Better Bread from Vigorous Grain?

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Abstract

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A high seed vigour is a desired trait in agronomy as it promotes the fast field emergence and homogeneity of harvest, which is necessary for producing quality food raw material. In this work, we studied the effects of the seed vigour on the bread quality. Relationships between the grain vigour, nutrients and anti-nutrients, contents, and bread volume were evaluated using the samples acquired from official variety trials. In the trials, the grain vigour was perceived as the grain ability to germinate in stress conditions, i.e. at 10°C in a solution of polyethylene glycol (PEG 6000 at osmotic pressure – 2 bars, the so called permanent wilting point). The results showed that the locations and years changed the bread volume but not the rank of the varieties tested in the trait. A higher grain vigour was significantly related to a higher falling number during two of the three trial years. The vigour was negatively related to the lipase activity. Moreover, other decomposing enzymes showed a similar tendency as was that found in the samples with the most diverse vigour. The grain samples with 80–90% vigour produced the greatest bread volume. The grain with a vigour below or above this range produced less voluminous loaves. The varieties of the highest quality produced the most voluminous bread from the samples reaching the grain vigour of 90–95%. The standard germination test was not related to the vigour and was less responsible for the bread quality. High-quality varieties had higher contents of total polyphenols than the varieties of lower quality, and the polyphenol content was correlated with the vigour (r^2 = 0.19**) and bread volume (r^2 = 0.08*). It can be concluded that the grain vigour is genetically controlled, thus the bread quality could be improved by grain breeding.

Keywords: wheat; grain vigour; bread volume; polyphenols; cultivars

A generally accepted measure of objective bread quality is the volume of bread baked from an invariable amount of wheat flour. The bread volume is related to quantity and quality of gluten (gliadins and glutenins). Cereals naturally contain a wide variety of polyphenols that have many protective ef-

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fects on human health, including the improvement of the function and redox state of immune cells in unhealthy or aged subjects – they are considered as antioxidants (Alvarez *et al.* 2006). Polyphenols are concentrated in the outermost layers of bran (Liyana-Pathirana & Shahidi 2007).

There is no visible difference between the viable and non-viable grain, but due to the natural deterioration caused by aging the grain vulnerability to external challenges increases and the viable grain survival capacity decreases. Grains can be distinguished by the germination test which is the official seed testing procedure of ISTA (International Seed Testing Association). However, not all grains germinating under optimal water and temperature conditions, in which the test is conducted, are able to emerge and develop into satisfactory plants under field conditions. Therefore, this ability is evaluated by tests of vigour. However, so far only few of those have been recommended for routine testing. We have been using a test during which grains germinate under the temperature and drought stress (Chloupek *et al.* 1997, 2003).

Grain vigour is negatively influenced by the following factors: premature harvest, late harvest (especially during rainy weather), unsuitable agronomy procedures and environmental conditions, self-heating of moist grain or drying of too moist grain, mechanical damage during harvest, drying and cleaning, and ageing of grain. Grain aging is directly proportional to its level of vigour (the lower the vigour the faster the aging) at the time of harvest and the storage conditions. A lower vigour has been related to a lower field emergence rate (Chloupek *et al.* 2003).

The advantages of fresh food are well-known, e.g. a higher content of vitamin C in freshly harvested potatoes in comparison to those long-term stored ones. Based on these facts, we wondered whether the same applies to wheat. Thus the objective of our study was to find out whether flour and bread made from fresh wheat grain (characterised by a high vigour) had similar advantages for consumers. Therefore, the contents of appropriate enzymes, polyphenols, wet gluten, nitrogen compounds, falling number of flour, and sensory characteristics of bread were evaluated for this purpose.

MATERIAL AND METHODS

22 varieties and candivars[1] of winter wheat (*Triticum aestivum* L.) from 2000–2002 harvests grown on four locations were tested for the bread volume in the National Variety Office Brno. The results of the variety testing for the bread volume were analysed by variance analysis to assess the importance of the respective factors for this trait. Four of the cultivars (Ludwig, Samanta, Sulamit, and Svitava) were evaluated also in the subsequent experiments.

