Baking Quality Parameters of Wheat in Relation to Endosperm Storage Proteins

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Summary

Wheat storage proteins of twelve winter wheat cultivars grown at the experimental field of the Agricultural Institute Osijek in 2009 were studied for their contribution to the baking quality. Composition of high molecular weight glutenin subunits (HMW-GS) was analyzed by SDS-PAGE method, while the proportions of endosperm storage proteins were determined by RP-HPLC method. Regarding the proportion of storage proteins, results of the linear correlation ($p<0.05$) showed that protein (P) and wet gluten (WG) content were highly negatively correlated with albumins and globulins (AG) and positively with α-gliadins (GLI). A strong negative correlation between AG and water absorption (WA) capacity of flour was found, while α-GLI had positive influence on this property. Dough development time (DDT) was positively significantly correlated with HMW-GS and negatively with AG. Degree of dough softening (DS) was strongly positively affected by γ- GLI and gliadins to glutenins ratio (GLI/GLU) and negatively by total GLU and HMW-GS. Dough energy (E) and maximum resistance (R_{MAX}) were significantly positively affected by Glu-1 score and negatively by GLI/GLU ratio. Resistance to extensibility ratio (R/EXT) was significantly negatively correlated with total GLI. Bread volume was significantly negatively influenced by AG.

Keywords: wheat cultivars, baking quality, endosperm storage proteins, SDS-PAGE, RP-HPLC

Introduction

Wheat (*Triticum aestivum* L.) seed storage proteins represent an important source of food and energy and are involved in the determination of baking quality. The protein content in the wheat grain is highly dependent on genotype but it is also influenced by environmental conditions such as nitrogen, water access and temperature during growth especially through the grain filling period (Dupont and Altenbach, 2003; Johansson et al., 2004; Torbica et al., 2007). Mature wheat grains contain 8 - 20 % protein which are traditionally classified into four different groups according to their solubility: albumins and globulins as non-gluten proteins and gliadins (ω-, α- and γ-) and glutenins (HMW-GS and LMW-GS) as gluten proteins (Wieser and Kieffer, 2001). Gluten is a very large complex composed mainly of polymeric glutenins and monomeric gliadins and constitute 78 - 85 % of total wheat proteins. In the evaluation of baking quality the glutenin polymers which are covalently linked into large elastic networks are of particular importance. It is well established that flour with higher gluten strength contain favorable HMW-GS (1 and 2 at the Glu-A1 locus, 7+8 at the Glu-B1 locus and 5+10 at the Glu-D1 locus) and higher proportions of HMW-GS. During the dough formation the gliadins act as a 'plasticizer', promoting viscous flow and extensibility which are important rheological characteristics of dough (Payne et al, 1987; Shewry et al., 2001; Pena et al., 2005; Wieser, 2007; Torbica et al., 2007). Albumins and globulins constitute 10 - 22 % of total wheat protein. These proteins have metabolic activity or structural functions in grain and do not play a critical role in flour quality although some authors have noticed their relationship with baking quality (Horvat et al., 2007; Gao et al., 2009). The objective of this study was to evaluate the baking quality of bread wheat cultivars in relation to composition of HMW-GS and endosperm storage proteins content.

Materials and methods

Wheat cultivars

Experiments have been performed with 11 Croatian bread wheat cultivars (Srpanjka, Žitarka, Divana, Felix, Zlata, Ilirija, Ružica, Sana, Seka, Golubica and Olimpija) and French cultivar Soissons which was included in set because it is well distributed in Croatian wheat market. Cultivars were grown at the experimental field at the Agricultural Institute Osijek during 2008/2009 growing season.

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Wheat baking properties

The wheat quality parameters were defined by grains crude protein content (Infratec 1241, Foss Tecator) and wet gluten content of flour (ICC Standard No 155). The dough rheological properties were examined by the Brabender farinograph and extensograph (ICC Standard No 115/1 and 114/1, respectively). The baking test was done according to ICC standard No 131.

