ISSN 1330-7142
UDK = 633.11: 664.641.12

THE IMPORTANCE OF HIGH-MOLECULAR-WEIGHT GLUTENIN SUBUNITS FOR WHEAT QUALITY EVALUATION

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Original scientific paper
Izvorni znanstveni članak

SUMMARY

High-molecular-weight glutenin subunits (HMW-GS) composition was analyzed by sodium-dodecyl-sulfat-polyacrilamid-gel electrophoresis (SDS-PAGE), while the quantitative determination of total HMW-GS was obtained by reversed phase- high performance liquid chromatography (RP-HPLC). Considering HMW-GS composition, the most frequent subunits at Glu-A1 locus were N, at Glu-B1 locus 7+9 and at Glu-D1 locus 2+12. The cultivars with the GS 5+10 at Glu-D1 locus have shown better technological characteristics in contrast to cultivars with the GS 2+12. The cultivars Žitarka, Srpanjka, Barbara, Klara and Golubica in spite of presence HMW-GS 2+12 have shown very good and good technological properties because they had optimal proportions (>10%) of total HMW-GS. The results of the linear correlation analysis between quality parameters and HMW-GS composition have shown significant (P<0.05) positive influence of HMW-GS (Glu-1 score) on sedimentation value (r=0.55), Gluten Index (r=0.72), dough energy (r=0.61), maximum resistance (r=0.64) and resistance to extensibility ratio (r=0.58). The influence of HMW GS proportions on technological parameters, compared to HMW-GS composition, was more pronounced on protein content (r=0.82), dough development time (r=0.70), degree of softening (r=-0.90), dough energy (r=0.74) and loaf volume (r=0.65).

Key-words: wheat quality, HMW- GS, SDS-PAGE, RP-HPLC

INTRODUCTION

The wheat quality is essentially determined by composition and quantity of gliadin and glutenin proteins which are major components of gluten. The unique dough elasticity is mainly influenced by polymeric glutenins, while monomeric gliadins define viscous behaviour of dough (Uthayakumaran et al., 2000; Kuktaite et al., 2000; Shewry at al., 2002; Lasztity, 2003).
HMW-GS and low-molecular-weight glutenin subunits (LMW-GS) explain the variation in all quality parameters better than gliadin components (Antes and Wieser, 2001; Wieser and Kieffer; 2001; Johansson et al., 2004). The HMW-GS are encoded by polymorphic genes at Glu-1 loci (Glu-A1, Glu-B1 and Glu-D1) present on the long arms of the group 1 chromosomes (Payne and Lawrence, 1983). Although they account only 10% of the wheat storage proteins, HMW-GS are one of the most important genetic factors in determining the dough-forming properties. In particular, HMW-GS 5+10 and 2+12 at the Glu-D1 locus have a major effect on dough strength (Jurković et al., 2000; Johansson et al., 2002). The relationship between gluten proteins composition and amount and quality parameters has been taken into consideration by wheat breeders of Agricultural Institute Osijek in order to create new cultivars with optimal gluten strength (Drezner, 1995; Horvat et al., 2002). The aim of this study was to investigate the influence of composition and proportions of HMW-GS on wheat quality.

MATERIAL AND METHODS

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15 wheat samples differing in bread-making quality were collected from experimental field of Agricultural Institute Osijek. Cultivars Žitarka, Srpanjka, Barbara, Klara, Golubica, Monika, Kata, Hana, Ana, Demetra and OS Crvenka were created at Agricultural Institute Osijek. Cultivars Divana (Jošt-Seed, Križevci) and Sana (Bc institute, Zagreb) are Croatian improver and yield standard, respectively. The crude protein content of sample grains was measured by NIT technology (*Infratec 1241, Foss Tecator*). The following wheat quality parameters of flour (ash content 0.55%) were determined: Zeleny sedimentation value (ICC No 115/1), wet gluten content and Gluten Index according to Gluten Index method (ICC No 155), farinograph (ICC No 115/1) and extensograph (ICC No 114/1) properties. The baking test was done according to standard ICC method No 131. Composition of HMW GS was analyzed by sodium-dodecyl-sulfat-polyacrilamid-gel electrophoresis (SDS-PAGE) using Phast System (*Pharmacia LKB*). Prior to electrophoresis, total proteins were extracted from 50 mg of whole-meal flour with SDS-PAGE sample buffer (2X stock buffer: 0,125 M Tris-HCl, 4% SDS, 20% glycerol, 0,2 M DTT, 0,02% Bromphenol Blue, pH 6,8). HMW-GS bands were analyzed according to the nomenclature of Payne and Lawrence (1983) and Glu-1 quality scores were calculated. Gluten proteins were analyzed by reversed phase- high performance liquid chromatography RP-HPLC system (*Integral 4000, Perkin Elmer*), following the quantitative extraction procedure of Wieser et al (1998). The separation of glutenin components was carried out on Supelcosil LC 318 column. Column temperature was maintained at 50°C. A linear elution gradient of acetonitrile was applied to separate glutenin components. Eluted proteins were monitored at 210 nm. Glutenin proteins were eluted according to different surface hydrophobicity in the series glutenin-bound gliadins (ω(b)-), HMW-GS and LMW-GS. Statistical analyses were done using SAS 8.e., Stat Software.

