PRODUCTION OF BIOETHANOL FROM STARCH BASED AGRICULTURE RAW MATERIAL

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Abstract
Bioethanol can be used for food production and to partially replace fossil fuel. Bioethanol is mainly produced from renewable biomass that contains sugars, starch or lignocellulose. The main raw materials for production of bioethanol are cereals, maize (*Zea mays*), sugarbeets (*Beta vulgaris saccharifera*) and other plant species. During the trial that took place in State Stende Cereals Breeding Institute during a three year period (from 2009/2010 to 2011/2012) we examined the suitability of grain from winter wheat (*Triticum aestivum* L.), triticale (×*Triticosecale* Wittm) and rye (*Secale cereale* L.) for the production of bioethanol. Three varieties of each species were used in the trial. During the trial period the grain yield, the ethanol outcome (L t^{-1}) and the ethanol yield (L ha^{-1}) were determined. It was established that during three years wheat and triticale provided the highest starch content (more than 700 g kg^{-1}) of the grain as well as the highest ethanol outcome (L t^{-1}). These species provided both high grain yield (more than 9 t ha^{-1} on average) and the highest ethanol yield (3300 – 4665 L ha^{-1}). The choice of variety was also important as both the grain starch content and the grain yield depend on the genotype of the variety.
Key words: winter cereals, starch, yield, ethanol outcome.

Introduction

The economical use of energy resources and the use of renewables in the energy production is a topical issue in the entire world. Intensive use of fossil fuels polluting the atmosphere with gas emissions is mentioned as one of the reasons for rapid climate change. One way to use the renewables is to produce bioethanol that can be used for food production and to partially replace fossil fuel. Bioethanol is mainly produced from renewable biomass containing sugars, starch or lignocellulose (Ethanol production…, 2006). The main raw materials for production of bioethanol are sugar cane (*Saccharum* spp.), cereals, maize (*Zea maize*), sugar beet (*Beta vulgaris saccharifera*) and other plant species. European Union member states mainly use cereals and sugar beet for that purpose. Sugar and starch processing technologies are mainly used in the production of bioethanol. Technologies for ethanol production from cellulose are used less frequently.

The limiting factor for the production of bioethanol from raw materials containing starch and sugar is the extensive use of water, fertilisers and pesticides in the cultivation of raw materials. An important issue is also the necessity to use these raw materials for food production. For example, in the USA where bioethanol is mainly produced from maize, the price of beef, pork and poultry, eggs, bread, cereals and milk increased for 10 – 20% as a result (Brown, 2008). Taking into account the conditions in Latvia, the cereals with higher starch content in the grain could be more suitable for the production of bioethanol as they are less suitable for food production. Winter cereals: rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.) and triticale (×*Triticosecale* Wittm) provide comparatively high grain yield, but the grain yield is one of the most significant indicators for a high bioethanol yield (L ha^{-1}). According to the data of Serbian researchers triticale and wheat are considered to be the most suitable raw materials for the production of bioethanol (Mojović et al., 2009).

The purpose of the study was to evaluate the suitability of winter cereals: wheat, triticale and rye as the starch based raw materials for the production of bioethanol in the conditions of Latvia. **Work task** resulting from the purpose was to set grain yield and starch content in it as well as the ethanol yield and ethanol outcome from different species of winter cereal crops.

Materials and Methods

The field study was carried out at State Stende Cereals Breeding Institute for three years: 2009/2010, 2010/2011, 2011/2012. The following varieties were examined during the study: winter wheat varieties ’Mulan’, ‘Skalmeje’ and the line ‘99-115’ created at State Stende Cereals Breeding Institute; winter rye varieties ‘Matador’, ‘Placido’ (F1), ‘Dankowskie Nowe’; winter triticale varieties ‘SW Valentino’, ‘Dinaro’ and the line ‘0002-26’, created at State Priekuli Plant Breeding Institute. The study was carried out in sod-podzolic or podzolic-gley soil depending on the year. The fields were chosen according to the crop rotation established at the Institute (Table 1). All the years the soil reaction was slightly too acid for cultivation of wheat, but suitable for the cultivation of rye. The variants were randomly placed in 4 replicates with the harvest reference area of 12 m^2. All three years white mustard (*Sinapis alba*) for green manure was used as a pre-crop. All three years the sowing

