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BAKING QUALITY AND HIGH MOLECULAR WEIGHT GLUTENIN SUBUNIT COMPOSITION OF EMMER WHEAT, OLD AND NEW VARIETIES OF BREAD WHEAT

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ABSTRACT

Emmer wheat [(*Triticum diccocum* (Schrank) Schuebl] is a tetraploid wheat species that used to be wide-spread in former times. Lately, it has become a more interesting crop, as it has specific parameters. There are only few bred varieties and landraces available. The particular parameters of Emmer wheat baking quality were studied and evaluated in two different localities in 2007, 2008 and 2010. The research was carried out with six landraces of the emmer wheat (spring forms), in comparison with old and modern varieties of bread wheat (*Triticum aestivum* L.). High molecular weight glutenin subunits (HMW-GSs) were analysed and characterised as well. The emmer wheat had a high proportion of crude protein in grain (16.05-19.00%). On the other hand, the landraces reached lower values of the usual indicators of the baking quality, such as a gluten index (23-39) and Zeleny sedimentation index (14.58-17.58 ml), than the old and modern bread wheat varieties. HMW-GSs characteristics also confirmed the unsuitability of emmer wheat for the baking industry. Therefore, the emmer wheat grain is considered a valuable material having a high concentration of proteins and might be good for unbaked products, such as pasta, biscuits, müsli or various types of mush.

Key words: emmer wheat, bread wheat, landraces, baking quality, HMW glutenin subunits

INTRODUCTION

Cultivated emmer wheat, *T. dicoccum* [syn. *T. turgidum* L. subsp. *dicoccon* (Schrank) Thell.], is a tetraploid species (BA - genomes) belonging to the *Triticum* L. genus (Zaharieva et al., 2010). Emmer wheat had a long tradition of being grown and used in the human diet (Marconi and Cubadda, 2005). It has rarely been bred and nowadays only old cultivars, landraces and wild forms can be found. Because of increasing requirements for richness, diversity and good quality in food products, interest in this species of wheat is increasing (Peleg et al., 2007). The areas devoted to emmer wheat have been decreasing even in countries with a less well developed farming sector (Marconni and Cubadda 2005). It is still grown in extreme mountain conditions in small areas of the Balkan, the Caucasus and in India and Ethiopia (Yenagi et al., 1998), Turkey (Karagoz, 1996), and in Italy and Spain (Troccoli and Codianni, 2005). Tolerance to drought is one the most important advantages of this cereal (Zaharieva et al., 2010). This is an important aspect of crop growing (Bucur et al., 2006; 2010). Landraces of emmer wheat do not compare to the modern bred varieties of wheat in profitability (Ehdaie et al., 1991). The varieties of emmer wheat are however less demanding and more adaptable (Dengcai et al., 2003; Kotschi 2006), therefore, they are suitable for low-input or organic farming systems (Marino et al., 2009). When grown in marginal areas, they provide a lower, but a more stable yield level (Collins and Hawtin 1999), and they may contribute, as organic products, to a higher economic profit than other conventional varieties of cereals (Pardo et al., 2009). Emmer wheat was used in foodstuff production in the past (Marconni

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and Cubadda 2005). The suitability of emmer wheat grains to modern technologies is a serious issue which should be considered.

An important part of emmer wheat seed are storage proteins. These proteins are generally considered a suitable tool for diversity studies and cultivar identification and as excellent markers of the gluten strength in durum and common wheat (Rodriguez-Quijano et al., 1990; Nakamura 2001; Branlard et al., 2003; Bradová and Šašek 2005; Gregova et al., 2007). A major class of wheat storage proteins comprise glutenins that are divided into two groups according to their molecular characteristics i.e. high (HMW-GSs) and low (LMW-GSs) molecular weight glutenin subunits. The HMW-Gs are coded at the *Glu-1* loci, located on the long arm of group-1 homologous chromosomes; whereas the LMW-Gs are coded at the *Glu-3* loci, located on the short arm of the same homologous group (Jackson et al., 1996). As far as we know, HMW-GS composition has not been studied in emmer wheat yet, especially in relation to bread making quality.

