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WHEAT TECHNOLOGYCAL QUALITY IN RELATION WITH THE COMPOSITION OF GLUTEN PROTEINS-GLIADINS AND HMW-GLUTENINS

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INTRODUCTION

- Cereals , especially wheat are basic food in Europe and in the most part of the world.

- Proteins are the principal factors of wheat quality.
- Bread-making quality depends on the quality and quantity of wheat proteins (*Finney 1945, Hoseney and Finney 1971, Pomeranz 1985*).

- Gluten, the elastic and plastic mass formed from the protein fractions gliadin and glutenin during flour mixing water is a reliable indicator of flour strength.

I. Classification of cereal proteins

Ozborn (1907) **has first classified the wheat flour proteins into 4 main fractions according to their characteristics:**

а) **Albumins**-disolve in water and coagulate on heat,

б) **Globulins**-disolve in neutral salt solurtion,

в) **Glidains/Prolamins**- disolve in 75% alcohol, and

г) **Glutenins/Glutelins**-do not disolv in alcohol, but dispolve in diluted acid or diluted base.

- The terms *prolamin* and *glutelin* refer to the *coresponding protein fractions with other cereals (Ozborn, 1907*) :

  barley (*hordaine*), maize (*zein*) and

  rye (*secaline*), oat (*avenine*)

- **Other classification of proteins is based on:**
  - chemical composition,
  - cerel morhpology and
  - biological function

- For characterisation proteins were separated by colon chromatograhpy, electrophoresis and centrifugation.
- These techniques have shown that fractions obtained by separation are heterogenic, composed of **many protein components.**

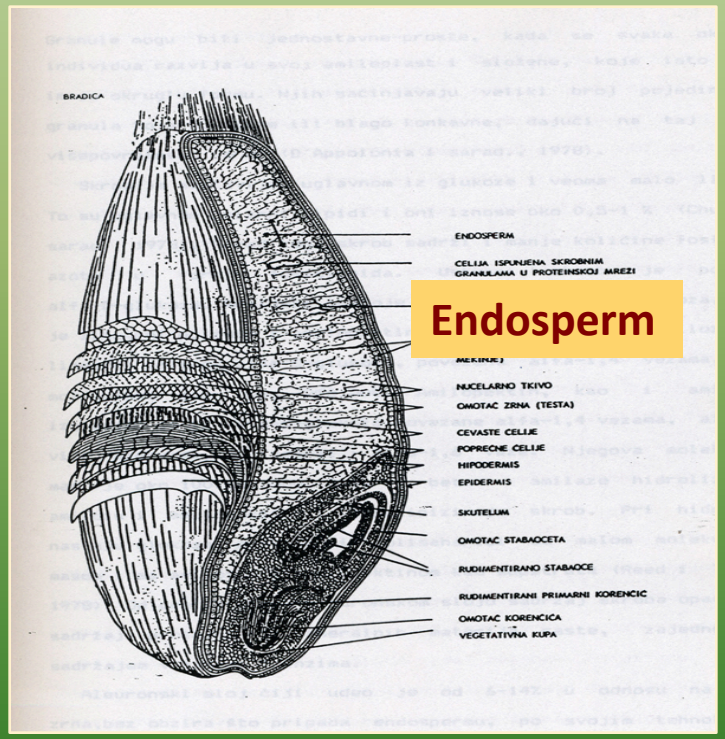
WHEAT STORAGE PROTEINS-PROTEINS OF ENDOSPERM

Gluten proteins

a) Gliadins

б) Glutenins

- compose the major part of wheat storage proteins (*Kasarda, 1976*)



Endosperm

Wheat kernel cross section

The genetic studies on chromosomal locations of the genes that control the synthesis of gluten proteins have determined:

- **Gliadins are controlled by the complex gene loci , enableing their genetic classification by blocks of gliadins (*Metakovsky et al, 1984*) .**
- **The genes for gliadin synthesis are determined on the chromosoms 1A, 1B and 1D, 6A and 6D.**

- **The synthesis of HMW subunits of glutenins is controlled by the gene loci Glu-A1, Glu-B1 and Glu-D1 located on the long arms of chromosomes 1A, 1B and 1D, respectively (*Payne et al., 1988*).**

- **The glutenin loci have many allelic variants (*Payne and Lawrence, 1983*).**

Instrumental methods for wheat quality determination

Electrophoretic methods

- **Polyacrilamide gel elecrophoresis (PAGE) applied to gliadin proteins was adopted in many countries as a routine laboratory procedure for wheat varieties identification (***Autran et Bourdet, 1973, Bushuk and Zillman, 1978, Lookhart et al., 1982; Metakovskyet al., 1984***).**

- **HMW glutenins were also used for the identification of wheat varieties by sodium dodcel sulphate poliacrilamyde gel electrohporesis (***Laemmli, 1970, du Cros et al., 1980, Kasarda, 1986***).**

Chromatographic methods

- **Refersed-phase high performance liquid chromatograhpy (RP-HPLC), (*Bietz, 1983*).**

Research on gluten proteins

- **Many reserachers pointed out to the possibility of relationship between wheat quality properties and gluten proteins-gliadins and HMW glutenins (*Payne et al. 1979, Wrigly et al.1982, Menkovska, 1987,1988, 1989, 1993,1995, MacRitchie et al.1991, and other*).**

- **As immediate products of DNK gluten proteins give electrophoregrams and chromatograms characteristic for each particular wheat variety -*"Finger print"* of the variety (*Bitz, 1983, Lookhart et al. 1982*).**

The aim of investigations

- To determine the composition of gluten proteins of domestic bread-wheat varieties and of their milled products;
- To identify wheat varieties on the basis of composition of the gluten proteins;
- To find relationship between the composition of gluten proteins of wheat varieties and technologycal quality characteristics of the kernel, flour and bread.