Table 1

The characteristics of soil

Year	Type of soil	Granulometric content	pH KCL	Content of organic matter, g kg^{-1}	P, mg kg^{-1}	K, mg kg^{-1}
2009/2010	Sod-podzolic	Clay loam	5.8	24	100	150
2010/2011	Podzolic-gley	Clay loam	5.8	23	82	111
2011/2012	Podzolic-gley	Clay loam	5.3	19	36	105

rate was 200 germinating seeds per m^{-2} for hybrid rye ('Placido'), 400 germinating seeds per m^{-2} for open pollinated rye varieties and triticale, and 450 germinating seeds per m^{-2} for wheat. The sowing was carried out in the second ten-day period of September (18.09.2009, 14.09.2010, 15.09.2011). Before sowing plots were fertilized with NPK fertilizers at the rate: N 12-15 kg ha^{-1}, P 20-26 kg ha^{-1} and K 75-79 kg ha^{-1}.

Ammonium nitrate (N 340 g kg^{-1}) was used as a top-dressing in spring:

- after the renewal of vegetation growth:
  - for winter wheat – 90 kg N ha^{-1};
  - for triticale and rye – 60 kg N ha^{-1};
- at the plant growth stage (GS) 31 – 32 for all examined varieties of winter cereals - 60 kg N ha^{-1}.

Fungicide Osiris e.c. (epoxiconazole, 37.5 g L^{-1}, metconazole, 27.5 g L^{-1}) in the amount of 1.5 L ha^{-1} was used during the vegetation period in order to prevent the diseases of winter wheat and triticale. To keep the weeds at bay the herbicide Mustang s.e. (florasulam, 6.25 g L^{-1}, 2.4 – D, 300 g L^{-1}) in the amount of 0.55 L ha^{-1} (sowing year 2009 and 2010) and Mustang Forte s.e. (florasulam, 5 g L^{-1}, aminopyralid, 10 g L^{-1}, 2.4 – D, 180 g L^{-1}) in the amount of 0.6 L ha^{-1} (sowing year 2011) were used. The retardant Medax Top (prohexadione-calcium, 50 g L^{-1}, mepiquat chloride, 300 g L^{-1}) in the amount of 0.75 L ha^{-1} was used only during the season 2011/2012 when due to increased humidity, there was a risk that the winter cereals would lodge. The crop was harvested at the GS 88–92 on 8 August 2010, 4 August 2011 and 6 August 2012.

After the samples were dried on platform driers, they were cleaned with MINI PETKUS MP100. The yield of winter cereals (t ha^{-1}) was then recalculated at 14% humidity and 100% purity. The quality analysis of grain was carried out at the Grain technology and agrochemistry laboratory of State Stende Cereals Breeding Institute. The content of crude protein (LVS 277:2000) and starch (LVS EN ISO 10520) was determined.

Experimental extraction of bioethanol. The practical extraction of ethanol from all the studied species and varieties took place in the Microbiology and Biotechnology Institute of the University of Latvia. The method was based on the fermentation of saccharised wheat, triticale and rye samples using yeasts *Saccharomyces cerevisiae*, which was followed by the calculation of the ethanol outcome and the fermentation speed.

Saccharification. 20 g of ground cereals sample and 80 g of water were weighed and mixed in the retort. Liquefaction was carried out by using 54 mL alfa-amylase preparation "Talzyme AL90" (JP Biotechnology) for 40 minutes at 90 °C. The pH was adjusted to 5.0 and the mixture was cooled to 60 °C.

Saccharification was carried out by using 400 mL glucoamylase preparation "Talzyme GL60" (JP Biotechnology) for 40 minutes at 60 °C. Liquefaction and saccharification was performed by a constant stirring of the sample.

Fermentation. After saccharification the sample was cooled to 30 °C. *Saccharomyces cerevisae* inoculum in the amount of 2 mL was added. The fermentation was carried out in retorts at 30 °C for 1–2 days. The changes in retort weight were measured during the fermentation. When the weight remained constant (thus indicating the end of fermentation process), the sample was taken and ethanol concentration was determined by chromatography. The ethanol outcome from the theoretical result and the productivity of ethanol formation (g kg^{-1} h^{-1}) were calculated.

The theoretical ethanol outcome g kg^{-1} from grain was calculated from the determined starch content by using formulas (1) and (2):

$$C\ glikoze = \frac{Cciete \times 180.16}{162.16}, \text{ where} \quad (1)$$

C glikoze – outcome of glycose, g kg^{-1};
C ciete – starch content, g kg^{-1}

$$C\ et.teor = \frac{C\ glikoze \times 2 \times Metanols}{M\ glikoze \times k}, \text{ where} \quad (2)$$

C et. teor. – theoretical ethanol outcome, g kg^{-1};
M etanols – molar mass of ethanol;
M glikoze – molar mass of glycose;
K – dilution factor, 5.

