents to conceptualise ideas about family education and discover the main advantages and disadvantages in the city and rural areas. By answering closed inquiry questions, the respondents evalueted family edaucation and partnership with Professional necessity in Likert scale.

IBM SPSS 20 packet was used for quantitative data analysis. The descriptive statistics indicators were defined and statistical Mann Whitney criteria was calculated, analysing differences of family education comprehension of respondents living in cities or rural areas.

Using quantitative and qualitative methods, the gained results were compared and integrated results were obtained providing a concept about significant family education comprehension aspects in rural area and brought forward assumptions for further question research.

Results and Discussion

Comprehension aspects of family education

Comparing given answers with J. Tomas, M. Arcus, and R. McPherson etc. approach for family education concept with emphasis to continuous disturbance overcoming knowledge and ability perfection subordinate to physical, psychological and social development period of family life, analogical view was marked.

Respondent comprehension about family life focuses on conceptual knowledge about disability production axis. There is knowledge that helps to understand children needs (64%), knowledge that turns to solution (19%) and knowledge whose production is specially organised in collaboration with specialists in a seminar form (17%). As the knowledge production aim, an opportunity to deal better with disability created consequences both to family, and child (65%) and optimal development of children with special needs (35%) appears in respondent opinions. Importance of family life development is not enough conscious in family education comprehension as underlined J. Tomas and M. Arcus (1992). As a negative attitude to family education component manifests a disbelief of necessary information of respondents who are living in rural area because previous experience displays situations in which families have to overcome lack of knowledge in solitude without professional help (82%).

In general, there are no fundamental diferences in comprehension of family education concept between respondents that are living in the city or rural area because both city inhabitants (64%), and rural area inhabitants (63%) consider knowledge about children special needs as the fundamental family education components. Although the significant differences do not appear between respondents who are living in the rural area and city in family education concept comprehension, there are no differences depending on education level. People living in rural area and having children with special needs are more pessimistic about family education opportunities to change situation than those who are living in the city. It alludes to presently dominant negative or in drift left families, where children are with special needs, experience of disability's overcoming in the rural area; more over, it underlines disadvantages in existent family and professional collaboration system that has not been solved since 1999 when Concept of Family with children support was worked out.

Components of family education comprehension

Base of family education concept that in literature is related with knowledge and ability production and development to an optimal overcoming of disability (McPherson, 1998), is explicit represented in family education concept comprehension of research participants (M=9.2) (McPherson, 1998). As an equivalent the family knowledge about themselves, children who have special needs in ability development and knowledge for mutual psychological support administration in family apart from specialists involvement (M=8) were evaluated. This statement complies with E. Kozleski, P. Engelbrecht and R. Hess (2008) inter-culture research about increase of family collaboration in activities and decision making, advancing a family autonomy.

The medical knowledge (M=7.1) and social ability production (M=4.9) are evaluated lower; that can partly be explained with a special medical care for children with special needs in various disability cases needed, so it will determine social activities present experience (see Fig. 1).

Comparing of rural area and city conditions

The conditions in the rural area cannot be unequivocally interpreted as a disadvantage for children with special needs and families satisfied existence. Although in researches (Darling and Gallagher, 2004; Skinner and Slifkin, 2007; Hornsby and Witte, 2010) dominates an opinion that families where children are with special needs have a chance to meet needs in the city environment easier, the research points out the factors that could be specifically positive to special needs satisfaction in the rural area (see Table 1).

Advantages of rural environment include traditional societies that are typically preserved in rural areas and could define stability that in respondent opinions balance on rural social isolation comprehension as a value. These opinions point to rural inhabitant insecurity in globalised today's society. An emphasis on an isolation advantage determines families where children are with special needs confrontation with lack of tolerance and negative society's attitude that can be avoided in rural environment from, keeping local connections. An advantage is also ecological factors that positively relate to health maintenance opportunities. Natural farm economical contribution and relative material rural inhabitant equality evaluation can be criteria to region backwardness