The objective of this study was to analyse the suitability of emmer wheat as an alternative crop for profitable organic food production. In this paper we report (1) the results of the research comparing the crude protein content, (2) analysis and evaluation of the technological quality using standard methods of baking quality analysis and (3) composition of high molecular weight glutenin subunits (HMW-GSs). Comparing the above-mentioned characteristics between the emmer wheat landraces, and old or modern bread wheat varieties was another objective of the study.

MATERIAL AND METHODS

The evaluated varieties came from the Gene bank in the Crop Research Institute (CRI) in Prague-Ruzyně (Prague). Six genetic resources of emmer wheat (*Triticum dicoccum* SCHRANK) were chosen (Table 1). Four old varieties of bread wheat (*Triticum aestivum* L.) were added to the study. They are not grown nowadays, even though they were grown between 1917 and 1975 (Table 1). Two modern bread wheat varieties (*Triticum aestivum* L.), Vánek (2005) and SW Kadrilj (2006), were chosen as control varieties. All varieties are spring ones. Varieties were sown in a randomised, complete block design on experimental plots in CRI Prague, and at the University of South Bohemia in České Budějovice (CB) during 2007, 2008 and 2010. The seeding rate was adjusted for a density of 350 germinable grains per m^2.

Table 1. List of studied varieties

ECN[1]	BCHAR[2]	Name	Origin	Variety	R[3]
Triticum dicoccum (SCHRANK) SCHUEBL					
01C0200117	412064	Horny Tisovnik	CZ	var. *rufum* SCHUEBL	-
01C0200947	412048	Ruzyne	-	var. *rufum* SCHUEBL	-
01C0201262	412051	Tapioszele 1	-	var. *serbicum* A. SCHULZ	-
01C0201282	412017	Tapioszele 2	-	var. *rufum* SCHUEBL	-
01C0203989	412013	Kahler Emmer	D	var. *dicoccum*	-
01C0204501	412013	No. 8909	-	var. *dicoccum*	-
Landraces of *Triticum aestivum* L.					
01C0204158	635100	Kundan	IND	var. *meridionale* MANSF.	-
01C0200051	635104	Rosamova přesívka	CZ	*milturum* (ALEF.) MANSF.	1917
01C0200008	635090	Praga	CZ	*lutescens* (ALEF.) MANSF.	1968
01C0200100	635090	Jara	CZ	*lutescens* (ALEF.) MANSF.	1975
Controll varieties of *Triticum aestivum* L.					
01C0204800	635090	Vanek	D	var. *lutescens* MANSF.	2005
01C0204877	635000	SW Kadrilj	S	var. *lutescens* MANSF.	2006
Remarks: [1] ECN = identificator; [2] BCHAR = taxonomical code; - = unknown; [3]registration					

Land and climatic characteristics of the research locations

The years were characterised by a higher temperature compared to the mean year temperature and mean temperature in the growing season. 2007 was a warmer year and the highest temperature was registered at the station in Prague during 2008 (1°C higher than the average for July). In April 2007, there was a precipitation deficiency which led to a considerable decrease in field emergence. The precipitation was balanced and stable in the following season. 2008 was characterised by an average level of precipitation. 2010 was characterised by a slightly lower temperature, whereas the precipitation rate in the growing period was above average.

Location 1: Crop Research Institute in Prague (50°08´N, 14°30´E) altitude of 340 metres above sea level; mean annual temperature of 7.9°C; mean annual precipitation rate of 472 mm. Proportion of mineral nitrogen is satisfactory, pH is alkaline, proportions of phosphorus, potassium and magnesium are satisfactory.

Location 2: University of South Bohemia in České Budějovice (48°98´N, 14°45´E) altitude of 388 metres above sea level; mean annual temperature of 8.2°C; mean annual precipitation rate of 620 mm. Proportion of mineral nitrogen is low, pH is slightly alkaline, proportions of phosphorus, potassium and magnesium are satisfactory.

Laboratory analyses

Baking quality: The following parameters were tested after the harvest and dehulling of the grains, according to methods recommended by the International Association for Cereal Chemistry (ICC). The methods used included: crude protein content (ICC 105/2), sedimentation index - Zeleny test (ICC 160/1), wet gluten content (ICC 106/2), gluten index (ICC 155) and starch content (Ewers polarimetric method). For the milling, standard mills and particle size of flour or scrap according to above mentioned norms were used.

