Effect of growing conditions on starch and protein content in triticale grain and amylose content in starch

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ABSTRACT

The effect of growing conditions on starch and protein content in triticale grain and amylose content in starch was studied on winter triticale cvs. Kitaro, Lupus, Lamberto and Ticino harvested in 2006 and 2007 in Humpolec and Pernolec, Czech Republic. Dry matter of the investigated triticale grain contained 62.4–70.9% of starch. The starch content and amylose content in starch were significantly affected by cultivar and year. Statistical analysis did not confirm that starch content and amylose content in starch were significantly influenced by growing variant or location. The protein content was significantly affected only by year. The differences in protein content among cultivars, growing variants and locations were not significant. The protein content was negatively correlated with starch content (r = –0.83). Statistical analysis of data also confirmed a positive correlation between the starch content and amylose content in starch (r = 0.45).

Keywords: triticale; starch; amylose; amylopectin

Triticale (*X Triticosecale* Wittmack) is a man-made cereal. It is an amphiploid hybrid derived from wheat (*Triticum* L.) and rye (*Secale* L.). At present, triticale is the most widely grown cereal crop in some regions of Poland and Germany (Kulp and Ponte 2000). Winter types are more common in moderate climate in Europe, whereas spring types are more suited for warm and dry regions, such as Australia (Dendy and Dobraszczyk 2001).

Triticale is the crop with a potential of growing almost worldwide. It could be expected to become more important in association with progressive desertification, soil salinity and increasing acidity (Kulp and Ponte 2000). It is also suitable for growing in marginal regions and on all soil types. Its genetically determined resistance allows decreasing the amount of applied pesticides. Triticale is also resistant to numerous contaminants present in the soil (Zhang et al. 1998).

Triticale grain is mostly used for feeding. High grain yield, high saccharides content and activity of amylase enzymes predetermine the triticale grain for distilling and brewing (Kulp and Ponte 2000). According to Dendy and Dobraszczyk (2001), Kulp and Ponte (2000) and Petr et al. (1991), the food use of triticale is increasing. A main advantage of triticale use for this purpose is its unique nutritional quality. High proportions of albumins and globulins, and simultaneously a lower proportion of prolamin protein (gliadins) enhance digestibility of triticale-based products. High content of essential amino acid lysine, being about 20% higher than in wheat grain, is also important. The aleurone grain layer contains a large amount of minerals and cover layers are rich in fibre.

Triticale is used in food products, most commonly as part of multi-grain breads and specialty items. Cookies, muffins, tortillas, and pancakes are among the products that are particularly good with triticale instead of wheat. Nutritional quality is generally higher in triticale than in wheat (Doxastakis et al. 2002).

Supported by the Ministry of Agriculture of the Czech Republic, Project No. 1G57056.

General grain characteristics, i.e. structure, appearance and basic composition, are similar to those of parental cereals. Chemical composition of triticale grain is close to that of wheat, particularly in contents of protein, starch, non-starch polysaccharides and fats.

Starch is the major storage nutrient of plants. Grain dry matter contains from 55 to 75% (w/w) of starch (Velíšek 1999, Kulp and Ponte 2000). Native starch is a mixture of two homopolysaccharides composed of α-D-glucopyranosyl units – amylose and amylopectin. They usually occur in the 1:3 weight ratio.

Amylose is defined as a linear molecule of (1→ 4) linked α-D-glucopyranosyl units. The amylose molecule forms a helix with one reducing end (Whister and BeMiller 1999, Kulp and Ponte 2000). Amylose molecular weight is in the range between 180 and 1000 kDa (Velíšek 1999).

Amylose content in common cereal starches varies from 20 to 30% and is affected by climatic and soil conditions during grain development. In wheat, for example, it is decreased by high temperatures whereas low temperatures have an opposite effect (Kulp and Ponte 2000).

Amylopectin is highly branched and consists of chains (1→4) linked α-D-glucopyranosyl units joined through (1→6) linkages. Amylopectin is one of the largest biopolymers with typical molecular weight 10–200 MDa (Massaux et al. 2008). Amylopectin is present in all known starches, constituting about 75% of most common starches. Some starches consist entirely of amylopectin and are called waxy starches.