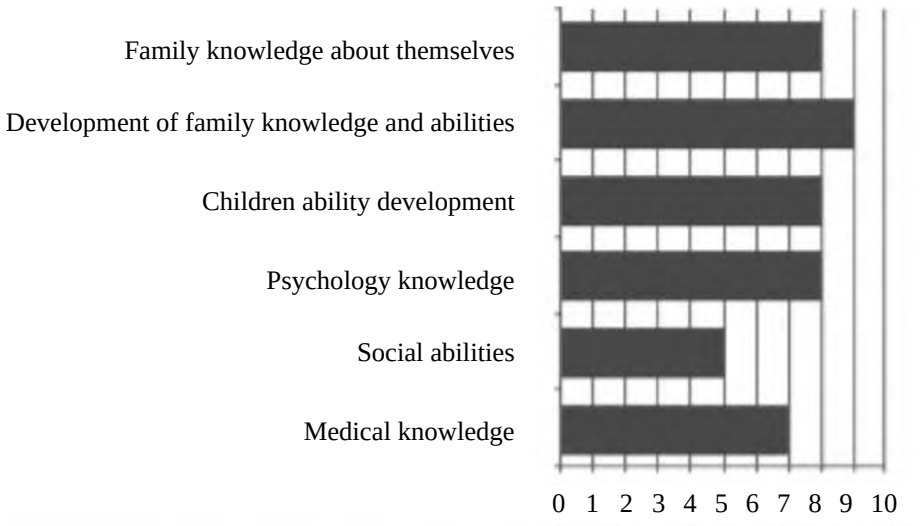

Figure 1. Evaluation of family education components in 10 points Likert scala.

from the city environmental trends, it purports about existence in agrarian society structures with an orientation to past socialism ideology. This situation also points to investments deficit and present government mechanism inefficiency.

Families having children with special needs percentage have to face with environmental barriers. Family real experience differs from inclusive education and even integrative education ideals. In rural regions there is a tendency not to enrol student with special needs in education institutions as well as there are various barriers – unavailable or hardly available benefits in common poorly developed infrastructure, lack of specialists, general unemployment and poverty, lack of information, transport and education problems. Analogical data were gained in foreign countries concluding that poverty together with mobility barriers and infrastructure features creates difficulties to satisfy special needs. The analogical data were gained in foreign countries according to which the conclusion that poverty in combination with barriers for mobility and infrastructure factors defies to satify special needs was drawn (Skinner and Slifkin, 2007).

City environment is friendlier to families where children are with special needs but the barrier analysis shows that political interest in conceptual solution of problems in cities also influences special needs satisfaction.

In statistical analysis, families who have children with special needs and live in rural or city environment and their ability to deal with difficulties that disabilities have created is compared. In accordance with the empirical division indicators, data proceeding, reposing in descriptive statistic, non parametric statistic method – Mann Witney U criteria because $Skewness_{calculate}|-0.792| > Skewness_{critical\ value}\ 0.533$

Table 1

Comparison of rural and city environment factors

Rural environment		City environment	
conductive factors	barriers	conductive factors	barriers
▪ traditional environment;	▪ favours are unavailable or are too far;	▪ available medical and rehabilitation favours;	▪ country disinterest;
▪ ecological environment, food	▪ lack of necessary specialists;	▪ guaranty with specialists;	▪ society intolerance;
▪ personalised education environment that define little classes at schools;	▪ no contact with families with analogical problems;	▪ favour and specialist choice opportunities;	▪ ecological factors (fresh air, food);
▪ special transport to education institutions;	▪ general unemployment and poverty;	▪ environment sustainability to children with movement disabilities (sidewalks etc.);	▪ high prices of goods and favours.
▪ tolerant society;	▪ lack of information;	▪ specialised kindergarten and schools;	
▪ protection from society;	▪ poor developed infrastructure;	▪ integration in comprehensive schools;	
▪ lack of psychological pressure;	▪ education institutions do not want to enrol students with special needs;	▪ opportunity to out-of-school interest development;	
▪ relative material equality;	▪ in problem case it is impossible to change education institution;	▪ culture environment availability.	
▪ economical contribution of natural farms.	▪ transport irregularity or lack;		
	▪ adjustment to school bus.		
Conductive factors in 43% of respondent views	Barriers in 57% of respondent views	Conductive factors in 84% of respondent views	Barriers in 16% of respondent views