In 2004, 2005, and 2006, forty five samples, i.e. nine cultivars – Akteur (E[2]), Darwin (A), Globus (B), Hedvika (B), Ludwig (E), Rheia (B), Samanta (B), Sulamit (E) and Svitava (B), harvested on five locations (Table 1) were evaluated. In 2004, the vigour was 71–98% (the average was 91.8%), in 2005, it was 55–97% (average 81.3%), and in 2006, the vigour reached a very high figure of 85–99% (the average was 94.1%), thanks to the favourable weather. All the grain samples were sown in the official state variety testing field where *candivars* are grown in four replications.

Ten selected samples that were most diverse in regard to the grain vigour (11 to 98%, the average was 74%) harvested in 2004 were evaluated in the Federal Institute for Food and Nutrition in Detmold, Germany. The unusual differences in vigour were caused by the differences in precipitation amounts during individual harvest periods – a high precipitation resulted in a low vigour, harvest conducted during the periods of low precipitation preserved a high vigour. The Sulamit variety was sown on six locations, Darwin on two, and Akteur and Hedvika on one each, i.e. only one variety was of lower quality. The samples were evaluated for the activity of selected enzymes and the contents of some nutrients and antinutrients. Concerned were the following: α-amylase, protease (essayed through the production of yellow 4-nitro-anilin concentration produced from colourless N-benzoyl-L-arginin-4-nitroanilid, L-BAPA), peroxidase, and lipase (triacylglycerol acyl hydrolase EC 3.1.1.3 which split triacylglycerols with fat acids longer than 12C).

In 2005 and 2006, ten samples of five selected varieties harvested on two locations were evaluated

[1]Candidate for variety or for cultivar (Jensen 1988), i.e. the lines tested for official registration. It means that the breeding was completed and the developed lines are distinguishable, uniform, and stable.

[2]*E* – elite suitable, *A* – very good suitable, *B* – good suitable, *C* – unsuitable varieties for bread making

for the contents of wet gluten, nitrogen compounds, falling number, and sensory characteristics in the Institute of Chemical Technology in Prague. Furthermore, flours of nine varieties harvested in 2005 and 2006 on three locations were evaluated for the contens of polyphenols in the Food Research Institute Prague.

The grain vigour was evaluated as the percentage of grains that had germinated within 14 days at 10°C in a solution of polyethylenglycol 6000 at osmotic pressure of –2 bars which is considered the limit for the persistent wilting of plants as described for barley (Chloupek *et al*. 2003). The grain vigour was not closely related to germination measured in optimal conditions for the germination (20°C and optimum water conditions). The time between harvest and the vigour evaluation was long enough to overcome the seed dormancy.

The grain samples were milled on the Chopin laboratory mill to particle size of 160 μm; the extraction rate corresponded to white flour of standard quality, containing 0.55% of ash. The grain vigour and bread volume were estimated at the site of the Mendel University of Agriculture and Forestry in Brno. The volume was assessed by putting the loaf in a calibrated container which was then filled up with oil rape seed; the more voluminous loaf, the less seed was necessary. The nitrogen compounds content, falling number, Zeleny-test, and gluten content in flour were estimated in the Plant Breeding Station Branišovice. The total amount of total phenolic compounds was measured in March and September 2007. The samples were harvested on the locations in Čáslav, Hradec nad Svitavou, and Žabčice, as shown in Table 1. The seeds were extracted in the Soxhlet Apparatus with an ethanol-water mixture (80:20 V/V) for 20 hours. The extract was adjusted to a volume of 250 ml and 5 ml aliquots were then pipetted into 50 ml volumetric flasks. After diluting the individual aliquots with 80% water-ethanolic solution to approximately 30 ml, to each 2.5 ml of Folin-Ciocalteau reagent was added, the blend was agitated and mixed. Then 7.5 ml of 20% sodium carbonate solution was added, the volume was made up to the mark with distilled water, and after a thorough agitation, the mixture was left for two hours to allow for the quantitative formation of blue colour. The same procedure was used for blank but instead of 5 ml of sample solution, 5 ml of 80% ethanol was used. After two hours standing, the solution was centrifuged at 2000 cpm for 12 min and the total amount of phenolic compounds was measured spectro-photometrically at the wavelength of 765 nm and expressed as gallic acid (Lachman *et al*. 1997). The evaluation was done in the Food Research Institute Prague. The polyphenols contents in nine varieties as averaged from six environments are presented in Table 3, and their contents in six environments as averaged from nine varieties are displayed in Table 4. Table 5 shows the statistical evaluation.