By using formula (3) the practically acquired ethanol outcome % was calculated in comparison to the theoretically calculated ethanol outcome.

$$Cet(\%no\ teor) = \frac{Cet \times 100}{Cet.teor}, \text{ where} \quad (3)$$

Cet (% of the theoretical) – practically acquired ethanol outcome, %, was calculated in comparison to the theoretically calculated ethanol outcome, %

Cet – concentration of ethanol, determined in the fermentation environment, g kg^{-1}

In the laboratory, the ethanol outcome from grains was determined, and the ethanol outcome was calculated in grams from one gram of grains (formula (4))

$$C = \frac{Cet}{Cgraudi}, \text{ where} \quad (4)$$

C – ethanol outcome from grain, g g^{-1}

C_{graudi} – amount of grain in the fermentation environment (grain in g 100 g^{-1} of fermentation environment, in this case – 20 g 100 g^{-1})

The productivity of ethanol formation depends on the concentration of ethanol determined in the fermentation environment and the time of fermentation (formula (5))

$$Cpr = \frac{Cet \times 10}{t}, \text{ where} \quad (5)$$

Cpr – productivity of ethanol formation g kg^{-1} h^{-1}

t – fermentation time, h

Ethanol outcome is expressed in litres by using formula (6)

$$Cl = \frac{C}{0.789} \times 1000, \text{ where}$$

Cl – ethanol outcome, L t^{-1}

0.789 – density of ethanol, g cm^{-3}

Two factor dispersion analysis method was used for the mathematical analysis of data.

The weather conditions during the trial years were varied. During all three years there was adequate humidity and temperature during the germination and tillering in autumn. During the wintering period the most unsuitable conditions were observed in the winter of 2010/2011. The dry spring of this year also affected the development and yield of certain varieties. Hot summers of 2009/2010 and 2010/2011 affected the formation of crude protein in grain whereas the starch content in grain was higher in 2011/2012 when there was sufficient humidity and the temperatures were lower. The increased amount of precipitation affected harvesting in all the years.

Results and Discussion

Starch content in grain. According to the data of scientific literature, the grain of winter cereals is suitable for the production of bioethanol due to the high starch content. According to the data, the starch content is 57-66% in the grain of rye (Boese, 2006), 670-690 g kg^{-1} in the grain of triticale (Krejčirova and Capouhova, 2008), 640-730 g kg^{-1} in the grain of wheat (Ethanol production..., 2006; Kindred et al., 2008; Sedláček et al., 2008; Saunders, 2011). The results obtained during the three years in Stende show that in the conditions of Latvia the starch content was 692.17 – 726.07 g kg^{-1} for winter wheat, 685.50 – 722.07 g kg^{-1} for triticale and 613.10-633.30 g kg^{-1} for rye (Table 2). According to the results of other authors, the starch content in grain depends on both the variety and the climate conditions (Kučerov, 2007; Krejčirova and Capouchova, 2008). Similarly to the descriptions in the scientific literature the impact of variety and the weather conditions during the year of cultivation was also observed in the trial in Stende. The highest starch content in the grain was observed in 2011/2012, when the humidity level was high and temperature range was around the long-term average during the formation of the grain. The starch content of winter wheat was similar in all the trial years, whereas for triticale and rye the grain starch content was substantially higher in 2011/2012 if compared with that of the previous year (Fig. 1).

According to the research data from the Czech Republic, the starch content in triticale grain was 673.3 – 693.9 g kg^{-1} (Krejčirova and Capouchova, 2008). A similar result was obtained in the research of State Stende Cereals Breeding Institute – 703.1 g kg^{-1} on average. Comparing starch content in the grain of different species, the highest average starch content was observed in wheat – 710.4 g kg^{-1}. The average starch content in triticale grains was slightly lower (703.1 g kg^{-1}). The lowest average starch content was observed in rye grain – 627.0 g kg^{-1}; it was 11.7% lower than that of wheat grain. The research of other authors also shows that the starch content in rye grain is lower than that of triticale (Wang et al., 1997).

