

The Effect of Year, Site and Variety on the Quality Characteristics and Bioethanol Yield of Winter Triticale

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ABSTRACT

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Results of three-year trials (2003 to 2005) conducted with four winter triticale varieties to investigate the effect of sites and years on quality characteristics and ethanol yield showed a statistically highly significant effect of sites ($P < 0.01$) on the quality parameters of test weight, falling number, protein content, starch content, grain yield and ethanol yield. Climate conditions showed a statistically highly significant effect ($P < 0.01$) on test weight, thousand grain weight, falling number, Zeleny sedimentation test, starch content, grain yield and ethanol yield. Percentage of complete grains and protein content were non-significant. The effect of the variety was highly significant ($P < 0.01$) on the quality parameters of test weight and percentage of complete grains and there was a significant effect ($P < 0.05$) on falling number, Zeleny sedimentation test and starch content. Regarding the bioethanol yield, the most suitable variety according to the measured values was the variety Modus (average 41.19 la/100 kg dry matter). The variety Disco provided the largest yield on the site Hradec nad Svitavou in 2005 (46.7 la/100 kg dry matter), which agrees with starch content. Year 2003, when the starch content in grain was at the lowest level (63.41%), resulted in the lowest bioethanol yield.

Key words: Bioethanol yield, quality characteristics, sites, triticale, varieties.

INTRODUCTION

Basic growing goals in all crops include an achievement of maximal production in sufficient quality. Triticale shows a number of advantages for the grower. The main distinguishing features are as follows: higher grain yield even in unfavourable conditions, higher test weight, resistance to soil-climatic conditions, tolerance to dryness, tolerance to more acid soils and a lower requirement of nutrient substances. Also, it does not need as much fertilizer when compared to types and varieties providing the same yields. Triticale has a lower susceptibility to diseases and pests¹⁶ which attack rye and wheat and this reduces the necessity of chemical protection against harmful agents.

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The main reason for the exploitation of cereals for the production of industrial alcohol is to acquire the largest possible quantity of renewable energy per unit of area. Cereals appear to be very advantageous in this respect. Moreover, to cultivate them, we do not need to provide as large an amount of energy as in sugar beet production¹⁷.

Based on analysis and results of numerous authors, triticale and wheat appear to be the most suitable choices for the production of bioethanol from cereals, and they provide more alcohol than rye when grown under comparable conditions^{2,18}.

In bioethanol production, the main criterion is a high content of starch and at least 60% is required. Other major factors are amylolytic activity, mainly alpha-amylase, viscosity index (FN), degree of starch gelation and some additional factors. There are correlations between ethanol yield and the viscosity index and between the amylogram value and saccharification temperature. On the other hand, ethanol yields are reduced by the protein content, which usually shows a negative correlation to starch. It is important to know the fermentable components and the autoamylolytic quotient (AAQ)⁷. The autoamylolytic enzymes of triticale are characterized by higher amylolytic activity in comparison to others cereals such as wheat and rye, which are positive for starch degradation. The content of starch depends on weather conditions and this affects the ethanol yield. There was variability of correlation between the starch and ethanol yield.

Ethanol yield is also influenced by the content of pentosans, rate of polymer digestion which relates to the viscosity index, and the ratio of soluble toward insoluble pentosans. Many authors highlight other variable factors that are dependant on cultivation conditions and climatic factors, and on the functional activity of enzymes²².

The triticale grain contains sufficient amounts of starch, which are easily susceptible to the enzymatic action. There is high activity of triticales' own amylolytic enzymes, mainly alpha-amylase, and this is crucial in starch saccharification^{1,15}. Considering the currently prevalent "cold" technique of saccharification, by means of bacterial enzymes (mostly imported), the processing of triticale is economically beneficial as it enables the reduction of the consumption of enzyme preparations by up to 50%²⁰.

This study contributes to the evaluation of year, site and importance of variety for a number of quality parameters for bioethanol yield from winter triticale grain.