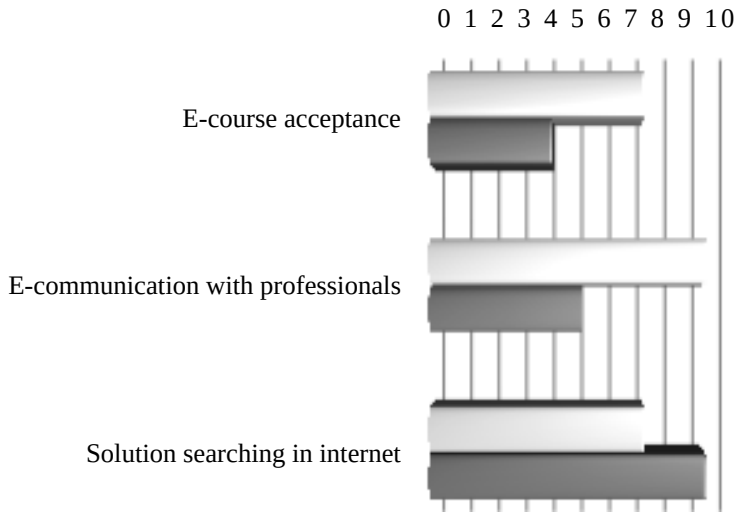

Figure 2. Evaluation of e - solutions in 10 points Likert scale: □ - in city; ■ - in rural area.

was chosen. Two sided importance level 0.003<0.05 confirms that there exists a statistically significant difference between ability of rural area and city environment inhabitants to overcome the difficulties that disabilities have created.

Family education as a partnership comparing in rural area and city environment

Evaluating statistically necessity of family education, there were not established statistically significant differences between respondent opinions of rural area or city environment because accounted two sided level is 0.099>0.05. It means that families where children are with special needs see an opportunity to greater family independence from specialist favours both in the rural areas and city environments.

Respondent comprehension about family and professional partnership in a disability consequence prevention characterises statistically significant higher partnership evaluation to respondents that are living in cities (0.045<0.05). In a rural area orientation to the traditional relationships with professionals in which families are information receivers than coordinators (Skinner and Slifkin, 2007) prevail. It can create the barriers to family education development and introduction in rural area environment although present situation confirms a necessity to activate and use family resources in the rural area.

The collaboration with professionals must have new characteristics, knowledge accordingly to society technological opportunities. Development of favourable infrastructure in rural areas can be a further perspective but the usage of technological opportunities – today's opportunity. Information obtained in e-environment, e-communication with specialists and online consultations, family education organisation in a type of e-course are some of future potentials (Zaidman-Zait and Jamieson, 2007).

Surveying the respondents from e-solutions that could increase family autonomy and with disability compared difficulties prevention of knowledge mobility, searching in the internet is accepted for information searching and searching of problem solution in rural areas (M=9). Other solution types of e-communication (mails, skype etc.) with professionals in order to obtain the information or do online consultations are more acceptable in families that are living in cities. It is necessary to talk with rural area inhabitant about importance of e-opportunities, showing advantages of information and decreasing family isolation.

Conclusions

1. The essential steps in political action are made in the last decade, including family education content the family consulting and education and also marking platform of institutional realisation. But in reality, there is lack of coordinate institutional mechanism that provide efficient but simple and understandable family education algorithm with clear divided institution and professional responsibility sector and reasoned functions.
2. In family education concept it is necessary to include aspects of knowledge and possibilities that help families and their members to deal efficiently with physical, social and psychological consequences that disabilities have created and turn to family autonomy and development of decision making.
3. Family partnership with professional is differentiated from other environmental structure collaboration forms, underlining an involvement of political and institutional resources, interaction of family and school, medical care and rehabilitation specialists to equal partnership and activity bases.

4. Family orientation to traditional relationship with professionals in rural area where families are information receivers but not coordinators, social isolation is determined as a value that protects from social intolerance, comprehension that could make a risk to family isolation in the rural area where children are with special needs.
5. Technological opportunities of knowledge society can develop new solutions in family educations that could be economically suitable and attainable with infrastructure improvements, using existent professionals and activating family resources. Information obtained in e-environment, e-communication with professionals and online consultations, family education organisations as e-courses can be future opportunity potentials.

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