PRODUCTION OF BIO-ETHANOL FROM WINTER CEREALS

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Abstract

Renewable energy resources play an important role in energy production both in Latvia and in the world. Bio-ethanol is used as a substitute for oil products in various countries of the world. It is produced from the plants containing starch: cereals, potatoes, beet, maize. The task of the research was to evaluate the suitability of different varieties of winter wheat, triticale and rye for extraction of bio-ethanol in Latvia. The research was carried out at the State Stende Cereals Breeding Institute in 2009, and the following varieties and lines of cereals were examined: winter wheat varieties 'Mulan', 'Skalmeje', and the line '99-115', developed at the State Stende Cereals Breeding Institute; winter triticale varieties 'SW Valentino', 'Dinaro', and the line '0002-26', developed at the State Priekuli Plant Breeding Institute; winter rye varieties 'Matador', 'Placido' F_1, and 'Dankowskie Nowe'. The content of crude protein and starch of grains, the grain yield, and the bio-ethanol yield were determined. The highest bio-ethanol yield was acquired from the winter wheat and triticale varieties that had the highest starch content and the lowest crude protein content of grains. The best results were obtained from winter wheat line '99-115' and variety 'Mulan' (409.4 and 406.8 L t^{-1}), triticale variety 'Dinaro' (423.3 L t^{-1}), and winter rye variety 'Matador' (370.1 L t^{-1}.)

Key words: winter wheat, winter triticale, winter rye, crude protein, starch.

Introduction

The renewable energy resources have the priority in the future energy production. The tendency in the world is to promote the use of biofuels that significantly reduce the greenhouse gas emission. By ratifying the Kyoto Protocol and introducing the Action plan for energy efficiency, the European Union (EU) made the commitment to achieve the long-term goals, including the goal to reduce the general level of EU greenhouse gas emission and increase the share of renewable energy in the entire energy consumption of the EU till 2020 (Directive..., 2009). Latvia, similarly to the majority of countries in the world, has also committed itself to implement international obligations related to the prevention of the global climate change.

The use of bio-ethanol for renewable energy resources is one of the solutions for saving the environment and reducing the global warming. It also reduces the necessity to import oil products and provides diversification of fuels as well as ensures the security of supply.

Bio-ethanol is increasingly used as a substitute for oil products in various countries of the world. According to the data for the year 2009, the total amount of bio-ethanol produced in the world was 74 billion litres. The biggest producers of bio-ethanol were the United States of America and Brazil. The EU is mentioned as the third biggest producer of bio-ethanol. In 2009 it produced 3.7 billion litres of bio-ethanol. Among the EU countries, France and Germany are the ones that produce the greatest amount of bio-ethanol. Latvia produced 15 millions litres of bio-ethanol in 2009 (Production of..., 2009).

Bio-ethanol is extracted from plants that contain starch, for example, cereals, potatoes (*Solanum tuberosum* L.), beet (*Beta vulgaris saccharifera* L.), maize (*Zea mays* L.). In Latvia, bio-ethanol is produced from cereals. The research data from other countries shows that cereals are suitable for production of bio-ethanol (Clarke et al., 2008; Wang et al., 1997, 1998; Müllerovs and Mikulins, 2008).

The aim of the research was a suitability of several varieties of winter wheat (*Triticum aestivum* L.), triticale (*Triticosecale* Wittm), and rye (*Secale cereale* L.) for extraction of bio-ethanol in Latvia.

Materials and Methods

The research was carried out at the State Stende Cereals Breeding Institute in the vegetation period of 2009/2010. Soil at the trial site was sod-podzolic loam with the following characteristics: pH KCL 5.6 – 6.0, content of organic substance 22 – 26 g kg^{-1}, content of plant-available P – 100 mg kg^{-1}, K – 150 mg kg^{-1}.

Field experiments were carried out in the breeding crop rotation fields, placed randomly in 4 replications with the plot area of 12 m^2.

The following varieties and lines were examined during the research: wheat varieties 'Mulan' and 'Skalmeje', and the line '99-115', developed at the State Stende Cereals Breeding Institute; winter triticale varieties 'SW Valentino', 'Dinaro', and line '0002-26', developed at the State Priekuli Plant Breeding Institute; winter rye varieties 'Matador', 'Placido' F_1, and 'Dankowskie Nowe'.