The bread samples were baked in the *Panasonic* automatic oven that is commonly used by plant breeders for the assessment of wheat quality, according to J. Stragliati and M. Taylor (Nickerson Company, Maintenon, France). Bread was made from flour (300 g), dry yeast (3 g), margarine (10 g), sugar (10 g), salt (5 g) and water (25°C, 190 ml) as described by Hanišová *et al*. (1995). The wheat quality test is broadly used in plant breeding even in such cases where constant water absorption without optimising the mixing time is used in the measurement process.

The values of the vigour and volume were evaluated by the analysis of variance, and their mutual relationships were assessed by the linear correlation analysis. The percentage of the total variation factors was expressed by the comparison of the mean squares roots, i.e. by standard deviations for the factors. The percentage of the unexplained variation is the measure of accuracy for each trait

Table 1. Characteristics of locations where the samples for our evaluation were grown

Locations	Altitude (m)	Long-term	
		annual precipitation (mm)	averaged annual temperature (°C)
Čáslav	260	555	8.9
Hradec/S.	450	616	7.4
Oblekovice	242	435	9.3
Věrovany	207	502	8.7
Žabčice	180	480	9.2

Table 2. Analysis of variance of 22 varieties for bread volume made from samples harvested in three years on four locations

Source of variation	Degrees of freedom	Percentage of total variation and significance
(a) Cultivars	21	17.6**
(b) Locations	3	12.6**
(c) Years	2	41.9**
Interaction a × b	63	3.6
a × c	42	4.6
b × c	6	15.5**
Unexplained variation	126	4.2

**$P = 0.01$

evaluated. The evaluation was carried out manually using a calculator according to the textbook guide (Chloupek 1996) because the data was limited.

RESULTS

Factors influencing bread volume

The results (Table 2) showed the prevailing effect of the years, followed by the varieties, interactions of locations and years, and locations. It is generally known that the traits genetically controlled by quantitative trait loci, such as the bread volume, are mostly influenced by years and locations. The bread volume is also massively influenced by the gluten quantity and quality. The quantity is controlled by only few loci. The gluten content, on the other hand, is controlled genetically, but also very significantly, by the nitrogen availability. This explains the prevailing factors. The insignificance of the interaction between the varieties and the locations and years is important because it proves that good varieties are not affected by locations and years. This finding is important for breeders and producers. However, the combination of selected varieties and particular locations can produce grain for superior bread, as will be shown later.

Grain vigour and flour characteristics

The effect of locations prevailed in most parameters, but as concerns the vigour, only in the year of a generally high vigour. In 2004, the vigour

Table 3. Content of polyphenols, grain vigour, and bread volume with nine varieties as averaged from six environments (three locations in two harvest years)

Cultivar	Content of polyphenols (mg/kg)	Grain vigour (%)	Bread volume (ml)
Akteur	1714[a]	89.7[b]	1868[bc]
Darwin	1730[a]	86.3[ab]	1853[abc]
Globus	1712[a]	84.8[ab]	1860[bc]
Hedvika	1650[a]	91.2[b]	1668[a]
Ludwig	1621[a]	89.2[b]	1888[c]
Rheia	1656[a]	86.2[ab]	1802[abc]
Samanta	1597[a]	86.3[ab]	1685[ab]
Sulamit	1648[a]	80.2[a]	1970[c]
Svitava	1591[a]	85.3[ab]	1690[ab]

Average values in a column marked, with the same letter are not significantly ($P_{0.05}$) different did not significantly correlate with any of the traits evaluated (bread volume, content of nitrogen compounds, falling number, Zeleny-test, content of gluten, and gluten index). In all 45 samples harvested in 2005, the linear correlation analysis showed that, a greater grain vigour was significantly related to a higher falling number (r^2 = 0.12*), and to the wet gluten content (r^2 = 0.79**), the content of nitrogen compounds (r^2 = 0.46*), the falling number (r^2 = 0.72**), and less important subjective sensory characteristics (r^2 = 0.41*).