Storage proteins characterization

Composition of HMW-GS was previously analysed by SDS-PAGE (Hoefer SE 600). HMW-GS were identified and consequently the Glu-1 score was calculating according to the Payne and Lawrence nomenclature (1983). The wheat proteins extraction and RP-HPLC separation were based on Wieser et al. (1998) method. Perkin Elmer LC 200 chromatograph was used with a Supelco Discovery Bio Wide Pore C18 column (300 Å pore size, 5 µm particle size, 4.6 × 250 mm i.d.). Solvents were composed of water and acetonitrile (ACN), containing 0.1 % (v/v) trifluoroacetic acid. 20 µl samples were injected for analyses. Albumins and globulins were eluted with a linear gradient from 20 to 60 % ACN, while gliadins (ω-, α- and γ-) and glutenin fractions (HMW-GS and LMW-GS) were eluted with a linear gradient from 24 to 58 % ACN over 30 min at 1 ml/min, using a column temperature of 50 ºC. All samples were detected by UV absorbance at 210 nm and were analysed at least in two replicates. The obtained chromatograms were analysed by Total-Chrom software package (Perkin Elmer Instruments, USA). Summed peak areas under albumins-globulins, gliadins and glutenins chromatograms were used as a direct measure of total extractable wheat proteins content and consequently the proportions (%) of all storage protein fractions were calculated and expressed per milligram of flour.

Statistics

Statistical analysis was performed using STATISTICA 8.0 (StatSoft Inc., USA) software. Principal component analysis (PCA) was used to establish similarities among cultivars with respect to analysed baking quality attributes.

Results and discussion

Allelic variations at *Glu-1* loci in wheat samples separated by SDS-PAGE are shown in Table 1. It was found that HMW subunits 2* at the Glu-A1 locus, 7+9 at the Glu-B1 locus and 5+10 at the Glu-D1 locus were dominant in analysed cultivars. The high frequency of favourable HMW-GS 2* at the Glu-A1 locus and 5+10 at the Glu-D1 locus in cultivars confirmed that breeders of Agricultural Institute Osijek in the last decade have made a considerable effort to improve of cultivars gluten strength properties (Horvat et al., 2009). The domination of HMW glutenins combinations 2* 7+9 5+10 in European winter wheat cultivars have been also reported by Tohver et al. (2007), Denčić et al. (2008), Tsenov et al. (2009).

Table 1. Wheat cultivars origin and HMW-GS composition at the Glu-1 loci

CULT.	ORIGIN[a]	YEAR OF RELEASE	HMW-GS			GLU-1 SCORE[b]
			GLU-A1	GLU-B1	GLU-D1	
SANA	Bc	1983	2*	6+8	2+12	6
ŽITARKA	PIO	1985	N	7+8	2+12	6
SOISSONS	FRA	1987	2*	7+8	5+10	10
SRPANJKA	PIO	1989	N	7+8	2+12	6
DIVANA	JS	1995	2*	7+9	5+10	9
GOLUBICA	PIO	1997	N	7+9	2+12	5
SEKA	PIO	2006	1	7+9	5+10	9
FELIX	PIO	2007	2*	7+8	5+10	10
ZLATA	PIO	2007	2*	7+9	5+10	9
ILIRIJA	PIO	2008	2*	7+8	5+10	10
RUŽICA	PIO	2008	1	7+9	2+12	7
OLIMPIJA	PIO	2009	2*	7+9	5+10	9

[a]PIO=Agricultural Institute Osijek - Croatia; JS= Jost-Seed - Croatia; Bc=Bc Institute - Croatia, FRA=France
[b]According to the Payne and Lawrence nomenclature (1983)