The **previous crop** in the experiment field was white mustard used for green manure.

Soil tillage. The pre-crop – mustard – was worked into the soil, using the KUHN plough VARIMASTER.

The sowing rate for population rye ('Matador', 'Dankowskie Nowe') and triticale was 400 germinating seeds per 1 m^2, for hybrid rye ('Placido' F_1) – 200 germinating seeds per 1 m^2, and for winter wheat – 450 germinating seeds per 1 m^2. The sowing was carried out on 18 September of 2009. Complex fertiliser N4-P20-K20 in the amount of 300 kg ha^{-1} was used as a basic fertiliser. In the spring of 2010 ammonium nitrate (N 34%) was used as top-fertiliser in the following amounts:

at the renewal of vegetation:
- for winter wheat – 90 kg N ha^{-1};
- for triticale and rye – 60 kg N ha^{-1};

at the growing stages 31 – 32 for all examined winter cereals – 60 kg N ha^{-1}.

The winter cereals were harvested on 8 August using the harvester WINTERSTEIGER DELTA, which also determined the weight of grains (kg), and moisture (%). After drying the samples, they were cleaned with MINI PETKUS MP100. The yield of winter cereals (t ha^{-1}) was recalculated at 14% of moisture and 100% purity.

The analyses of grain quality were carried out in the Laboratory of Grain Technology and Agro-chemistry of the State Stende Cereals Breeding Institute. The content of crude protein and starch was determined by using INFRATEC ANALYZER 1241.

The ethanol was extracted at the Institute of Microbiology and Biotechnology of the University of Latvia. A modified method was used for the extraction of ethanol. The method is based on the fermentation of the grain sample with alcohol yeasts (*Saccharomyces cerevisiae*) (Vigants et al., 2008).

The formulas (1)-(3) recommended by the Institute of Microbiology and Biotechnology of the University of Latvia were used in order to calculate the theoretical ethanol outcome (L t^{-1}) and yield (L ha^{-1}).

Theoretical outcome of ethanol from starch:

$$Et=\frac{C\times 1.11\times 2\times 46}{180.16} \quad (1),$$

where: Et – theoretica l outcome of ethanol, g g^{-1};
1.11 – factor for starch recalculation into glucose;
2 – stechiometrical factor for the summary reaction equation of glucose conversion into ethanol;
46 – molar mass of ethanol;
180.16 – molar mass of glucose;
C – starch, g g^{-1}.

Outcome of ethanol, L t^{-1}:

$$E=\frac{Et}{0.789}\times 1000 \quad (2),$$

where: E – outcome of ethanol, L t^{-1};
E_t – acquired outcome of ethanol, g g^{-1};
0.789 – ethanol density, g ml^{-1}.

Ethanol yield, L ha^{-1}:

$$E_r=E\times R \quad (3),$$

where: E_r – ethanol yield, L ha^{-1};
E – outcome of ethanol, L t^{-1};
R – grain harvest, t ha^{-1}.

The meteorological data obtained at the Stende station of hydrometeorology were compiled for the period of the vegetation and hibernation period. The weather conditions during the season of 2009/2010 were suitable for germination and tillering of winter cereals. The thick cover of snow at the beginning of the winter was beneficial for wintering. Rather dry and hot weather was registered during summer. It reduced the potential yield of winter cereals because grain was dried before full maturation. During the harvest the amount of precipitation increased, hampering the harvesting of grain and leaving an impact on their quality.

The statistical evaluation of data was carried out by using methods of dispersion, correlation and regression analysis, as well as descriptive statistics.

Results and Discussion

Grain yield

The grain yield in trials in 2009/2010 was comparatively good. It ranged from 8.46 to 8.90 t ha^{-1} (Table 1). The average grain harvest of the examined species of winter cereals did not have significant differences ($p>0.05$).