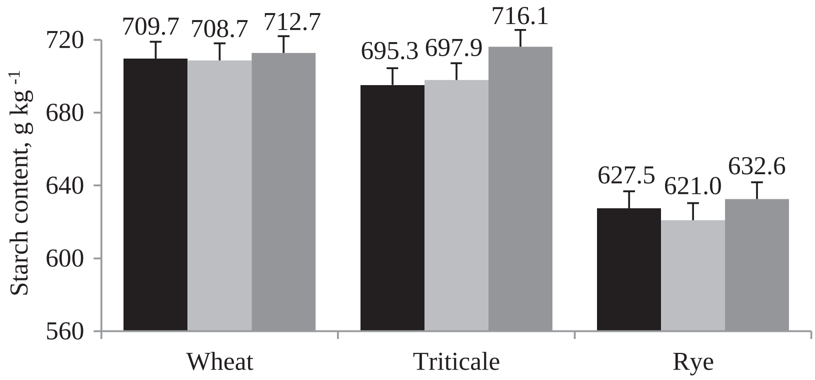

Figure 1. The changes in starch content (g kg^{-1}) of cereals grain in all three trial years depending on the species where 2009/2010 ■, 2010/2011 ■, 2011/2012 ■, LDS $_{0.05}$=9.24.

Table 2

Yield, starch content, ethanol outcome of winter cereals in Stende in the years 2010 – 2012

Variety	Yield, t ha^{-1}	Starch content, g kg^{-1}	Ethanol outcome, L t^{-1}
Wheat			
Skalmeje, min – max average	8.16-10.83 9.50	692.17-718.80 709.77	384.03-435.15 417.97
99-115 average	8.71-9.84 9.27	713.13-726.07 **718.09**	409.38-440.64 **428.67**
Mulan min – max average	8.29-11.48 **9.72**	693.07-721.07 703.13	406.84-426.49 415.50
$LDS_{0.05*}$	0.535	7.88	7.40
Triticale			
SW Valentino min – max average	7.88-11.05 9.18	685.50-712.63 695.28	408.75-439.37 424.24
Dinaro min – max average	8.81-9.31 **9.39**	698.50-722.07 **708.28**	423.32-449.94 **433.67**
0002-26 min – max average	7.32-10.17 8.86	693.80-713.67 **705.69**	403.68-442.33 **426.28**
$LDS_{0.05*}$	0.547	5.89	4.77
Rye			
Placido min – max average	8-42-10.75 **9.74**	626.53-632.37 **628.88**	365.02-409.38 393.32
Matador min – max average	7.30-10.79 8.62	613.10-633.30 623.84	370.09-424.59 **400.08**
Dankowskie Nowe min – max Average	7.41-10.67 8.53	622.20-632.10 **628.38**	366.92-418.25 **396.92**
$LDS_{0.05*}$	0.591	7.85	4.77

*LDS refers to the average results of varieties.

The starch content in grain is a genetically determined feature. The highest average starch content during the three years was observed in the new winter wheat line 99-115 (718.09 g kg^{-1}), for triticale – in the variety ‘Dinaro’ and line 0002-26, 708.28 and 705.69 g kg^{-1} respectively, for rye – in varieties ‘Placido’ and ‘Dankowskie Nowe’, 628.88 and 628.38 g kg^{-1} respectively (Table 2).

Grain yield. One of the preconditions for selection of raw materials for bioethanol production is the high yield of species and varieties. Of all the examined species the highest average three year yield was obtained from winter wheat – 9.50 t ha^{-1}. Average yield of triticale and rye was slightly lower – 9.14 and 8.96 t ha^{-1} respectively. Certainly, the level of yield is also influenced by the choice of varieties. Evaluating the winter wheat varieties, the highest yield was observed for varieties 'Mulan' and 'Skalmeje', 9.72 and 9.50 t ha^{-1}, respectively. For triticale the highest yield was provided by the variety 'Dinaro' – 9.39 t ha^{-1}, the average yield of 'SW Valentino' was only slightly lower – 9.18 t ha^{-1}. For rye the highest yield was provided by the hybrid variety 'Placido' – 9.74 t ha^{-1}, which is almost identical to the average yield of the wheat variety 'Mulan'. In general, a high average grain yield was obtained from all species and varieties of winter cereals, but in 2010/2011 the winter hardiness of certain varieties was the reason for reduced grain yields.