RESULTS AND DISCUSSION

HMW-GS as polymeric glutenin fractions have the most important role in defining bread-making quality (Lasztity, 2003). The grain samples were different in HMW-GS (Table 1) and their crude protein content varied between 13.4% (cultivars Kata and Sana) and 17.8% (cultivar Divana) (Table 2).

Table 1. HMW GS composition and proportions of wheat cultivars
Tablica 1. Sastav i udjeli HMW podjedinica glutenina kultivara pšenice

Cultivars *Kultivari*	HMW-GS *Visokomolekularne podjedinice glutenina*			Glu-1 score *Glu-1 bodovi*	Proportion of HMW-GS (%) *Udio HMW podjedinica glutenina (%)*
	Glu-A1 locus				
Žitarka	1	7+8	2+12	8	10.43
Srpanjka	N	7+8	2+12	6	11.79
Barbara	N	7+8	2+12	6	11.51
Klara	1	7+8	2+12	8	10.49
Golubica	N	7+9	2+12	5	11.19
Monika	N	7+9	2+12	5	10.26
Kata	N	7+9	2+12	5	8.57
Sana	N	6+8	2+12	4	7.89
Libellula	1	20	2+12	6	9.81
Hana	1	7+8	5+10	10	10.27
Ana	1	7+9	5+10	9	10.53
Demetra	1	7+9	5+10	9	10.85
OS Crvenka	N	7+9	5+10	7	9.92
Bezostaja	N	7+9	5+10	7	11.82
Divana	N	7+9	5+10	7	13.82
					LSD 0.05 0.713

Typical SDS-PAGE electrophoregram was shown in Figure 1. The most frequent GS at Glu-A1 locus were N (60%), at Glu-B1 7+9 (53%) and at Glu-D1 2+12 (73%) (Table 1).

Glu-1 quality score ranged from 4 (cultivar Sana) to 10 (cultivar Hana) but as well as in our previous investigation (Jurković et al., 2000) the composition of HMW-GS at the Glu-D1 locus couldn't explain all variations of quality parameters.

Table 2. Quality parameters of wheat cultivars
Tablica 2. Pokazatelji kakvoće kultivara pšenice

Cultivar	P[a]	SED	WG	GI	DDT	R	DS	E	Rmax	Ext	R/Ext	V
Žitarka	14.1	41	41.9	83	3.3	3.8	87	77	270	212	0.9	510
Srpanjka	14.2	44	34.7	98	2.0	6.6	62	122	503	185	1.8	516
Barbara	14.3	54	41.6	84	3.7	5.1	81	86	318	211	1.0	516
Klara	14.7	57	39.3	90	4.2	5.2	78	92	348	207	1.0	520
Golubica	15.5	61	44.1	63	5.0	6.4	62	81	277	224	0.8	509
Monika	14.4	32	37.8	66	3.3	3.5	107	43	188	172	0.8	443
Kata	13.4	37	38.2	61	2.8	3.0	104	49	182	203	0.7	478
Sana	13.4	37	35.9	57	2.5	3.2	118	48	180	197	0.7	486
Libellula	14.7	22	37.9	57	2.0	2.3	132	44	150	211	0.6	391
Hana	14.5	62	37.0	86	2.5	4.0	65	99	430	176	1.7	513
Ana	13.6	51	30.8	97	4.3	5.7	70	119	420	211	1.3	483
Demetra	14.2	63	34.8	96	4.5	5.9	71	135	477	222	1.3	520
OS Crvenka	15.1	41	39.9	84	4.5	5.2	76	91	334	208	1.1	489
Bezostaja-1	15.2	55	37.0	93	5.5	7.2	40	126	477	198	1.6	575
Divana	17.8	47	44.0	90	9.1	12.0	11	142	441	248	0.9	678
CV %	7.2	25.7	9.2	18.5	44.4	43.7	38.2	37.5	35.8	9.6	34.5	13.3