The vigour in 2006 samples also correlated with the falling number (r^2 = 0.17**), a higher content of gluten (r^2 = 0.10*), and the tendency to a higher content of nitrogen substances (r^2 = 0.06). Germination was not significantly related to any of the characteristics only negative correlation was observed with the content of nitrogen substances (r^2 = 0.12*). Therefore, the vigour appears to be is more important for the bread quality than germination.

Activity of some enzymes in relation to flour and bread quality

The grain vigour was negatively related to the lipase activity (r^2 = 0.78**). Furthermore, insignificant negative correlations were found with peroxidase-, protease- (BAPA), and α-amylase-activities. It appears that all the enzymes evaluated were less active in flours made from vigorous grain.

Grain vigour and bread volume

The grain vigour greatly varied during the experimental years, as stated above. The relationship between the grain vigour and bread volume was non-linear as can be seen in Figure 1. The bread volume from 2005 harvest increased with the vigour rising up to 86–89%, the more vigorous samples producing less voluminous bread. The samples with a vigour of 55–74% produced bread of an average volume of 1711 ml, the samples with a vigour of 86–89% produced bread volume of 1810 ml, but with the most vigorous samples (90–97%), the volume was only 1579 ml.

As presented in Figure 1, 2006 samples with a small range of vigour (85–99%) showed a negative correlation with the bread volume (r^2 = 0.12*). There were no significant correlations of the vigour with either germination or the weight of 1000 grains. No correlation existed between the vigour and germination (r^2 = 0.04). The figure shows that the grain with the vigour of 85–90% produced the larges bread volume. Since the grain and/or flour of different varieties, locations, and different harvest years as well are mixed for the bread production, also combined samples from both harvest years

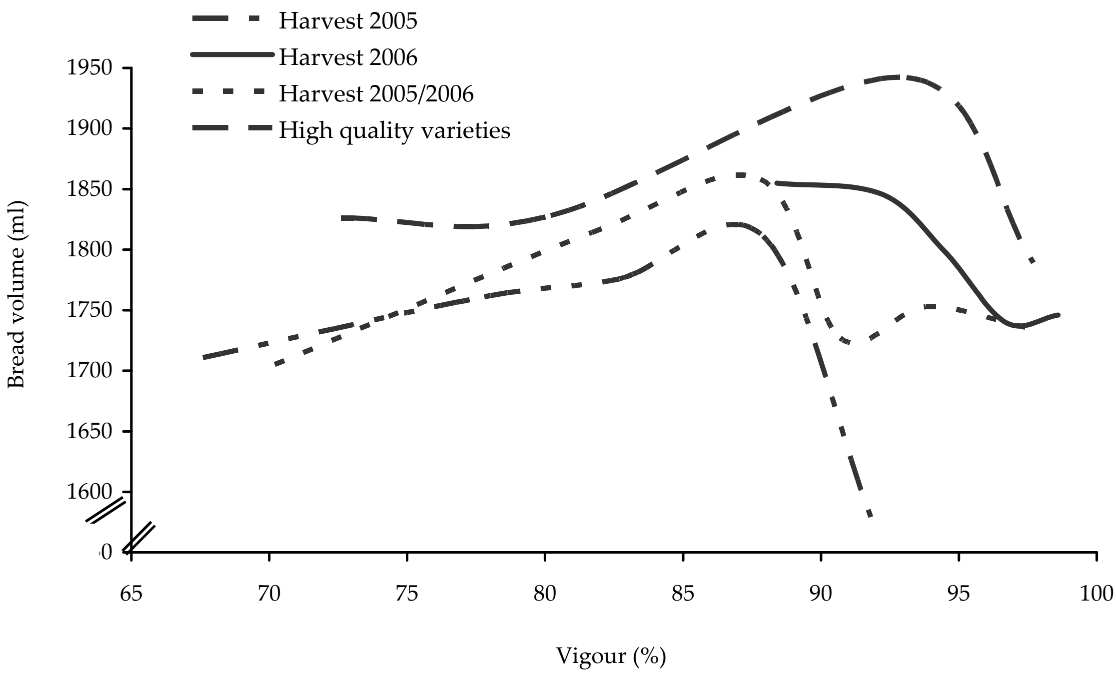

Figure 1. Relation between grain vigour and bread volume in similarly numerous groups

Table 4. Content of polyphenols, grain vigour, and bread volume from three locations and two harvest years as averaged from nine varieties