MATERIALS

- Ten wheat varieties (*Triticum Aestivum*) from domestic origin, bred at the Institute of Agriculture in Skopje.

- Technological quality of the wheat varieties varied widely from excellent, good to poor.

- Wheat varieties were clustered in three technological groups acccording to the quality parameters of the accepted wheat classification: class, milling and baking properties (*Sharic et al, 1989*).

Table 1. List of analysed wheat varieties

Varity	Abrev.	Breeding center	Year	
Partizanka	P	NS	1973	
Yugoslavia	J	NS	1980	
Nova Skopjanka	NS	SK	1990	
Radika	R	SK	1989	
Lihnida	L	SK	1990	
Orovchanka	O	SK	1982	
Babuna	B	SK	1987	
Super Zlatna	SZ	ZG	1977	
Skopjanka	S	SK	1982	
Pelagonia	Pe	SK	1974	

METHODS

- **Physical and chemical properties** of the kernel and milling flours were determined by the standard methods for quality determination of wheat as milling raw material (*Official Paper of SFRY No.74, 1988*). Protein content (Nx5.7 %db) was determined by micro-Khjedal method.

- **Milling properties** were analised by the Buhler experimental mill 202, according to the standardized method of the Institute for food engineering in Novi Sad, Serbia.

- **Milling fractions** were clustered in **four groups** according to the simlaraties in their origin and the participation of the particular anatomic parts of the kernel.

Methods continued

- **Rheological properties** of milled flours were analised by micro-Brabender farinograph using 10 g flour sample.
- **Enzymatic activity** of milled flours was determined by the standardized method Hagber falling number.
- **Breadmaking performance** was done by micro laboratory baking method (50 g flour).

Methods continued

Determination of composition of gluten proteins

- **Gliadins** were determined by modified classical method **of polyacrilamide gel electrophoresis in acid solutions A-PAGE** according to Bushuk and Zillman (1978) and vertical electrohporesis at high voltage according to Novoselskaya et al (1983).

- Identification of the variants of **gliadin blocks** was performed using the wheat standard Bezostaya-1, the known gliadin patterns of other wheats and the catalog of known gliadin blocks (*Metakovsky et al, 1984*).