For HMW-GS analyses, glutenins were extracted from single crushed wheat kernels using a 0.25M Tris-HCl buffer (pH: 6.8) containing 5% (*v/v*) β–mercaptoethanol, 2% *(w/v*) SDS, 10% (*v/v*) glycerol, and 0.02 (*w/v*) bromophenol blue; the extract was heated at 100°C for 2 min and centrifuged for 2 minutes at 15,000 RPM. The electrophoretic patterns of HMW-GSs were determined by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), using the Laemmli buffer system (Laemmli, 1970).

The acrylamide/bisacrylamide concentration (*T*) and the cross linker (*C*) used were as follows: T = 10% and C = 2.60%. Electrophoresis was performed at a constant current of 30mA/gel at 10°C for the time required in order for the tracking marker dye to migrate off the gel. Protein in the gels were fixed for 1 hour with 10% (*w /v*) trichloroacetic acid solution and subsequently stained with 0.5% (*w/v*) Coomassie Brilliant Blue R-250 solution, 25% (*v/v*) methanol, and 10% *(v/v)* acetic acid. Destaining was carried out with running water. Particular alleles of HMW-GSs were identified according to the published catalogue of Payne and Lawrence (1983) (Table 2).

Statistical data processing

The statistical data were processed by instruments of basic statistics, analysis of variance including the evaluation of a possible influence of each factor (ANOVA), where the Statistica 9.1 (StatSoft, Inc.) program was used. Correlations and interactions were evaluated by the regression and correlation analyses. The Tukey HSD test provided a comparison of varieties and classification of varieties into statistically different categories, according to the obtained values.

RESULTS

Mean values of three-year research, carried out in two different localities, indicated high crude protein content in grains of all the evaluated emmer wheat landraces (Table 3). Protein content was much lower in old and control bread wheat varieties. The difference between the highest value (Tapioszele 2 - 19.00%) and the lowest value of the crude protein content in grain (SW Kadrilj - 13.55%) reached 5.45%. The results of our research also showed that the emmer wheat varieties had a less stable concentration of crude protein in grain (CV = 12%) than the control varieties (CV = 9%). Table 4 (analysis of variance - ANOVA) presents a possible explanation of such results. Locality was a dominant factor (76%), as the two localities differed in the content of nitrogen in the soil. The correlation analysis (Table 5) indicated a logical ($P<0.001$) negative correlation ($r = -0.83$) between the crude protein content and the starch content (Table 3).

Fluctuation in wet gluten content in emmer wheat (Table 3) might have been induced by an application of a method of analysis, inadequate for this species. Most of the studied and evaluated landraces reached higher values of wet gluten content than old and control bread wheat varieties. As there was a positive correlation (Table 5) between the wet gluten content and crude protein content ($r = 0.64$), the wet gluten content was significantly influenced ($P<0.01$) by the locality (37%) and by the year (32%) (Table 4).

HMW-GSs are usually used as an indication of the genetic potential of bread wheat varieties, from the point of view of baking quality. This was done using the *Glu-1* score (Payne et al., 1987), which allocates scores for the respective subunits (alleles) in each of the wheat's three genomes (*Glu-A1, Glu-B1, Glu-D1*). This is mainly associated with the HMW glutenin subunits encoded at the Glu-D1 locus, which is obviously absent in emmer wheat. The bread making quality of emmer wheat was also low, as described by two other characteristics (gluten index and Zeleny sedimentation). The composition of HMW-GSs and mean values of the gluten index and the Zeleny test (for each variety) are shown in Table 2. Results showed that the emmer wheat varieties differed from the bread wheat varieties in the polymorphism of HMW-GSs.

As all the emmer wheat varieties had lower values of gluten index and Zeleny sedimentation than the control varieties (Table 2), they are also supposed to have consequently worse baking quality. The factor of locality played an insignificant role for both varieties and all the tested and evaluated characteristics (Table 4). The values of gluten index seriously fluctuated in the test. This was caused ($P<0.01$) by the influence of the locality (41%) in particular (Table 4). The values of the Zeleny test were influenced ($P<0.01$) by an interaction of locality and year.