[a]P=protein content (%); SED=sedimentation value (ml); WG=wet gluten (%); GI=Gluten Index; DDT=dough development time (min); R=resistance (FU); DS=degree of softening (FU); E=dough energy (cm^2); Rmax=maximum resistance (EU); Ext=extensibility (mm); R/Ext=resistance to extensibility ratio; V=loaf volume (cm^3/100 mg flour)
P=udio bjelančevina (%); SED=sedimentacijska vrijednost (ml); WG=vlažni gluten (%); GI=Gluten indeks; DDT=razvoj tijesta (min); R=otpor (FJ); DS=stupanj omekšanja (FJ);E=energija tijesta (cm^2); Rmax=maksimalni otpor (EJ); Ext=rastezljivost (mm); Ext/R=omjer otpora i rastezljivosti; V=volumen kruha (cm^3/100 mg brašna)

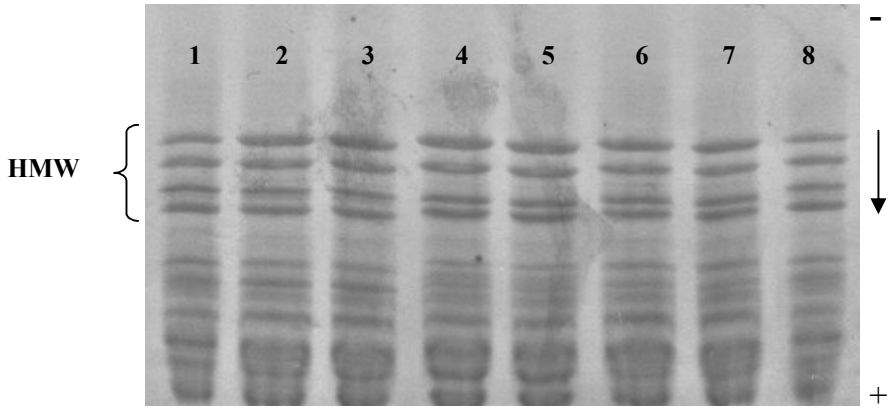

Figure 1. SDS-PAGE electrophoregram (cultivars 1&8 Chinese Spring; 2&3 Žitarka; 4&5 Golubica; 6&7 Kata)
Slika 1. SDS-PAGE elektroforegram (kultivari 1&8 Chinese Spring; 2&3 Žitarka; 4&5 Golubica; 6&7 Kata)

Cultivars Ana, Demetra, OS Crvenka, Hana, Divana and Bezostaja with HMW-GS 5+10 at the Glu-D1 locus and with the highest Glu-1 quality scores have shown better technological

characteristics in contrast to cultivars with HMW-GS 2+12 (Table 2). However, cultivars Žitarka, Srpanjka, Barbara, Klara and Golubica in spite of presence HMW-GS 2+12 have shown very good and good technological properties (Table 2) being in agreement with our previous investigation (Horvat et al., 2002). Therefore in this study we were interested to find out weather HMW-GS proportions affect wheat quality, too. The proportions of HMW-GS analyzed by RP-HPLC were summarized in the Table 1 and typical RP-HPLC chromatogram was shown in Figure 2.