Table I. The list of varieties, country of origin, breeder and year of registration.

Variety	Country of origin	Breeder	Registration in Czech Republic
Disco	Poland	DANKO Hodowla Roslin, Sp. z o. o.	1997
Kolor	Czech Republic	SELGEN, a. s., ŠS Úhřetice	1996
Modus	Germany	NORDSAAT Saatzeitgesellschaft mbH	1998
Presto	Poland	DANKO Hodowla Roslin, Sp. z o. o.	1990

Table II. Percentages of the precipitation normal in the period (April–August), in sites Staňkov (A), Hradec nad Svitavou (B), Krásné Údolí (C), Libějovice (D) (evaluated according Kožnarová and Klabzuba¹²).

Month	Precipitation normal (mm)				Percentage of precipitation normal ^a											
					2003				2004				2005			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
April	39	41	45	39	42 bn	76 n	54 bn	64 n	56 bn	104 n	52 bn	99 n	82 n	81 n	88 n	130 n
May	59	63	58	64	61 n	184 van	75 n	120 n	100 n	55 bn	123 n	122 n	134 an	113 n	104 n	139 an
June	67	80	65	89	86 n	14 exn	88 n	90 n	116 n	85 n	109 n	122 an	99 n	67 bn	112 n	105 n
July	70	79	76	91	105 n	112 n	137 an	49 bn	120 n	70 n	87 n	82 n	126 n	195 van	115 n	197 van
August	62	72	67	71	33 vbn	25 vbn	22 vbn	32 vbn	66 bn	101 n	70 n	72 n	99 n	122 n	86 n	164 an

^a ebn – extraordinary below normal, vbn – very below normal, bn – below normal, n – normal, an – above normal, van – very above normal, ean – extraordinary above normal.

Table III. Deviation of the temperature normal in the period (April–August), in sites Staňkov (A), Hradec nad Svitavou (B), Krásné Údolí (C), Libějovice (D) (evaluated according Kožnarová and Klabzuba¹²).

Month	Temperature normal (°C)				Deviation of temperature normal (°C)											
					2003				2004				2005			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
April	7.6	7.0	5.6	7.8	0 n	-0.7 n	0.2 n	-0.2 n	1.4 n	1.1 n	1.2 n	0.2 n	1.5 n	1.4 n	0.4 n	1.1 n
May	12.4	12.5	11.1	12.6	2.7 van	2.5 an	1.7 an	2.4 ean	-0.8 n	-1.8 bn	-1.6 bn	-1.1 n	0.7 n	-0.3 n	-0.9 n	0.4 n
June	16.1	15.2	14.1	16.1	4.1 van	3.6 van	4.9 van	3.5 ean	-0.7 n	-0.5 n	-0.7 n	-0.9 n	0.7 n	0.1 n	0.6 n	0.3 n
July	17.7	17.0	15.8	17.9	0.7 n	0.7 n	0.7 n	1.1 an	-0.6 bn	-0.5 n	-0.8 bn	-0.9 n	0.8 n	0.6 n	0.5 n	0 n
August	16.8	16.8	14.9	17.2	3.2 ean	2.2 van	4.3 ean	3.0 ean	1.3 an	0.6 n	1.4 an	1.3 an	-1.5 bn	-1.5 bn	-1.1 bn	-1.4 n

^a ebn – extraordinary below normal, vbn – very below normal, bn – below normal, n – normal, an – above normal, van – very above normal, ean – extraordinary above normal.

MATERIALS AND METHODS

Four triticale varieties were monitored between 2003 and 2005 in four sites. The sites are Central Institute for Supervising and Testing (UKZUZ) test stations. This guaranteed that field testing was carried out using consistent methodology. The samples of triticale grain were analysed at the Department of Food Technology, Mendel University of Agriculture and Forestry in Brno.