Location	Harvest year	Content of polyphenols (mg/kg)	Grain vigour (%)	Bread volume (ml)
Čáslav	2005	1478	80.3	1757
	2006	1743	91.3	1831
Hradec/S.	2005	1486	76.3	1649
	2006	1846	76.3	1649
Žabčice	2005	1553	85.6	1908
	2006	1839	94.9	1826

Statistical evaluation is given in Table 5

Table 5. Analysis of variance for the content of total polyphenols, grain vigour and bread volume in samples of nine varieties, harvested on three locations in two years

Source of variation	df	Mean squares with significance (percentage of variation) for		
		polyphenols (mg/kg)	grain vigour (%)	bread volume (ml)
Cultivars	8	15 710 (8)	62 (11)	67 337* (28)
Locations	2	33 462 (12)	201* (20)	47 691 (24)
Years	1	1 242 453** (70)	1 837** (60)	78 585 (30)
Unexplained variation	42	24 669 (10)	44 (9)	26 301 (18)

df – degrees of freedom, *significant at $P_{0.05}$ (probability of error max. 5%); **significant at $P_{0.01}$ (probability of error max. 1%)

were evaluated, i.e. for all 90 samples, altogether. In this case, the 85–90% vigour samples generated the largest volume again (Figure 1). The relationship between the vigour and volume was possibly linear, however, some other factors intervened in the course of the individual harvest years, perhaps related to the harvest, etc.

However, the relationship between the volume and vigour differed in four high quality varieties (three of the *E* and one of the *A* groups) since they had a higher bread volume in comparison to the other varieties (averages for 2005 and 2006 were 1843 and 1760 ml) respectively, but the same vigour as all nine varieties (87.5 and 87.7%, respectively). The vigour optimum for the bread volume was higher – 91–95%, as displayed in Figure 1. This could be explained by the enzymatic activity. The Hedvika (B) variety showed the lowest activity of protease and peroxidase, but the highest activity of α-amylase, and an average activity of lipase in comparison to the nine samples of the higher quality varieties grown on different locations.

Relationships between the grain vigour, bread volume, and of polyphenols content

The analysis of variance (Table 5) showed that the year factor was a significant source of variation in regard to the content of total polyphenols as well as the vigour. The bread volume, on the other hand, was significantly influenced by varieties. However, in regard to all three characteristics the years dominate as the main source of variation. The vigour estimation was the most accurate due to the percentage of the unexplained variation (9%), followed by the polyphenols estimation (10%), and the bread volume (18%). The measured characteristics were in the range of 1069–1995 mg/kg for the total polyphenols content, 55–97% for the grain vigour (which is common in the milling industry), and 1110–2120 ml/300 g for the bread volume.

Table 6. Similarly frequent group means differentiated by polyphenol content with appropriate grain vigour and volume of bread (54 samples, tracing to nine varieties grown in two years on three locations) in four similarly frequent groups

Averaged content of polyphenols (mg/kg)	Averaged vigour of grain (%)	Averaged volume of bread (ml)
1377	79	1725
1630	86	1846
1770	90	1767
1869	92	1898

The correlation analysis showed a highly significant correlation (r^2 = 0.19**) between the grain vigour and the content of total polyphenols. Nine varieties, three locations, and two years were used in the calculation in order to simulate the practice of the milling industry where a mixture of different varieties is milled to achieve the required quality of flour. Likewise, grain coming from different locations is mixed, too. Moreover, the bread volume was significantly related to the content of polyphenols (r^2 = 0.08*). No significant correlation was found between the grain vigour and bread volume (r^2 = 0.02), probably due to the non-linear relation mentioned above. The means of similarly frequent group differentiated due to the content of polyphenols with the appropriate means of other two characteristics are given in Table 6, to show the variation of the characteristics.

The E and A varieties assessed on the basis of the data obtained from six environments (two years and three locations) were only slightly better than those of the B group. Four cultivars of the E and A qualities contained 1678 mg of total polyphenols, and five varieties of the B quality had 1641 mg of total polyphenols in 1 kg of flour.