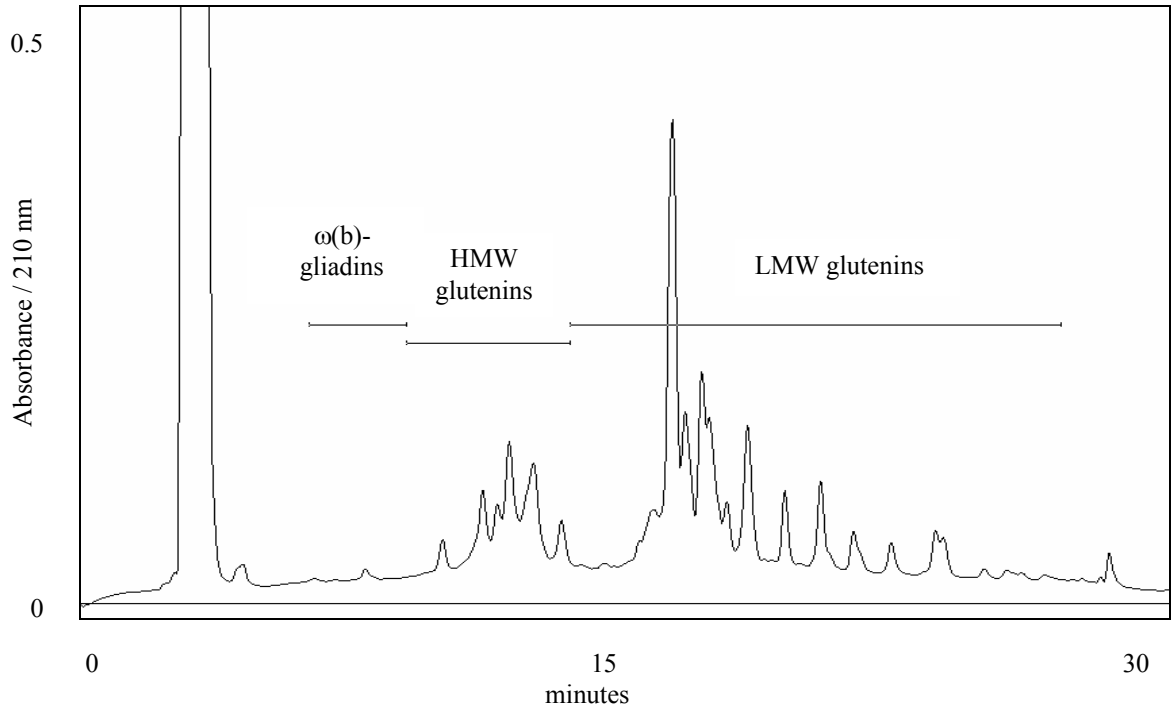

Figure 2. RP-HPLC chromatogram of glutenins (cultivar Žitarka)
Slika 2. RP-HPLC kromatogram glutenina (kultivar Žitarka)

Polymeric glutenin proteins play a critical role for flour technological properties. Cultivar Divana (N, 7+9, 5+10), as Croatian quality improver, had protein content 17.8% and also had the highest proportion of HMW-GS (13.82%). Cultivars Žitarka (1, 7+8, 2+12), Srpanjka (N, 7+8, 2+12), Barbara (N, 7+8, 2+12), Klara (1, 7+8, 2+12) and Golubica (N, 7+9, 2+12) with optimal proportions of HMW-GS (10.43-11.79%) have shown better technological parameters, in contrast to cultivars Sana (N, 7+9, 2+12) and Kata (N, 6+8, 2+12) with lower proportions of HMW-GS (7.89%-8.57%). At the same time, cultivars Monika (N, 7+9, 2+12) and Libellula (1, 20, 2+12) have shown lower technological characteristics in spite of higher proportions of HMW-GS. HMW-GS proportion of cultivar Monika (10.23%) has shown the same level of significance (LSD 0.05) as for cultivars Demetra, Ana, Klara and Žitarka), while HMW-GS proportion of cultivar Libellula (9.81%) has shown the same level of significance (LSD 0.05) as for cultivar OS Crvenka (9.92%) (Table 1). At these two cultivars, the presence of HMW-GS 2+12 had the dominant negative effect on their bread-making quality.
The results of the liner correlation ($P<0.05$) analyses between wheat quality parameters and HMW-GS composition and proportions are presented in Table 3. HMW-GS composition (Glu-1 score) has shown significant positive influence on sedimentation value ($r=0.55$), Gluten Index ($r=0.72$), dough energy ($r=0.61$), maximum resistance to extension ($r=0.64$) and resistance to extension ratio ($r=0.58$).