Table 1

Average grain yield and quality, and bio-ethanol outcome of winter cereals

Species	Crude protein, g kg^{-1}	Starch, g kg^{-1}	Yield, t ha^{-1}	Bio-ethanol outcome, L t^{-1}	Bio-ethanol yield, L ha^{-1}
Winter wheat	126.6	676.8	8.46	400.1	3386
Winter triticale	117.1	647.7	8.74	411.9	3607
Winter rye	104.8	614.9	8.90	367.3	3270
$LDS_{0.05}$	3.53	11.94	0.71	10.07	369.18

Evaluating the results of different varieties of the same species, the average yield of the examined wheat varieties and lines was 8.46 t ha^{-1} (Table 1). Better grain yield was obtained from the line '99-115' – 9.00 t ha^{-1}. Yield of varieties 'Skalmeje' and 'Mulan' was 0.81 and 0.80 t ha^{-1} lower (8.18 and 8.19 t ha^{-1}, respectively) in comparison to the yield of the line '99-115' (Table 2). The average yield of the examined triticale varieties and lines was 8.74 t ha^{-1}. A significantly ($p<0.05$) better grain yield was obtained from triticale variety 'Dinaro' – 9.65 t ha^{-1}. The variety 'Dinaro' also had a good yield during the research in previous years (Jansone et al., 2010). The yield of triticale variety 'SW Valentino' and the line '0002-26' was 0.93 and 1.79 t ha^{-1} lower (8.72 and 7.86 t ha^{-1} respectively).

Table 2

Grain yield and quality of varieties and lines of winter cereals

Varieties and lines	Yield, t ha^{-1}	Crude protein, g kg^{-1}	Starch, g kg^{-1}	Bio-ethanol outcome, L t^{-1}	Bio-ethanol yield, L ha^{-1}
Wheat					
Skalmeje	8.18	123.7	675.8	384.0	3141
Mulan	8.19	128.7	676.8	406.8	3332
L. 99-115	**9.00**	127.5	678.0	409.4	3684
$LDS_{0.05}$	1.16	9.56	38.89	-	-
Triticale					
Dinaro	**9.65**	111.1	666.5	423.3	4085
SW Valentino	8.72	115.8	642.8	408.7	3564
L. 0002-26	7.86	124.5	634.0	403.7	3173
$LDS_{0.05}$	0.78	4.48	10.26	-	-
Rye					
Placido	**9.69**	102.8	610.5	365.0	3537
Matador	8.48	101.8	617.0	370.1	3138
Dankowskie Nowe	8.54	110.0	617.3	366.9	3133
$LDS_{0.05}$	0.49	1.98	3.07	-	-

The hybrid rye variety 'Placido' F1 had significantly ($p<0.05$) higher level of yield in comparison to population rye varieties 'Matador' (-1.21 t ha^{-1}) and 'Dankowskie Nowe' (-1.15 t ha^{-1}).

Yield quality

According to the data of other researchers (Reaker et al., 1998; Clarke et al., 2008), a higher starch and lower crude protein content of grain has impact on the bio-ethanol outcome. In our research, the crude protein and starch content in the grain of the varieties and lines of winter cereals also was evaluated. Among the species the rye had the lowest average crude protein content – 104.8 g kg^{-1} (Table 1). The winter wheat had the highest average crude protein content (126.6 g kg^{-1}). Although the wheat grain contained the highest amount of crude protein, they also had the highest starch content of all the species – 676.8 g kg^{-1}. During the evaluation of the rye it was possible to find the same correlation in reverse: the grain that had the lowest crude protein content also had the lowest starch content – 614.9 g kg^{-1}. The quality parameters for triticale were in the middle between the wheat and rye quality parametrs. The starch content is dependent on the weather and cultivation conditions (Kučerov, 2007).

Evaluating the species separately, no substantial differences in the crude protein and starch content of grain were discovered among the examined varieties and lines of winter wheat. The crude protein content ranged from 123.7 to 128.7 g kg^{-1}, and the starch content ranged from 675.8 to 678.0 g kg^{-1}. Evaluation of the wheat varieties by taking into account the crude protein content of the grain showed they are suitable for baking high-quality bread.