Variety of winter triticale

The following varieties were entered in the study – Kolor, Presto, Modus and Disco. The list of varieties used in this study, as well as their origin (country, breeder, year of registration), are given in Table I (variety details).

Characteristics of the cultivation sites

Staňkov (site A). The site is situated in a cereal production region, 370 m above sea level. The type of soil is orthic luvisol and this soil is loamy (medium). The previous crop in year 2003 was rape, in the year 2004 it was field peas, and in the year 2005 it was field peas.

Hradec nad Svitavou (site B). The site is situated in a potato production region, 450 m above sea level. The soil type is orthic luvisol and this soil is clay-loam (heavy). The previous crop during the watch period was spring wheat.

Krásné Údolí (site C). The site is situated in a forage production region, 647 m above sea level. The soil type is eutric cambisol and this soil is loamy-sand soil (light). The previous crop in year 2003 was spring barley, in year 2004 it was field peas, and in year 2005 it was rape.

Libějovice (site D). The site is situated in a cereal production region, 460 m above sea level. The soil type is eutric cambisol and this soil is sandy-loam (medium). The previous crop in years 2003 and 2004 was winter wheat, and in year 2005 it was oats.

Characteristics of climatic conditions on the sites and temperature data for 2003–2004

Site A. Average yearly temperature +8.1°C, average yearly sum of precipitation 537 mm.

Site B. Average yearly temperature +7.4°C, average yearly sum of precipitation 616 mm.

Table IV. Mean values of the examined traits. Grain and ethanol yield for varieties in 2003–2005.

Examined traits	Variety				D _{0.05}
	Kolor	Presto	Modus	Disco	
Test weight (g · L ⁻¹)	711.1	735.6	687.5	705.4	2.284
Thousand grain weight (g)	43.15	40.94	40.69	44.32	3.330
Percentage of complete grains (%)	92.52	88.50	86.13	94.66	4.170
Falling number (s)	83.1	93.6	94.8	66.3	22.190
Zeleny-test (mL)	24.13	22.80	20.16	23.88	2.778
Protein content (%) of dry matter	10.47	10.59	9.89	10.38	0.878
Starch content (%)	66.71	68.59	68.56	67.60	1.506
Grain yield (t/ha)	6.50	6.91	6.51	6.79	0.773
Ethanol yield (la/100 kg dry matter)	40.08	40.34	41.19	40.10	1.817

Table V. Mean values of the examined traits. Grain and ethanol yield for sites Staňkov (A), Hradec nad Svitavou (B), Krásné Údolí (C), and Libějovice (D) in 2003–2005.

Examined traits	Site				D _{0.05}
	Staňkov	Hradec nad Svitavou	Krásné Údolí	Libějovice	
Test weight (g · L ⁻¹)	671.5	710.4	727.7	732.4	2.284
Thousand grain weight (g)	40.92	42.82	43.14	42.21	3.330
Percentage of complete grains (%)	88.15	89.50	93.75	89.96	4.170
Falling number (s)	63.6	85.8	107.2	81.2	22.190
Zeleny-test (mL)	23.33	22.01	23.61	22.44	2.778
Protein content (%) of dry matter	13.05	9.90	9.10	9.28	0.878
Starch content (%)	65.33	68.10	69.37	68.68	1.506
Grain yield (t/ha)	6.31	6.59	7.61	6.21	0.773
Ethanol yield (la/100 kg dry matter)	39.15	41.68	41.24	39.64	1.817

Table VI. Mean values of the examined traits. Grain and ethanol yield in years 2003, 2004, and 2005.

Examined traits	Year			D _{0.05}
	2003	2004	2005	
Test weight (g · L ⁻¹)	735.8	710.7	685.0	1.978
Thousand grain weight (g)	43.53	43.93	39.36	2.884
Percentage of complete grains (%)	88.01	90.75	92.26	3.611
Falling number (s)	91.38	97.98	64.03	19.217
Zeleny-test (mL)	26.16	21.85	20.53	2.406
Protein content (%) of dry matter	10.19	10.23	10.58	0.760
Starch content (%)	63.41	70.53	69.67	1.304
Grain yield (t/ha)	6.20	7.98	5.86	0.669
Ethanol yield (la/100 kg dry matter)	39.35	40.44	41.48	1.573

Site C. Average yearly temperature +6.3°C, average yearly sum of precipitation 602 mm.