DISCUSSION

Only fully mature caryopses, particularly those with a high protein concentration, can have a high vigour. This is apparent from the significant correlations between the content of nitrogen compounds and the vigour. The protein content in wheat was related to suitable precipitations of 500–550 mm (Garrido-Lestache *et al.* 2004), in particular during the time of protein accumulation (May) and at temperatures between 26 and 27°C. However, N-fertilisation had the main impact (Lopez-Bellido *et al.* 2001). Greater varietal differences in regard to the grain vigour were found in varieties with generally low vigour grown during unfavourable years. In favourable years, the influence of location prevailed (Chloupek *et al.* 2003); the present results show a similar tendency.

A living tissue has a reducing ability which is used in the official method of the International Seed Testing Association: The colourless trifenyltetrazoliumchlorid or -bromid are in the seed living tissues are reduced to the red stable trifenylformazan. The reducing ability can be diminished by free radicals produced during seed deterioration connected with the loss of vigour. Less vigorous grain showed a higher activity of enzymes that decompose the flour substances, which could explain the relation between the vigour and the bread volume, characterised by a curve. In our experiments, a lower volume of bread made from very vigorous grain was linked to a lower lipase activity, and can be also explained by the increase in specific volume resulting from a greater expansion of individual cells when a new form of lipase was added (Guys & Sahi 2006). Furthermore, there are other enzymes that influence the loaf volume; e.g. pentosanase (Koyuncu *et al.* 2008), glucose oxidase, and hemicellulase, in particular in combination with ascorbic acid. The effects are dependent on the amount of the enzyme and the initial quality of the wheat flour (Dagdelen & Gocmen 2007). However, no relationship was found between the enzyme antioxidant activities and the seed quality (Lehner *et al.* 2006). The gluten content correlated positively with the falling number (Pasha *et al.* 2007), and in our experiments also with the grain vigour.

The accelerated ageing of the wheat grain at 40°C and 100% relative humidity completely inhibited germination after 14 days. The content of free radicals in flour increased and that of carotenoids decreased during the process. Carotenoids are a group of more than 700 very common yellow, orange, and red lipophilic pigments that occur in all organisms capable of photosynthesis

(Velíšek *et al.* 2008). A similar carotenoid profile was found in the wheat species examined (Abdel *et al.* 2007).

Reduction of soluble proteins contents and degradation of glutenins and gliadins were observed, associated with a substantial increase of protease activity and a decrease of gluten flexibility (Galleschi *et al.* 2002). These results support our findings – less vigorous grain had a lower content of total polyphenols which are known antioxidants (Alvarez *et al.* 2006). This can also be supported by the work of Fujii *et al.* (2007) who confirmed that grape seed polyphenols might help prevent oxidative stress as well as reduce the production of free radicals. The content of free radicals increases during seed ageing as they probably consume polyphenols. The products prepared from germinated flours showed a significant decrease of polyphenols together with an increase in starch and protein digestibility (Reema & Sadana 2004). It appears that polyphenols are vital for the initial phase of the plant life – a proper germination and field emergence, and therefore, their content was low in germinated flour. This has been supported by Wilson and McDonald (1986) according to whom the seed deterioration was connected with the oxidative effect on the membrane lipids. Free radical attack can be reduced by scavenger and antioxidant compounds found in seeds (McDonald 2004) which can explain the relationship between the content of polyphenols and the grain vigour. This result was found with the best quality flour, i.e. flour of a low extraction rate, although most polyphenols are located in bran (Liyana-Pathirana and Shahidi 2007).

Not only the grain vigour but also the bread quality is directly affected by pests and diseases. The *Fusarium* fungi damage grains, which results in a substantial reduction of the loaf volume. The loss of dough functionality and loaf volume potential was attributed to the presence of fungal proteases (Nightingale *et al.* 1999). Alpha-amylase of *F. culmorum* may damage starch granules, inducing unsatisfactory bread quality (Wang *et al.* 2005). Certain insects deposit salivary enzymes into immature wheat grain while feeding. These enzymes survive in harvested wheat, destroy gluten structure in dough, and cause poor quality of bread. The levels of alpha-amylase differentiated the damaged grains from the rest (Every *et al.* 1992). However, insects that damage wheat during storage have similar effect as those in the field.