Starches and modified starches have an enormous number of food uses, including adhesion, binding, dusting, film-formation, foam strengthening, antistaling, gelling, glazing, moisture retention, stabilizing, texturizing, and thickening applications (Whister and BeMiller 1999). Nutritional and technological properties depend on the amylose to amylopectin ratio.

High amylose starch (> 40%) is used as thickeners and strong gelling agents. However, they are more susceptible to retrogradation. Amylose starch is exploited in the production of biodegradable plastic and food industry.

Amylopectin improves homogeneity, stability and texture of gelled starch, and enhances also stability of starch gel at frosting and defrosting of frozen foods (Massaux et al. 2008). Waxy cultivars are a raw material suitable for paper and textile industries, production of colours, adhesives and in civil engineering.

Total starch content in grain is, to a certain extent, negatively correlated with protein content (Kulp and Ponte 2000). Haberle et al. (2008) and Krejčířová et al. (2007) showed that protein content is affected by a growing system and by climatic and soil conditions.

Starch content in DM is negatively correlated with protein content.

The aim of this paper is to present results of two-year evaluation of the effect of growing conditions on starch content, amylose content in starch and protein content in triticale grain. A possibility to influence the amylose content in triticale starch by modifications of growing conditions, which can affect triticale food use, is also discussed.

MATERIAL AND METHODS

The research was carried out on winter triticale cvs. Kitaro, Lupus, Lamberto and Ticino harvested in 2006 and 2007. The cultivars were grown in two variants differing in nitrogen rate – 90 kg N/ha (L variant) and 120 kg N/ha (H variant). Triticale was grown in experimental fields at two locations in the Czech Republic.

The characteristics of the locations are given in Table 1. The sowing date in Humpolec was from 6 to 9 October and the seeding rate was 5 million viable seeds per hectare. Phosphorus (25 kg/ha) and potassium (60 kg/ha) were applied in the autumn. The stands were treated with the Maraton herbicide at a dose of 4 l/ha in November, fertilized with 45 kg/ha nitrogen in the early spring and with 45 kg/ha nitrogen at the end of tillering (L variant). In H variant, the treatment in the autumn was identical, i.e. 25 kg/ha phosphorus and 60 kg/ha potassium. In November, the stands were treated with the Maraton herbicide at a dose of 4 l/ha. The stands were fertilized with 30 kg/ha nitrogen in the early spring, 60 kg/ha nitrogen at the end of tillering and additional 30 kg/ha nitrogen before the end of stem elongation using a solid form. In May, the Juwel Top fungicide at a rate of 0.8–1.0 l/ha was applied. Triticale was grown in plots of 10 m^2. Grain samples for analyses were collected at harvest.

The sowing date in Pernolec was from 30 September to 11 October and the seeding rate was 5 million viable seeds per hectare. In November, the stands of both variants were treated with Affinity WG (2.25 l/ha) + Glean 75 WG 7 (l/ha) in 2005 and Syntop (1.5 kg/ha) in 2006. In April 2006, the stands were treated with the Mustang herbicide at a rate of 0.6 l/ha. In April 2007, her-

Table 1. Characteristics of Humpolec and Pernolec growing locations

Location	Humpolec	Pernolec
Altitude	525 m	530 m
Latitude	49°32' N	49°46' N
Longitude	15°32' E	12°41' E
Climatic region	mildly genial 4	mildly genial 4
Climatic subarea	B1 – mildly genial, mildly humid, upland	B5 – mildly genial, mildly arid, upland
Long-term average annual temperature	6.5°C	7.1°C
Long-term average annual total rainfall	667 mm	559 mm
Pedologic characteristics		
Taxonomical soil category	stagno-gleyic Cambisol	stagno-gleyic Cambisol
Textural class	sandy loam	sand-clay
Parent rock	paragneiss	orthogneiss
Topsoil depth	cca 25 cm	25–28 cm
Content of available nutrients		
P (mg/kg)	43	145
K (mg/kg)	187	130
Mg (mg/kg)	95	108
Ca (mg/kg)	1 411	1 643
pH/KCl	5.0	6.8
Humus	2.60%	2.78%

bicides Logran (8 g/ha) + Starane (0.4 l/ha) were applied. In addition, the stands of H variant were treated with Sportak HF (1 l/ha) in June 2006 and with Caramba (1.2 l/ha) + Retacel in May 2007. The levels and timings of fertilization with basic nutrients were identical to those at the Humpolec location.