Different HMW-GS alleles *Glu-A1* were noticed in the studied emmer wheat varieties, (Table 2), but this did not have visible effect on quality. Old bread wheat varieties (Kundan, Rosamova přesívka) containing the subunit 2* (*Glu-A1*) reached similar values and results of the Zeleny sedimentation test to the control varieties, being characterised by good baking quality.

Table 2. Characterisation of HMW-GSs

Variety	Gluten index	Zeleny test (ml)	HMW-GSs		
			Glu-A1	*Glu-B1*	*Glu-1D*
Triticum dicoccum (SCHRANK) SCHUEBL					
Horny Tisovnik	24	18	2^*	21	-
Ruzyne	37	15	1	7+8	-
Tapioszele 1	23	17	2^*	6+8	-
Tapioszele 2	28	16	1	7+8	-
Kahler Emmer	34	15	1	7+8	-
No. 8909	39	15	1	7+8	-
Old varieties of *Triticum aestivum* L.					
Kundan	79	56	2^*	7+8	2+12
Rosamova přesívka	50	52	2^*	7+9	5+10
Praga	36	34	1	7+9	5+10
Jara	32	41	1	7+9	2+12
Control varieties of *Triticum aestivum* L.					
Vanek	90	56	1	7+9	5+10
SW Kadrilj	80	57	1	14+15	5+10

Table 3. Basic Baking Quality Parameters (mean ± SD)

Variety	Crude protein (%)	Wet gluten (%)	Gluten index	Zeleny test (ml)	Starch content (%)
Triticum dicoccum (SCHRANK) SCHUEBL					
Horny Tisovnik	16.96±2.3deg	21.26±18.0f	24±36.0a	17.58±6.3a	64.13±2.1ab
Ruzyně	18.59±2.3ef	43.99±12.1acd	37±34.8a	15.17±5.0a	63.23±4.3ab
Tapioszele 1	16.05±2.5bcd	24.43±13.0ef	23±36.3a	17.42±7.6a	63.89±2.5ab
Tapioszele 2	19.00±2.4f	47.46±7.7a	28±35.2a	15.58±4.7a	61.78±3.0a
Kahler Emmer	18.86±1.8ef	47.64±6.4a	34±31.7a	14.58±5.0a	62.19±1.7ab
No. 8909	18.04±1.7efg	47.11±6.4ac	39±33.6a	14.58±3.4a	62.91±2.0ab
Old varieties of *Triticum aestivum* L.					
Kundan	15.06±1.7abcd	36.94±7.5bcd	79±6.7b	55.50±4.3b	62.92±2.7ab
Praga	14.79±1.6abc	39.83±12.3abcd	50±20.3a	51.83±10.5b	62.81±2.3ab
Jara	14.26±1.3ab	34.87±7.7bd	36±27.7a	33.75±12.3c	63.51±1.9ab
Rosamova	16.23±1.8cdg	46.31±8.8ac	32±13.6a	41.33±7.8c	61.95±2.4a
Control varieties of *Triticum aestivum* L.					
Vánek	13.90±1.4a	32.94±6.7be	90±6.5b	56.00±7.0b	64.23±1.2ab
SW Kadrilj	13.55±1.2a	33.52±5.8be	80±4.4b	56.83±9.5b	64.66±1.7b
Different letters indicate statistical differences between varieties for Tukey HSD test, $P<0.05$					

Table 4. Effect of Experimental Factors on Basic Baking Quality Parameters of Emmer (ANOVA)