The effect of years can be attributed to different weather conditions but also to different periods of time between the harvest and chemical evaluation of polyphenols. The polyphenol contents in 2005 and 2006 harvests amounted on average to 1506 and 1809 mg/kg, respectively. However, the reality of practice has an impact here as bakeries use flours of different ages. A significant effect of varieties and locations on the content of total polyphenols and its increase caused by controlled accelerated ageing was found with barley and pea grains (Lachman *et al.* 1997). In seeds, the ability to withstand desiccation during maturation includes the activation of antioxidant defence systems (Bailly *et al.* 2001), synthesis of dehydrins etc. However, our results that evaluated the naturally occurring grain vigour revealed a lower content of polyphenols in low-vigour samples. Varietal differences can be related to the colour of grain – pigmented varieties of sorghum had higher antioxidant levels (Dykes & Rooney 2006). The genes for phenol colour of wheat grain are located on the long arms of chromosome 2A and chromosome 2B, respectively (Watanabe *et al.* 2004). The colour can also be changed by polyphenol oxidase causing darkening and discoloration of wheat foods; the responsible gene(s) is (are) located on chromosome 2A (Anderson & Morris 2001). These data support the explanation of varietal differences regarding the content of polyphenols. Our results conform with those of Kováčová and Malinová (2007) who described a high correlation between a ferulic acid and total phenolic compounds. Both substances were positively related to the grain vigour, although ferulic acid only insignificantly.

Wheat grain, as a living organised unit, contains a natural multitude of enzymes and enzyme systems. For many of those, the physiological functions have not yet been clearly established. However, enzymes can be active also in non-physiological conditions. During wheat processing and in the dependence on the technological regimes, the activities of different endogenous enzymes may have considerable effects on the quality of the end-products.

Breeding could develop genotypes combining a high protein content, a high protein quality, and a low polyphenol oxidase level to produce varieties with superior bread quality (Habernicht *et al.* 2002). It is generally known that different varieties produce different bread qualities and this is the foundation for the classification of varieties into different classes based on the loaf volume that each variety is able to achieve. Some wheat varieties have some stable quality traits but some unstable ones, suggesting that the genetic factors involved in the genotype differ between traits by environment al interactions (Grausgruber *et al.* 2000). This finding was supported by Švec *et al.* (2007) who reported that the Bezostaja variety technological quality was more influenced by the harvest year than the American variety Jagger. A new single gene for the loaf volume on chromosome 3A was reported on recently (Law *et al.* 2005). A higher content of total phenolics in corn was found when it was grown organically and sustainably than when it was grown conventionally (Asami *et al.* 2003).

The highly significant correlation coefficient between the grain vigour and the content of polyphenols ($r^2 = 0.19^{**}$) determines only 19% of the variation (determination coefficient $D = r^2$). High values of the determination can be expected only between traits controlled by simple/few gene(s), when they are linked, i.e. positioned closely on a chromosome. But such complex traits like the grain vigour or the loaf volume are controlled by many minor genes (quantitative traits loci, QTLs) located on different chromosomes, and such a connection cannot be expected between the grain vigour and the bread volume, even if a single gene for the trait has been described, as stated above. However, the explanation of the 19% of polyphenols content variability by the grain vigour was significant. It is generally known that the grain vigour, bread volume, and polyphenols content are influenced by many factors. Therefore, they cannot be expected to be comprehensively determined by a single factor.

CONCLUSION

Our results can be seen as a contribution to the high quality bread production – bread from superior varieties grown in selected locations and of high grain vigour. Grains of superior cultivars – Akteur (E), Darwin (A), and Ludwig (E) grown in Žabčice had a vigour of 89% on average, 1611 mg/kg of polyphenols, andprovided the bread volume of 1955 ml in 2005. A vigour of 94%, 1876 mg/kg of polyphenols, and bread volume of 1940 ml in 2006 in comparison to other varieties grown on all locations, 79%, 1483 mg/kg, and 1748 ml in 2005, and 92%, 1802 mg/kg and 1810 ml in 2006, respectively.

Because in many years, the harvested grain is of a low vigour and in such years the varietal influence is higher than in other years (Chloupek *et al.* 2003), millers and bakers could use the grain vigour criterion to achieve a higher bread quality production in such years. For this purpose, we have identified suitable varieties (Hedvika, Akteur, and Ludwig) that have a significantly greater grain vigour in comparison to other varieties, and this may encourage plant breeders to use the grain vigour trait as the selection criterion.

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