The results obtained by evaluation of baking quality are summarized in Table 2. The protein and wet gluten content varied between 12.0 and 16.4 % and 26.0 and 37.0 %, respectively. Dough rheological parameters as indicators of gluten strength characteristics (DDT, DS, E, R_{MAX} and R/EXT) varied between weak to very strong which implies cultivars specific differences. The specific loaf volume of bread was in range 269-424 cm^3/100 g. The highest variability was noticed for farinographic properties DDT and DS as well as for extensographic parameters E and R_{MAX} (Table 2).
Regarding the quantitative results of storage proteins obtained by RP-HPLC method a specific differences among cultivars were also noticed (Table 3). Within the gliadins and glutenins groups, the α-type and γ-type of gliadins as well as LMW-GS were generally present in greatest amount what is in accordance with others (Wieser, 1998; Horvat et al., 2006). Cultivar Divana is the Croatian bread improver and has a distinct position in Fig. 1. Cultivar Divana showed the best baking quality due to favourable HMW-GS composition (2* 7+9 5+10) and the highest HMW-GS proportion (12.9 %) as well as the lowest AG (11.0 %) proportion. On the opposite side of the Fig. 1 is high yielded cultivar Sana which obtained characteristics of weak flour due to unfavourable HMW-GS (2* 6+8 2+12), very low total GLU (28.5 %) and HMW-GS (5.5 %) as well as high proportion of AG (17.0 %) (Table 2 and 3). Žitarka is Croatian standard for quality but with unfavourable HMW-GS (N 7+8 2+12) and high GLI/GLU ratio (1.9) did not show any superior quality attributes. Cultivar Srpanjka as Croatian standard for grain yield and the most frequent cultivar in wheat production area in Croatia in spite of less favourable HMW-GS (N 7+9 2+12) showed a good baking properties due to well-balanced GLI/GLU ratio (1.6) (Table 2 and 3). These cultivars classification due to obtained quality attributes are in agreement with our previous findings (Horvat et al., 2006, 2009).

Table 2. Baking quality parameters of wheat cultivars

CULTIVAR	P[a]	WG	WA	DDT	DS	E	R_{MAX}	R/EXT	V_{SPEC}
SANA	12.0[b]	26.6	59.2	1.7	125	42	191	1.0	321
ŽITARKA	14.0	33.0	64.1	3.2	76	43	195	1.1	339
SOISSONS	12.4	26.9	56.9	1.7	47	123	592	2.1	320
SRPANJKA	13.1	26.6	57.9	1.5	56	95	479	2.2	335
DIVANA	16.4	34.6	61.4	10.3	26	126	486	1.4	422
GOLUBICA	14.5	36.0	64.4	5.9	51	55	203	0.8	337
SEKA	13.3	26.0	59.0	1.5	46	84	424	1.8	269
FELIX	13.7	27.8	61.1	2.0	57	83	366	1.7	331
ZLATA	13.1	26.4	58.3	1.8	58	115	560	2.0	363
ILIRIJA	14.5	29.2	60.2	1.8	45	120	516	1.6	337
RUŽICA	13.9	33.1	59.5	3.3	26	96	431	1.6	331
OLIMPIJA	16.3	37.0	61.8	8.1	54	87	289	1.4	424
$\overline{X}$	13.9	30.3	60.3	3.6	56	89	394	1.6	343
CV[c] %	10.1	13.9	3.8	83.3	46.1	33.5	36.6	25.0	11.7

[a]P=crude protein content of grains (%, DM); WG=wet gluten (%); WA=water absorption (%); DDT=dough development time (min); DS=degree of softening (FU); E=dough energy (cm^2); R_{MAX}=maximum resistance to extension (EU); R/EXT=ratio of resistance and extensibility; V_{SPEC}=bread volume (cm^3/100 g of bread)
[b]Mean values (n=2 determinations)
[c]Coefficent of variation

Table 3. Proportion (%) of storage proteins in wheat cultivars

CULTIVAR	AG[a]	GLI				GLU			GLI/GLU
		Total	ω-	α-	γ-	Total	HMW	LMW	
SANA	17.0 [b]	54.5	3.7	23.7	27.2	28.5	5.5	23.0	1.9
ŽITARKA	11.6	58.0	4.6	31.9	22.3	30.5	9.0	21.5	1.9
SOISSONS	23.4	42.2	2.8	19.8	19.6	34.4	8.5	25.8	1.2
SRPANJKA	16.0	51.7	4.1	28.3	19.3	32.3	8.9	23.4	1.6
DIVANA	11.0	53.9	4.2	30.3	19.4	35.1	12.9	22.2	1.5
GOLUBICA	12.3	51.5	4.0	29.5	18.0	36.2	9.8	26.4	1.4
SEKA	16.9	48.1	2.6	26.3	19.2	35.0	8.9	26.1	1.4
FELIX	14.1	54.0	4.4	31.3	18.8	31.9	9.1	22.8	1.7
ZLATA	14.7	48.2	2.3	25.6	20.3	37.1	8.2	28.9	1.3
ILIRIJA	11.7	53.1	3.9	26.0	23.3	35.2	9.3	25.9	1.5
RUŽICA	15.4	49.6	3.1	26.5	19.2	34.9	8.6	26.3	1.4
OLIMPIJA	11.1	52.3	4.2	30.6	17.5	36.5	8.3	28.3	1.4
$\overline{X}$	14.6	51.4	3.7	27.5	20.3	34.0	8.9	25.1	1.5
CV[c] %	24.4	7.8	20.9	12.9	13.3	7.7	18.4	9.6	14.6