Site D. Average yearly temperature +7.9°C, average yearly sum of precipitation 563 mm.

Climatic characteristics in 2003–2005 are given in Tables II and III and evaluated according to Kožnarová and Klabzuba¹².

Quality parameters and methods

Samples of winter triticale were monitored for the following quality parameters and the following methods were used for analysis: test weight¹⁰ (g · L⁻¹) – ISO 7971-2, thousand grain weight (g) – weight of 1000 grains, percentage of complete grains (%) – percentage a part of grain above the sieve 2.5 × 22 mm, Falling number¹¹ (s) – ISO 3093, Zeleny⁹ test (mL) – ISO 5529, protein content – N × 5.7 (%) – Czech Standard⁵ 56 0512-12, starch content after Ewers (%) in dry matter) conversion factor 1.898 – Czech Standard⁴ 56 0512-16, grain yield – weight harvested grain from one hectare (yields per acre) and ethanol yield.

To determine the ethanol yield, a laboratory fermentation test was employed according to the procedure of Šimůnek et al.²¹ The enzyme Termamyl SC (Novozymes, Denmark) was used. It contains a thermostable alpha-amylase which hydrolyses the 1,4-alpha-glycoside bonds and the enzyme SAN Extra L amyloglucosidase which hydrolyses 1,4-, and also 1,6-alpha bonds in gelated starch and dextrans ((Novozymes, Denmark). Analysis of variance of multiple classifications was used for the result evaluation and the LSD method at 95% for more detailed evaluation. The results were processed in the programme Unistat, version 4.53 (Unistat Ltd., London, England).

RESULTS AND DISCUSSION

Average quality characteristics of identified ethanol yield including a statistical evaluation are shown in Tables IV, V, and VI.

According to the analysis of variance, **the test weight** was highly significantly ($P < 0.01$), influenced by the

year, site and variety. In the aggregate of the varieties and sites, the highest average value was in 2003 (735.8 g · L⁻¹), when the highest temperatures and lowest rainfall occurred during the vegetation season, while the lowest value was observed in 2005 (685.0 g · L⁻¹) where lower temperatures and highest rainfalls occurred. The Presto variety reached the highest test weight in the course of all recorded years, which also corresponds to the findings presented by Háp and Pelikán⁸.

In the **thousand grain weight** the differences were not significantly identified, with the exception of 2005, when the lowest average value was 39.36 g, which is in compliance with the test weight.

According to the average value, **percentage of complete grains** was very low in variety Modus (86.13%), which corresponds with the lowest values test weight and thousand grain weight in this variety.

The falling number (FN) was significantly influenced by the year and site, and quite clearly also by the variety. The very low value in 2005 (64.03 s) was influenced by low sub-normal temperatures and higher rainfall, which caused a higher activity of alpha-amylase; Variety Disco showed a similar low average value (66.3 s). In contrast, a relatively high average value (107.2 s) was reached on the site Krasné Údoli. The low FN is characterized by a high activity of amylolytic enzymes in the grain, which is a favourable property for the production of bioethanol. For this purpose⁶, the FN value must not exceed 150 s. Enzymatic activity is usually evaluated according to the activity of the amylolytic enzymes and is often closely associated with the activity of proteolytic enzymes; therefore, low FN values show evidence of a higher activity of the amylase complex and a stronger interference of starch⁸.