Meteorological data from both growing seasons confirm that the weather course was similar at both locations. The weather in Humpolec was warm in the growing seasons 2005–2006 and 2006–2007. The average annual temperature was about 1.9°C higher in 2005–2006 (about 2.7°C higher in 2006 to 2007) than long-term average annual temperature. The growing season 2005–2006 was wet. The annual total rainfall was about 126 mm higher than long-term average annual total rainfall. The highest rainfall was recorded in March, May and June. The growing season 2006–2007 was normal.

The weather in Pernolec was warm in the growing seasons 2005–2006 and 2006–2007. The average annual temperature was about 1.6°C higher in the growing season 2005–2006 (about 3.1°C higher in 2006–2007) than long-term average annual temperature. The growing season 2006–2007 was wet. The annual total rainfall was about 163 mm higher than long-term average annual total rainfall. The growing season 2005–2006 was normal.

Amylose content. The amylose content in starch was determined using an Amylase/Amylopectin Assay Kit of the Megazyme. The amylose content is given in percent (w/w).

Protein content. The protein content was assessed by a method according to ICC standard No. 167:2000. It was determined as total nitrogen content multiplied by 5.7. The protein content was converted to dry matter (DM) and expressed in percent (w/w).

Starch content. The starch content in grain DM was assessed using a method according to EN ISO 10520:1999, which determines a method for polarimetric assessment of starch content. The starch content is related to DM and given in percent (w/w).

Statistical analysis of data. The significance of the effect of cultivar, location, growing variant and year on grain characteristics was evaluated by analysis of variance (ANOVA) at a level of significance $\alpha = 0.05$. All statistical analyses were done using Statistica 7.1 software (StatSoft, Inc.).

Table 2. Starch, protein and amylose contents (%)

Cultivar	Location	Variant	2006			2007			2006–2007		
			starch*	amylose**	protein*	starch*	amylose**	protein*	starch*	amylose**	protein*
Kitaro	Humpolec	H	62.4	23.1	12.4	68.0	25.9	10.1	65.2	24.5	11.3
		L	64.0	22.9	11.3	68.5	23.7	9.1	66.3	23.3	10.2
	Pernolec	H	65.4	23.1	12.0	68.0	23.3	9.5	66.7	23.2	10.8
		L	66.0	25.5	11.5	68.8	26.4	8.6	67.4	26.0	10.1
Lamberto	Humpolec	H	63.8	24.1	11.1	67.2	25.1	10.3	65.5	24.6	10.7
		L	66.1	23.0	10.5	67.8	24.6	9.4	66.9	23.8	9.9
	Pernolec	H	65.4	24.3	11.8	68.5	21.7	8.4	66.9	23.0	10.1
		L	65.6	25.3	11.1	69.0	25.7	8.2	67.3	25.5	9.6
Lupus	Humpolec	H	65.1	21.7	10.9	68.9	24.3	9.5	67.0	23.0	10.2
		L	66.4	20.8	10.9	69.6	24.8	9.0	68.0	22.8	10.0
	Pernolec	H	65.1	23.1	11.7	68.8	22.7	8.6	66.9	22.9	10.1
		L	65.6	23.4	11.6	69.3	24.9	8.0	67.4	24.2	9.8
Ticino	Humpolec	H	67.2	23.8	11.7	68.8	25.5	10.5	68.0	24.7	11.1
		L	67.6	23.7	11.3	69.1	25.9	9.4	68.3	24.8	10.4
	Pernolec	H	66.8	25.3	11.5	69.3	26.1	9.1	68.0	25.7	10.3
		L	67.1	25.7	11.0	70.9	25.6	8.8	69.0	25.7	9.9
Total			65.6	23.7	11.4	68.8	24.8	9.2	67.2	24.2	10.0

*starch and protein content in dry matter; **amylose content in starch

RESULTS AND DISCUSSION

Starch content. The DM of triticale grains contained 62.4–70.9% of starch (Table 2). These values were close to the upper boundary of starch content reported for cereal grain (Velíšek 1999, Kulp and Ponte 2000). The starch content was significantly affected by cultivar and year (Table 3). It is consistent with findings reported by Labuschagne et al. (2007) and Massaux et al. (2008) that starch content is significantly affected by cultivar and year.

A comparison of cultivars (Table 3) documented that the highest mean starch content was found in cv. Ticino (68.4%) and the lowest in cv. Kitaro (66.8%).