[a]AG=albumins and globulins; GLI=gliadins; GLU=glutenins; HMW=high molecular weight glutenin subunits; LMW=low molecular weight glutenin subunits; GLI/GLU=gliadins to glutenins ratio
[b]Mean values (n=2 determinations)
[c]Coefficent of variation

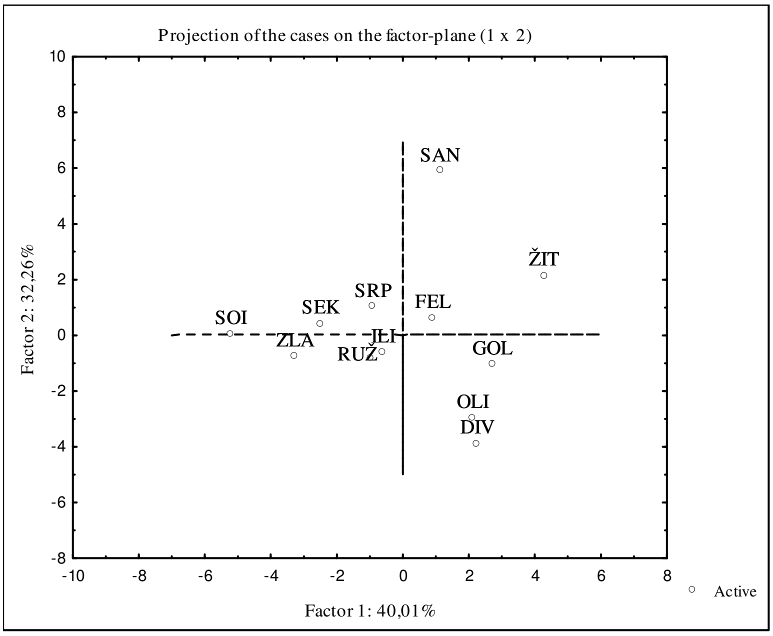

Fig. 1. Cultivar clusters on the plane of the two first principal components. Baking quality parameters, HMW-GS composition and RP-HPLC quantitative storage proteins data determine the clusters. Cultivars are marked with letters: SRP=Srpanjka; ŽIT=Žitarka; DIV=Divana; FEL=Felix; ZLA=Zlata; ILI=Ilirija; RUŽ=Ružica; SAN=Sana; SEK=Seka; GOL=Golubica; SOI=Soissons; OLI=Olimpija

The results of the linear correlation analyses ($p<0.05$) carried out on the storage proteins data and wheat baking properties are presented in Table 4. The P content was highly negatively correlated with AG and positively with α- GLI and HMW-GS. Consequently, the WG content was negatively correlated with AG and positively with α- GLI what is in accordance with Ivanov et al. (1998).

WA was negatively correlated with AG and positively with total, ω- and α- GLI. Gliadins are likely interacting with native and added lipids to form gas bubbles and have positive impact on loaf size and their content was linked to WA (Graybosch et al., 1993).

DDT was significantly influenced by the AG and HMW-GS what is in accordance with our previous findings (Horvat et al., 2006). Cultivars Divana and Golubica with the highest value of HMW-GS (12.9 and 9.8 %, respectively) showed a high value of this property (10.3 and 5.9 min, respectively). Jood et al. (2001) and Antes and Wieser (2001) showed that the addition of reoxided HMW-GS to base flour significantly increases dough strength.

DS as a measure of dough mixing tolerance was highly influenced by γ- gliadins, HMW-GS and GLI/GLU ratio what is in accordance with our previous findings (Horvat et al., 2006). Cultivar Sana and Žitarka with the lowest proportions of total GLU (28.5 and 30.5 %, respectively) (Table 3) and with the highest value of DS (125 and 76 FU, respectively) had shown a weak dough rheological properties (Table 2).