Evaluating triticale varieties and lines it was found that the crude protein and starch content of the grain depended greatly on the chosen variety. The variety 'Dinaro' that also had the highest starch content in the research of previous years (Jansone et al., 2010), had the highest starch content (666.5 g kg^{-1}) and the lowest crude protein content (111.1 g kg^{-1}). The variety 'SW Valentino' and the line '0002-26' were found to have significantly ($p<0.05$) lower starch content and substantially ($p<0.05$) higher crude protein content than 'Dinaro'. L. Krejčirova and I. Capouchova (2008) who have carried out research in the Czech Republic on the suitability of triticale for bio-ethanol production have acquired data on starch content of different triticale varieties. The starch content differed depending on the variety, the place, and the year of cultivation. The Czech research showed that the starch content of one variety ranged from 673.3 to 693.9 g kg^{-1}.

As regards the rye, the varieties 'Matador' and 'Dankowskie Nowa' were found to have significantly ($p<0.05$) higher starch content – 617.0 and 617.3 g kg^{-1} respectively. The starch content of the hybrid variety

'Placido' F_1 was slightly lower – 610.5 g kg^{-1}. The varieties 'Matador' and 'Placido' F_1 had the lowest crude protein content – 101.8 and 102.8 g kg^{-1} respectively. It was slightly higher for the variety 'Dankowskie Nowa' – 110.0 g kg^{-1}.

Ethanol outcome (L t^{-1}) and ethanol yield (L ha^{-1})

Grain ensures high ethanol outcome. Comparing the species, the trials showed that the highest ethanol outcome (L t^{-1}) was obtained from triticale – 411.9 L t^{-1}. Triticale can provide the ethanol yield in the amount of 3607 L ha^{-1} with the yield level of 8.74 t ha^{-1}, which is the highest result among all the examined species of winter cereals (Table 1). S. Wang et al. (1997) have stated that the bio-ethanol outcome from triticale grain is 362-367 L t^{-1}. F. Sosulski and K. Sosulski (1994) mention that the outcome of commercial bio-ethanol from wheat ranges from 324 to 364 L t^{-1}. It was found that the ethanol outcome of winter wheat (400.1 L t^{-1}) is 11.8 L t^{-1} lower than that of triticale, and with the yield level of 8.46 t ha^{-1} it is possible to obtain ethanol yield at the amount of 3386 L ha^{-1} .

The use of rye in production of bio-ethanol is economically rentable and a good alternative for production of biofuels. According to the data of different researches, the bio-ethanol outcome from rye can be at the amount of 362-409 L t^{-1} (Wang et al., 1997, 1998). During our research the ethanol outcome acquired from rye was 367.3 L t^{-1}. If the grain yield is 8.91 t ha^{-1}, rye can provide the ethanol yield at the amount of 3270 L ha^{-1}. According to the data of D'Appolonia (Д'Апполониа, 1978), the rye grain contains more starch granules of the size 30-40 and 20-30 μm. The size of starch granules influences the technology of grain processing for extraction of ethanol. The research has shown that the starch granules of the size >10 μm (type A) contain slightly higher amounts of amylose. In order to split amylose in ethanol production technology it is necessary to use more energy (Smith et al., 2006). This explains why rye varieties have lower ethanol outcome.

In order to produce bio-ethanol, it is customary to use wheat varieties suitable for fodder and technical purposes and have high starch content. The results of researches from other countries indicate that the highest ethanol outcome can be acquired from the soft wheat (*Triticum aestivum* L.) varieties (Smith et al., 2006; Sosulski and Sosulski, 1994). Out of all the examined varieties and lines the highest ethanol outcome was obtained from the new wheat line '99-115' and the variety 'Mulan' (409.4 and 406.8 L t^{-1} respectively). The line '99-115' provided the highest ethanol yield – 3684 L ha^{-1}, due to highest grain yield.

According to the data of the research carried out by J. Kučerov (2007), the ethanol yield of triticale can be affected by several factors: firstly, the choice of variety, because different varieties have different ethanol outcome, and secondly, the choice of geographical location and agro-ecological differences depending on the year. According to the results of our research it can be noted that the highest ethanol outcome and yield was obtained from triticale variety 'Dinaro' (423.3 L t^{-1} and 4085 L ha^{-1} respectively), which was logical, since this variety had the highest starch content and the best yield.