The starch content in DM was significantly affected by weather during the growing season in 2006–2007. Table 3 shows that the starch content in triticale grain harvested in 2007 was on average by 3.2% higher than that in 2006. It means that warm weather during the growing season in 2006–2007 had a significant positive effect on starch content.

Statistical analysis of data did not confirm that starch content was significantly influenced by growing variant or location. Mean starch content 66.8% in H variant and 67.6% in L variant did not significantly differ. The samples from Humpolec and Pernolec averaged 66.9 and 67.5% of starch, respectively. The difference between these values was not significant.

Amylose content. The amylose content in starch ranged from 20.8 to 26.4% (Table 2). The assessed values generally correspond to values reported for common cereals (Kulp and Ponte 2000). Furthermore, Table 2 shows that the highest content of amylose (26.4%) was found in cv. Kitaro grown in Pernolec, in L variant, in 2007. The lowest amylose content (20.8%) was in cv. Lupus grown in Humpolec, in L variant, in 2006.

The amylose content in starch was, as well as starch content, significantly affected by cultivar and year (Table 3). The confirmed significance of the effects of cultivar and year on amylose content in starch agrees with findings published by Labuschagne et al. (2007) and Massaux et al. (2008).

A comparison of the years (Table 3) documented that the amylose content in starch was higher in 2007 than that in 2006 with the difference of 1.1%.

Table 3. Effect of experimental factors on selected grain quality parameters of winter triticale (%)

Factor	Factor level	Starch*		Amylose**		Protein*	
Harvest year	2006	65.6	a	23.7	a	11.4	b
	2007	68.8	b	24.8	b	9.2	a
Cultivar	Kitaro	66.4	a	24.2	ab	10.6	a
	Lamberto	66.7	ab	24.2	ab	10.1	a
	Lupus	67.3	ab	23.2	a	10.0	a
	Ticino	68.3	b	25.2	b	10.4	a
Location	Humpolec	66.9	a	23.9	a	10.5	a
	Pernolec	67.5	a	24.5	a	10.1	a
Variant (crop management)	L	67.6	a	24.5	a	10.0	a
	H	66.8	a	23.9	a	10.6	a

*starch and protein content in dry matter; **amylose content in starch

This finding is in accordance with conclusions reported by Kulp and Ponte (2000) that the weather during the growing season can significantly affect the amylose content in starch.

Neither the effect of growing variant nor location was confirmed (Table 3). The conclusion by Nowotna et al. (2007) that the modification of nitrogen fertilization level can influence the amylose content in triticale starch was not confirmed.

Protein content. The protein content in DM varied between 8.0 and 12.4% (Table 2). The highest content (12.4%) was assessed in cv. Kitaro grown in Humpolec, in H variant, in 2006. The lowest protein content (8.0%) was found in a sample of cv. Lupus grown int Pernolec, in L variant, in 2007.

Statistical analysis of data confirmed that the protein content was significantly affected only by year (Table 3). Mean protein content was higher in 2006 (11.4%) than in 2007 (9.2%). The correlation between protein content and weather conditions is well-known and was published (Unrau and Jenkins 1964).

Table 3 shows that the differences in protein content among cultivars, growing variants and locations were not statistically significant.

Table 4. Correlation coefficients

Parameters	Amylose	Starch	Protein
Amylose	1.00	0.45*	–0.26
Starch	0.45*	1.00	–0.83*
Protein	–0.26	–0.83*	1.00

*statistically significant values

Correlations of parameters. Kulp and Ponte (2000) state that the starch content is in negative correlation with protein content, which was confirmed by the assessed value of correlation coefficient (–0.83) (Table 4).

In addition to the presented results, statistical analysis of data also confirmed a positive correlation between the starch content and amylose content in starch (0.45).

The results from a two-year evaluation of the effect of growing conditions on starch content, amylose content in starch and protein content in triticale grain documented a significant positive correlation between the starch content and amylose content, and a negative correlation between the starch content and protein content. The starch content in grain DM and amylose content in starch were significantly influenced only by cultivar and year. The effects of location and growing variant were not confirmed. So we did not succeed in proving that modification of the growing variant can influence amylose content in starch and thus to affect its usability for various modes of processing. The protein content was significantly affected only by year. The effect of other factors was not statistically significant.

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Received on June 23, 2009

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