E and R_{MAX} as good indicators of gluten strength were significantly positively affected by Glu-1 score and negatively by GLI/GLU ratio which is in accordance with other authors which noticed that increase in the GLI/GLU ratio caused decrease in dough strength (Uthayakumaran at al., 2000; Daniel and Triboi, 2000). Cultivars Divana, Zlata, Ilirija and Soissons with a favorable HMW-GS 2* and 5+10, the highest value of Glu-1 score and low values of GLI/GLU ratio (1.2-1.5) obtained dough E above 100 EU and very high values of R_{MAX} (486-592 EU) (Table 1, 2 and 3).

R/EXT ratio is also important parameter in defining gluten strength, because increase in extensibility caused decrease in strength and vice versa. According to obtained results it could be noticed that total GLI proportion had the negative influence on this parameter. Decrease in dough strength caused by addition of all groups of gliadin components in flour base is confirmed in literature (Uthayakumaran et al., 1999; Jood et al., 2001).

The bread V_{SPEC} was significantly influenced by AG. The bread V_{SPEC} was in high significant correlation with total P content and DDT (results were not shown). Wieser and Kieffer (2001) noticed that the correlation between bread volumes and the quantity of gluten proteins may be significantly improved if the processing baking parameters are optimized for each cultivar.

The obtained significantly positive influence of HMW-GS composition (Glu-1 score) and glutenin components content as well as negative influence of GLI/GLU ratio on dough rheological parameters were similar to those published in recent time (Tang et al., 2008; Kurtanjek et al., 2008; Horvat et al. 2009; Anderson et al., 2011). The noticed negative influence of AG as non-gluten proteins on some baking quality parameters are in agreement with results of another authors (Veraverbeke i Delcour, 2002; Horvat et al., 2007; Gao et al., 2009).

Table 4. Linear coefficient of correlation (p<0.05) between baking parameters and storage proteins of wheat cultivars

PARAM.	GLU-1 SCORE	AG[a]	GLI				GT			GLI/GLU
			Total	ω-	α-	γ-	Total	HMW	LMW	
P[b]	0.17	-0.77*	0.36	0.50	0.66*	-0.46	0.49	0.68*	0.07	-0.14
WG	-0.26	-0.66*	0.36	0.51	0.58*	-0.38	0.33	0.44	0.07	-0.05
WA	-0.38	-0.76*	0.68*	0.68*	0.77*	-0.13	-0.01	0.33	-0.23	0.36
DDT	-0.02	-0.58*	0.27	0.42	0.51	-0.40	0.37	0.63*	-0.02	-0.13
DS	-0.40	0.13	0.36	0.14	-0.15	0.75*	-0.74*	-0.75*	-0.30	0.69*
E	0.73*	0.17	-0.54	-0.38	-0.32	-0.34	0.60*	0.47	0.33	-0.69*
R_{MAX}	0.66*	0.40	-0.65*	-0.55	-0.48	-0.24	0.47	0.30	0.30	-0.65*
R/EXT	0.53	0.52	-0.62*	-0.49	-0.39	-0.30	0.24	0.03	0.24	-0.47
V_{spec}	0.13	-0.58*	0.31	0.45	0.45	-0.26	0.31	0.44	0.04	-0.07

[a]AG=Albumins and Globulins; GLI=Gliadins; GLU=Glutenins; GLI/GLU=gliadins to glutenins ratio

[b]P=crude protein content of grains (%, DM); WG=wet gluten (%); DDT=dough development time (min); DS=degree of softening (FU); R_{MAX}=maximum resistance to extension (EU); R/EXT=ratio of resistance and extensibility; V_{SPEC}=bread volume (cm^3/100 g of bread)

[*]significant at p<0.05

Conclusions

The obtained results reflected the contrast roles of analysed storage proteins on the wheat baking quality. Analysis of relationship between particular storage proteins and baking parameters showed that the influence of albumins and globulins, α- gliadins and HMW-GS was the most pronounced. Cultivars with favorable HMW-GS composition accompanied with higher proportion of HMW-GS produced flours with strong gluten characteristics.

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