Factor	*df*	Crude protein		Wet gluten		Gluten index		Zeleny test		Starch content	
		MS	%TV	MS	%TV	MS	%TV	MS	%TV	MS	%TV
Variety (1)	5	25^{**}	7	2748^{**}	32	811^{**}	1	33^{**}	3	15^{**}	4
Locality (2)	1	268^{**}	76	3114^{**}	37	26040^{**}	41	46^{**}	4	250^{**}	67
Year (3)	2	13^{**}	4	337^{**}	4	17700^{**}	28	248^{**}	20	41^{**}	11
1x2	5	1^{**}	0	397^{**}	5	231^{**}	0	4^{*}	0	9^{**}	2
1x3	10	3^{**}	1	106^{**}	1	631^{**}	1	29^{**}	2	14^{**}	4
2x3	2	43^{**}	12	1637^{**}	19	18096^{**}	28	863^{**}	70	37^{**}	10
1x2x3	10	1^{**}	0	161^{**}	2	271^{**}	0	14^{**}	1	7^{**}	2
Error	72	0	0	0	0	2	0	1	0	0	0
* statistically significant $P<0.05$; ** highly statistically significant $P<0.01$; ^{ns}not significant											

Table 5. Results of the Analysis of Correlation of Baking Quality Parameters of Emmer

Parameter		mean	SD	1	2	3	4	5	6
Crude protein	1	17.91	2.26						
Volume weight	2	734.49	30.11	0.13^{ns}					
Wet gluten	3	38.65	15.43	0.64^{***}	0.15^{ns}				
Gluten index	4	30.81	32.36	0.49^{***}	-0.40^{***}	-0.04^{ns}			
Zeleny	5	15.81	5.26	0.01^{ns}	-0.31^{**}	-0.45^{***}	0.67^{***}		
Falling number	6	354.85	78.74	-0.27^{**}	0.34^{***}	0.15^{ns}	-0.77^{***}	-0.67^{***}	
Starch content		63.02	2.64	-0.83^{***}	-0.09^{ns}	-0.48^{***}	-0.38^{***}	0.04^{ns}	-0.67^{ns}
Remark: Statistically significant * $P<0.05$; ** $P<0.01$; *** $P<0.001$; ^{ns} = not significant									

DISCUSSION

Some authors confirmed the fact, noticed also in our experiment, that protein content in the emmer wheat grains is higher (Marconni and Cubadda, 2005) than in the grains of modern bread wheat varieties (Jitareanu et al., 2005) (when both are grown in the same conditions). Many authors present the explanation that the emmer wheat landraces are more adaptable to the growing environmental conditions (Trčková et al., 2005), but can also be related to lower grain yields. From that, we can conclude that high crude protein content in grain is a clear advantage of the emmer wheat landraces when compared to old and modern control bread wheat varieties.

High molecular weight of glutenin subunits (HMW-GSs) plays in general a major role in determining the viscoelastic properties of dough, thereby determining bread making qualities (Šrámková et al., 2009). The HMW-GS alleles *Glu-A1* (subunits 1, 2*) (Vasil and Adrerson, 1997) and *Glu-B1,* (subunits 7+8, 7+9) have a favourable effect on dough properties (Cornish et al., 2005) Subunit 2*, encoded at *Glu-A1*, which is less frequently represented in modern common wheat, could be used in breeding for increased glutenin diversity of common wheat; and consequently could open up new use possibilities in the baking industry (Stehno et al., 2010). Application of the positive effect of the subunit 2* of the Kundan variety in breeding may be limited by the presence of the subunit 2+12 (*Glu-1D*), which is associated with worse baking quality (Vasil and Anderson, 1997). Rosamova přesívka seems to be an interesting breeding material among the old bread wheat varieties, as it also contains the subunit 2* (*Glu-A1*), which is usually connected with better baking quality (Horáková et al., 2010). This cultivar may be used as a genetic resource of this glutenin subunit in the breeding process; as it contributes to an improvement of the baking quality of wheat plants, and is rarely represented in the modern bred varieties.

On the other hand, the emmer wheat varieties differ from the bread wheat varieties in the polymorphism of HMW-GSs (Bradová and Šašek, 2005). When evaluating the baking quality using standard methods (the Zeleny sedimentation test, gluten index) and taking HMW-GS characteristics into account, it is obvious that the emmer wheat landraces are not suitable for classical bakery processing. Nevertheless, grain of the emmer wheat landraces is prospective material for the production of many traditional and new food products. It might be suitable for the production of non-yeasty products, such as mush, müsli, pasta, biscuits, etc.

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