The highest ethanol outcome among the rye varieties was obtained from the variety 'Matador' – 370.1 L t^{-1}. The highest ethanol yield was obtained from the rye variety 'Placido' F_1 – 3537 L ha^{-1}, because it had the highest grain yield (9.69 t ha^{-1}).

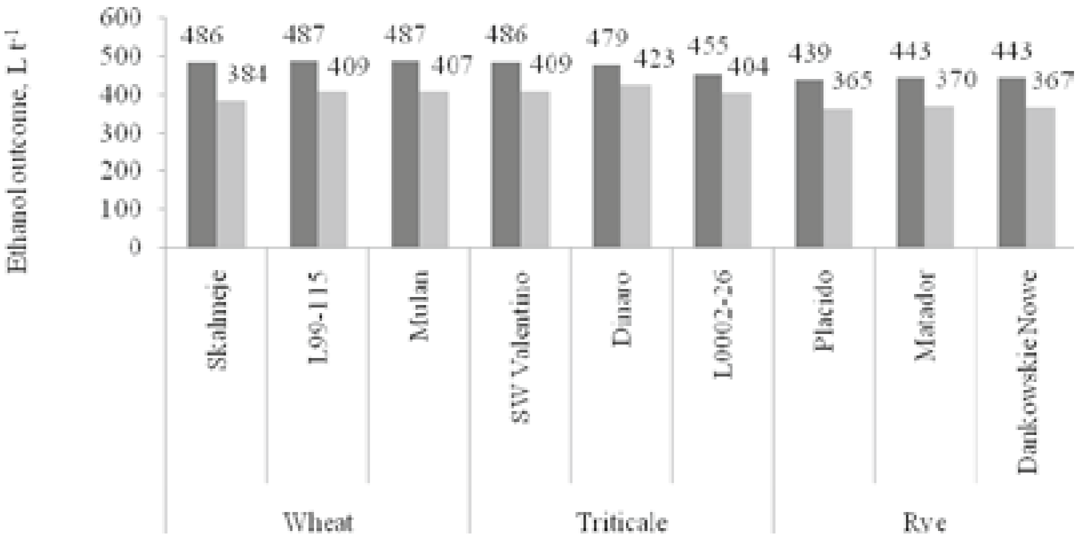

Figure 1. Theoretically calculated and practically obtained ethanol outcome, L t^{-1}, from winter cereals.
theoretically calculated ethanol outcome, L t^{-1}, practically obtained ethanol outcome, L t^{-1}

Evaluating the theoretically calculated and practically obtained ethanol outcome, the practically obtained outcome from all the examined varieties of winter cereals was 11-20% lower than the calculated outcome (Fig. 1). It can be explained by losses in the process of ethanol production (Dale and Tyner, 2006).

The most important qualitative characteristics that have to be taken into account while evaluating the suitability of species for bio-ethanol production, is the correlation between starch and crude protein. In order to determine the strength of correlation between starch and crude protein for each species, the analysis of data linear regression was carried out. Correlation between starch and crude protein content in triticale grains is described by a regression equation y = - 2.066x + 889.6. The determination coefficient R^2=0.744 shows that in 74% of cases the changes in starch content can be explained by the changes in crude protein content (Fig. 2).