Table 3. Correlations (r) of HMW-GS composition and proportions with wheat quality parameters
Tablica 3. Korelacija sastava i udjela HMW podjedinica glutenina i pokazatelja kakvoće pšenice

Parameters	Glu-1 score	% HMW
P[a]	0.06	0.82*
SED	0.55*	0.42
WG	-0.26	0.40
GI	0.72*	0.62*
DDT	0.16	0.70*
R	0.18	0.88*
DS	-0.41	-0.90*
E	0.61*	0.74*
MaxR	0.64*	0.48
Ext	0.07	0.45
Ext/R	0.58*	0.33
V	0.06	0.65*

[a]P=protein content (%); SED=sedimentation value (ml); WG=wet gluten (%); GI=Gluten Index; DDT=dough development time (min); R=resistance (FU); DS=degree of softening (FU); E=dough energy (cm^2); Rmax=maximum resistance (EU); Ext=extensibility (mm); R/Ext= resistance to extensibility ratio; V=loaf volume (cm^3/100 mg flour)
P=udio bjelančevina (%); SED=sedimentacijska vrijednost (ml); WG=vlažni gluten (%); GI=Gluten indeks; DDT=razvoj tijesta (min); R=otpor (FJ); DS=stupanj omekšanja (FJ);E=energija tijesta (cm^2); Rmax=maksimalni otpor (EJ); Ext=rastezljivost (mm); Ext/R=omjer otpora i rastezljivosti; V=volumen kruha (cm^3/100 mg brašna)
* $P<0.05$

CONCLUSION

The influence of HMW-GS quantity on technological parameter was more pronounced on protein content (r=0.82), dough development time (r=0.70), degree of softening (r=-0.90), dough energy (r=0.74) and loaf volume (r=0.65). These results indicated that HMW-GS composition could have predictive value in quality analysis of earlier wheat generations, but HMW-GS proportion must be taken into consideration for their more efficient quality estimation.
The results indicated that the presence of HMW-GS 5+10 contributes to very good bread-making quality, while better quality of cultivars with HMW-GS 2+12 is due to higher proportion of total HMW-GS at the Glu-D1 locus.

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ZNAČAJ VISOKOMOLEKULARNIH PODJEDINICA GLUTENINA U PROCJENI KAKVOĆE PŠENICE

SAŽETAK

Sastav visokomolekularnih podjedinica glutenina (HMW-GS) analiziran je natrium-dodecil-sulfat poliakrilamid-gel elektroforezom (SDS-PAGE), dok je njihova ukupna količina određena visokotlačnom tekućinskom kromatografijom obrnutih faza (RP-HPLC). U analiziranim kultivarima najzastupljenija podjedinica Glu-A1 lokusa je N, Glu-B1 lokusa podjedinice 7+9 te Glu-D1 lokusa podjedinice 2+12. Kultivari s HMW-GS 5+10 na Glu-D1 lokusu u prosjeku su pokazali bolja tehnološka svojstva u odnosu na kultivare s HMW-GS 2+12. Kultivari Žitarka, Srpanjka, Barbara, Klara i Golubica pokazali su, bez obzira na prisustvo HMW-GS 2+12, vrlo dobra i dobra tehnološka svojstva, jer je kvantitativnom analizom kod ovih kultivara utvrđena optimalna vrijednosti (>10%) ukupnih HMW-GS. Rezultati linearne korelacije između parametara kakvoće i sastava HMW-GS pokazali su značajan ($p<0,05$) pozitivan utjecaj HMW-GS (Glu-1 bodovi) na sedimentacijsku vrijednost ($r=0,55$), gluten indeks ($r=0,72$), energiju tijesta ($r=0,61$), maksimalni otpor ($r=0,64$), te omjera otpora i rastezljivosti tijesta ($r=0,58$). U odnosu na sastav HMW-GS, njihovi udjeli pokazuju značajniji utjecaj na udio proteina ($r=0,82$), vrijeme razvoja tijesta ($r=0,70$), stupanj omekšanja ($r=-0,90$), energiju tijesta ($r=0,74$) i na volumen kruha ($r=0,65$).

Ključne riječi: kakvoća pšenice, HMW- GS, SDS-PAGE, RP-HPLC

(Primljeno 14. ožujka 2006.; prihvaćeno 28. travnja 2006. - *Received on 21 March 2006; accepted on 28 April 2006*)