Ethanol outcome. The practical ethanol outcome is influenced by various factors, including the size of starch granules. The size of starch granules affects the grain processing technology for the production of ethanol. The wheat grain generally contains two types of granules: large disks of the size 15-35 µm and small spheres of the size 1 – 10 µm. Triticale starch granules are disks of the size 12 – 30 µm and small spheres of the size 1 – 10 µm. Rye starch granules are disks of the size 10 – 40 µm (KeShun Liu, 2011). British scientists have concluded that the grain with mixed type of starch granules is the most suitable for production of ethanol (Smith et al., 2006). The scientists found out that the starch content and grain size are not the decisive factors in the extraction of bioethanol; the important factor is the starch reactivity to starch hydrolysis (Mojević et al., 2009). The starch consists of two polymers: amylose and amylopectin. The wheat grains usually contain 20 – 30% amylose and 70 – 80% amylopectin (Šramkova et al., 2009). According to the data of Czech researchers, the amylose content in grains of triticale was 208 – 264 g kg^{-1} depending on the variety, location of cultivation and amount of nitrogen fertiliser (Burešová et al., 2010). In the ethanol processing technologies a higher level of energy is needed in order to gelatinise the amylose. Although winter wheat grain had the highest starch content among all the species, the highest ethanol outcome in the laboratory conditions were obtained from triticale – 428.06 L t^{-1}. The ethanol outcome from winter wheat was 420.72 L t^{-1}. Rye had the lowest starch content in grain and also the lowest ethanol outcome from 1 t of grain – 396.78 L t^{-1} (Fig. 2). According to the research data of S. Wang and his colleagues, the processing of rye in the bioethanol production is an economically sound and good alternative for the extraction of biofuel. According to different research data, rye can produce the bioethanol outcome of 362-409 L t^{-1} (Wang et al., 1997; 1998), and this result is similar to observations during the trial in Stende.

The highest ethanol outcome both acquired in the laboratory and calculated theoretically was obtained from wheat and triticale grain. The estimates show that the ethanol outcome acquired from rye grain is 12% lower than that acquired from wheat. Ethanol extraction in laboratory showed that the ethanol outcome from rye is 6% smaller than that from wheat (Fig. 2). Comparing the theoretically calculated and practically acquired ethanol outcome, it was found out that 82% ethanol was acquired from winter wheat grains, 85% – from triticale grains and 88% – from rye grains, if the comparison is made to the estimate based on the starch content in the raw materials. Depending on the species, the losses in the production process were 12 – 18% in comparison to the theoretical estimate.

Ethanol yield per one hectare (L ha^{-1}) is affected by ethanol outcome (L t^{-1}) and grain yield (t ha^{-1}).

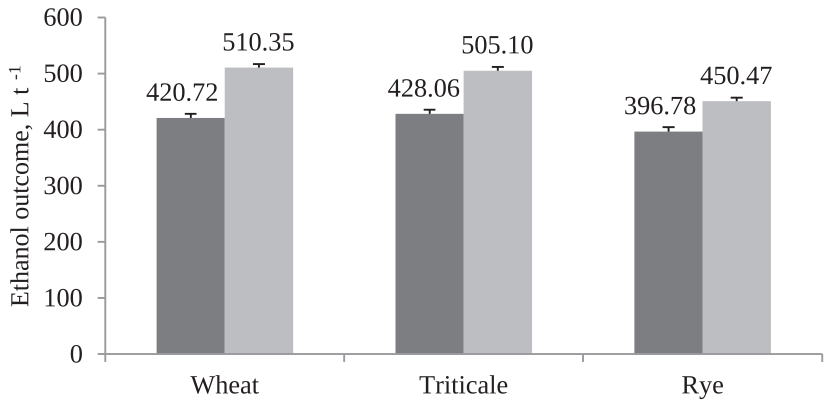

Figure 2. Average ethanol outcome per species, determined in laboratory and calculated theoretically, L t^{-1}, where LSD $_{0.05}$ for practical outcome = 7.40, LSD $_{0.05}$ for theoretical outcome =6.64,
practical, theoretical ethanol outcome.

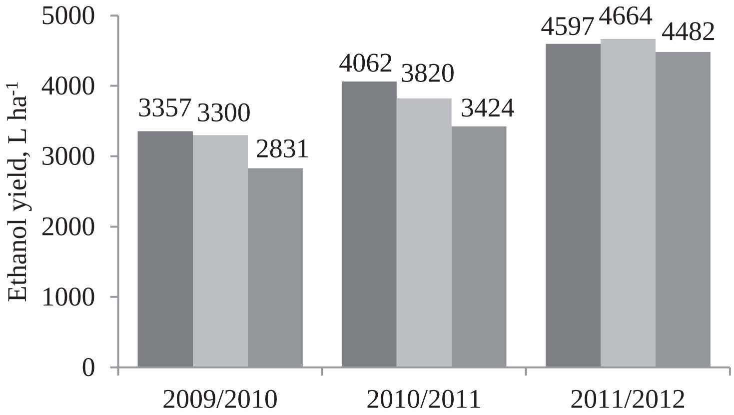

Figure 3. Ethanol yield (L ha^{-1}) depending on the species of cereals and trial year: wheat, triticale, rye.