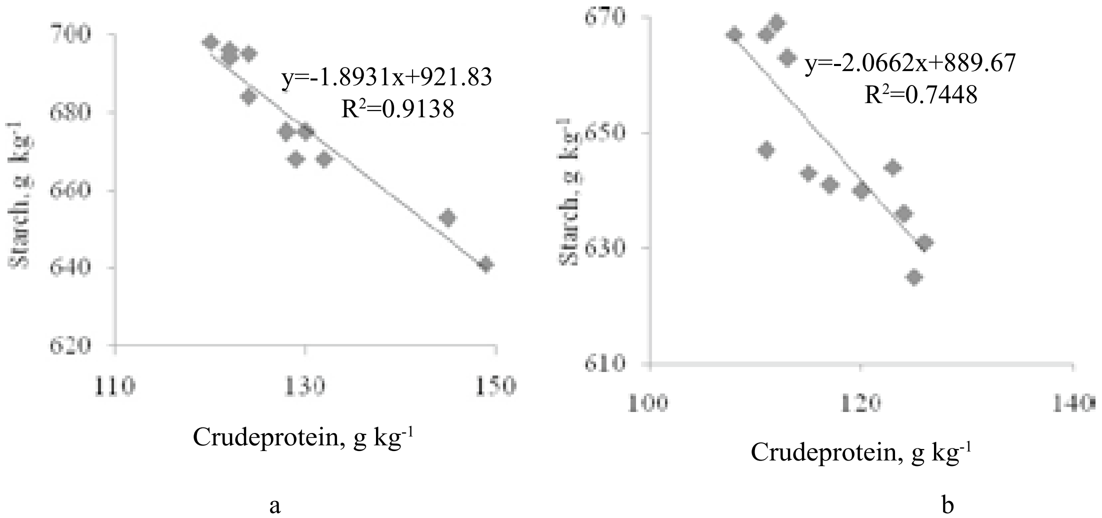

Figure 2. Correlations between crude protein and starch content in wheat (a) and triticale (b) grains.

Correlation between crude protein and starch content in wheat grain is described by a regression equation y = -1.893x + 921.8, R^2=0.913 (Fig. 2), but a significant correlation between these indices was not observed for rye (p>0.05) in this research period.

Correlation analysis was carried out comparing quality indicators of the winter cereals' yield and ethanol outcome. A positive correlation was determined between grain yield and ethanol yield ($r = 0.795 > r_{0.05} = 0.468$), between ethanol outcome and starch content ($r = 0.7122 > r_{0.05} = 0.468$), and between ethanol outcome and ethanol yield ($r = 0.633 > r_{0.05} = 0.468$), as well as a negative correlation between grain yield and crude protein content of grain ($r = -0.550 > r_{0.05} = 0.468$) was found for all the species of winter cereals. Evaluating the correlations it was confirmed that in order to obtain high ethanol outcome it is necessary to use the varieties of winter cereals with high starch content. A good grain yield is also necessary for obtaining a high ethanol yield. The analysis of correlations within the species shows that rye displayed deviations from the general tendency. The analysis of correlations for rye showed that there is a close correlation only between grain and ethanol yield ($r = -0.832 > r_{0.05} = 0.811$). No correlations between ethanol outcome and other indicators were found for rye. Such finding suggests paying attention to further investigations on the size of starch granules.

Conclusions

1. The average amount of grain yield of the examined winter cereal species did not differ significantly: it ranged from 8.46 to 8.91 t ha^{-1}. Evaluating the examined varieties and lines of each species, the best yield for wheat was obtained from the line '99-115' (9.00 t ha^{-1}), for triticale – the variety 'Dinaro' showed a significantly (p<0.05) better grain yield (9.65 t ha^{-1}), and for rye the best grain yield was obtained from the hybrid variety 'Placido' F_1 (9.69 t ha^{-1}).
2. In order to extract bio-ethanol it is important to use species and varieties of winter cereals with low crude protein content and high starch content. The results of the research showed that winter triticale practically provided the highest ethanol outcome (411.9 L t^{-1}) and ethanol yield (3607 L ha^{-1}). The average starch content of winter triticale was lower than that of winter wheat, while the grain yield was lower than the average yield of rye.
3. Of all the examined varieties and lines of winter wheat the highest bio-ethanol outcome was obtained from the new line '99-115' (409.4 L t^{-1}) and the variety 'Mulan' (406.8 L t^{-1}). The line '99-115' provided the highest ethanol yield (3684 L ha^{-1}), because this line had the best grain yield. As regards winter triticale, the highest ethanol outcome and yield was acquired from the variety 'Dinaro' (423.3 L t^{-1} and 4085 L ha^{-1} respectively), because this variety was observed to have

the highest starch content and the best grain yield. The highest ethanol outcome from winter rye was obtained from the variety 'Matador' (370.1 L t^{-1}), and the highest ethanol yield – from the hybrid rye variety 'Placido' F_1 (3537 L ha^{-1}), which provided the best grain yield.

4. Having evaluated the correlations between the grain quality indices, yield and ethanol outcome, it was found that the highest ethanol yield for winter wheat and winter triticale can be acquired from the varieties that provide the highest grain yield, high starch content and low crude protein content of the grain. As regards rye, the research has to be continued.

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