The average **crude protein content** in the recorded years was almost level (about 10.2%) and approached a protein content in the triticale of 10.8% as presented by Kunteová¹³, Aufhammer et al.³ pointed out that the production of ethanol requires a low protein content, maximum 11%, which the measured average values fulfilled. There was not a substantial difference between the varieties; only variety Modus showed a slightly lower protein content. Bigger differences appeared when the values of individual sites were compared. The highest value was measured in Staňkov (14.95%) in 2005, and the lowest average protein content was measured in Krásné Údolí (9.10%).

The starch content in the years 2003–2005 oscillated within a wide range of 61–74%, with the lowest values being identified in 2003 (average 63.41%), as opposed to the average of 70% measured in 2004 and 2005. Lower rainfall and higher-than-normal temperatures limited photosynthesis in 2003 and, as a consequence, the starch content in all of the sites. A considerable difference can even be seen between the particular varieties and sites. Presto and Modus varieties showed a markedly higher average starch content. The average starch content only reached 64% and was recorded in the site Staňkov in 2005, where the highest protein content was indicated. In contrast, the same year brought a very low protein content and an average starch content exceeding 72% in Krasné Údolí, which corresponded with a negative correlation between starch content and protein content. The starch

content was influenced by all the observed factors of year, site ($P < 0.01$) and variety ($P < 0.05$).

Grain yield

A high average yield (7.98 t) was achieved in 2004, which was by far the most productive year. In that year the increased yield exceeded the value of 10 t in variety Modus on the site Staňkov. Petr¹⁴ confirmed that the yield potential of triticale is significant; top yield reaching 7.5–8.0 tons per hectare. In 2005, the average yield approached 6 t and no considerable differences were found between the varieties.

The yield was also significantly affected by the previous crop type¹⁴. This was proven by a higher grain yield, for example on the site Krásné Údolí in 2004, where field peas were used as the previous crop in comparison with the yields in sites Libějovice and Hradec nad Svitavou, where cereals were used as the previous crop.

Bioethanol yield

According to analysis of variance, the differences in bioethanol yield were influenced by the year and the location conditions (Table VII). The results were converted to litres of 100% alcohol, which could be obtained from 100 kg of dry matter. The “la” abbreviation thereafter shall be used.

The low average bioethanol yield (39.35 la) in 2003 was influenced by an abnormally low content of starch (63.41%), as a result of high temperatures and low rainfalls at the time of grain maturation in all locations; with 2005 being the most favourable year (41.48 la). Year had a highly significant effect ($P < 0.01$) on the bioethanol yield.

The site Staňkov provided the lowest starch content, especially in years 2003 (63.45%) and 2005 (64.42%), which corresponded to the lowest average bioethanol yield. The low starch content in both years negatively affected the grain maturation by deficiency of rainfall and high temperatures. Favourable results were reached on the sites Hradec nad Svitavou (41.68 la) and Krásné Údolí (41.19 la). These sites had a highly significant effect ($P < 0.01$) on bioethanol yield.

As for the triticale varieties, Modus had the most favourable results; even without statistical significance, however; with the highest total average in bioethanol yield (41.19 la), which corresponded to the starch content in the above mentioned years. Rosenberger¹⁹ also presented high

Table VII. Influence of year, variety and site on the parameters of quality and ethanol yield according to analysis of variance.^a

Examined traits	Variety	Site	Year
Test weight	**	**	**
Thousand grain weight			**
Percentage of complete grains	**		
Falling number	*	**	**
Zeleny-test	*		**
Protein content		**	
Starch content	*	**	**
Grain yield		**	**
Ethanol yield		**	**

^a * $P < 0.05$, ** $P < 0.01$

bioethanol yields with Modus, having compared wheat yields (44.7 la), rye yields (42.8 la) and triticale yields (46.1 la). The variety Disco showed the best yields in 2005 (46.7 la/100 kg dry matter); and reached high yields in this year on the other sites except for Staňkov. Šimůnek et al.²¹ arrived at similar values of bioethanol yield, observing yields as high as 46.9 la/100 kg dry matter in selected triticale varieties.

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