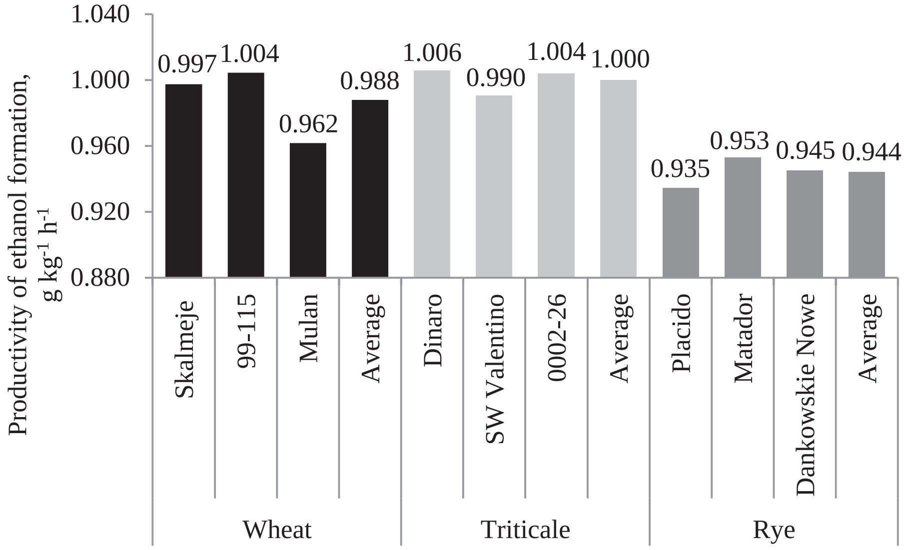

Figure 4. Productivity of ethanol formation in winter cereals, g kg^{-1} h^{-1}.

The lowest grain yield was harvested in 2009/2010, when wintering of cereals was problematical, water scarcity was observed in June during the formation of grain, the temperature in July was higher than the average long-term data, while in August a considerable amount of precipitations encumbered harvesting. Taking into account those circumstances, it was natural to have the lowest ethanol yield (Fig. 3).

The year 2011/2012 on the contrary provided the highest grain yield for all the examined species of winter cereals; consequently, the ethanol yield was considerably higher (4482 – 4597 L ha^{-1}) than in the previous two trial years.

Productivity of ethanol formation. The productivity of ethanol formation is ethanol outcome from the used substrate per hour. The productivity of ethanol formation is affected by starch content and composition of grain as well as the ferments used in the process. Grain fermentation is one of the factors promoting the productivity of ethanol formation. The scientific literature describes the impact of pentozane content. Pentozane is a polysaccharide. The research has proved that rye grain contains higher level of pentozane that forms a soluble compound. It leaves an unfavourable impact on fermentation process. Finnish study in which the impact of fermentation on rye bran was examined proved that fermentation process was also influenced by higher pentoze content (Katina et al., 2007). Pentoze practically does not decompose with *S. cerevisiae* yeast fungi. It means that specific ferments have to be added to substrates in order to ensure higher ethanol outcome in the fermentation process (Juodeikiene et al., 2011).

During our trial the same technology was applied to all the species for the extraction of ethanol in the laboratory. The three year data show that on average among all examined species by applying this technology the lowest productivity of ethanol formation is observed in rye 0.944 g kg^{-1} h^{-1}. According to the trial results, the average productivity of ethanol formation is higher for triticale and winter wheat – 0.988 and 1.00 g kg^{-1} h^{-1} respectively.

Conclusions

Evaluating three species of winter cereals as starch based raw materials for production of bioethanol, it was found out that winter wheat and triticale are the most suitable species for this purpose. A considerably higher starch content was observed in their grain, wheat and triticale had the higher average grain yield during the trial years and provided higher ethanol outcome per 1 t of grain. The choice of varieties in the framework of one species also had an essential role. Additionally, weather conditions affected the level of grain yield and starch content in grain. The productivity of ethanol formation was lower in rye grain, it might have been affected by the starch content and the impact of ferments on the grain.

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