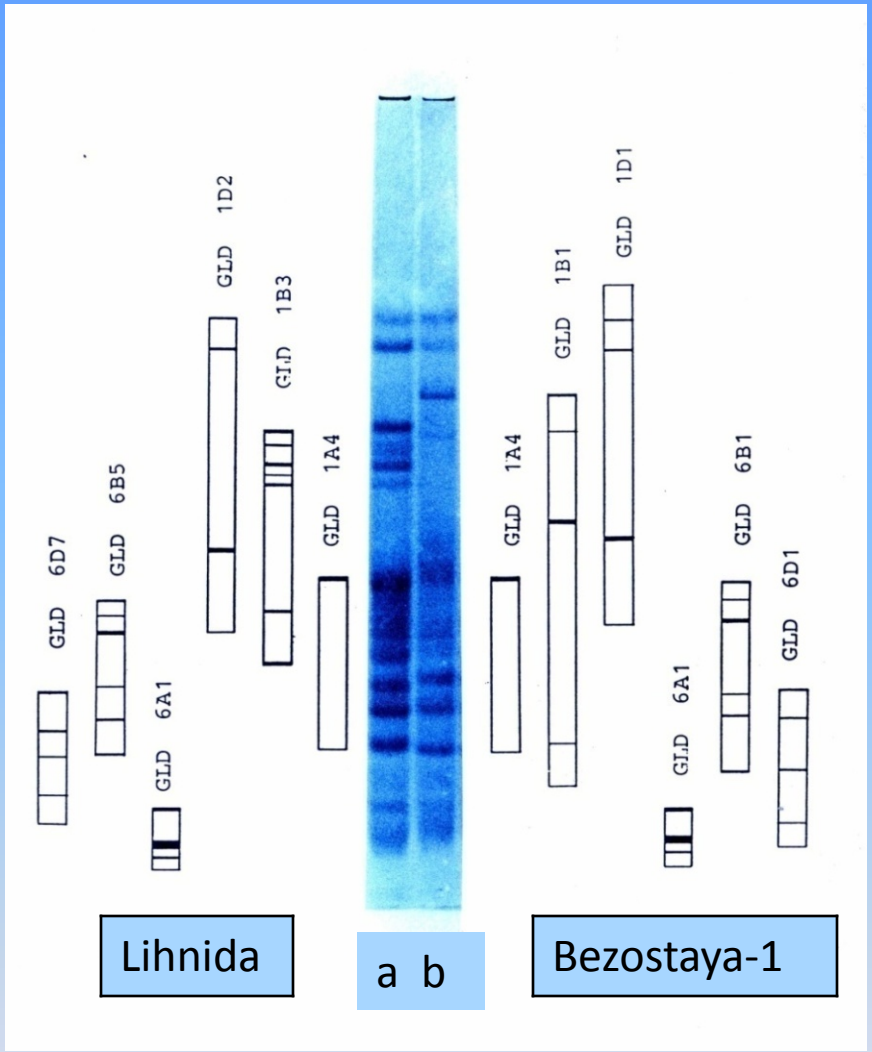
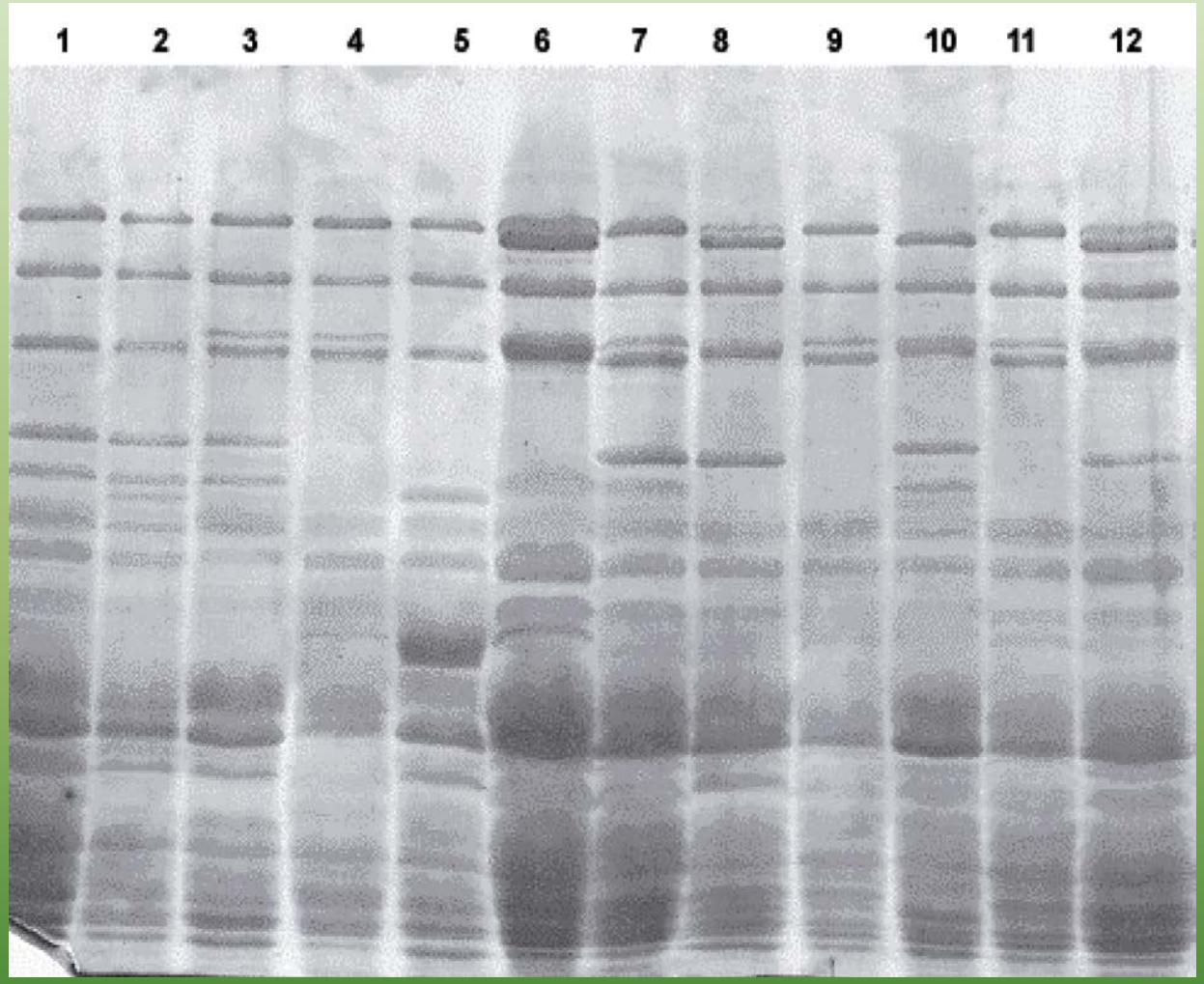


Fig. 1. Electroforegrams (PAGE) and shemes of gliadin blocks of wheat variety Lihnida (a) and Bezostaya-1 (b)

Determination of composition continued

- **HMW glutenins were determined using sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) according to *Kasarda et al (1986*). Their composition was determined according to *Payne and Lawrence* (1983).**

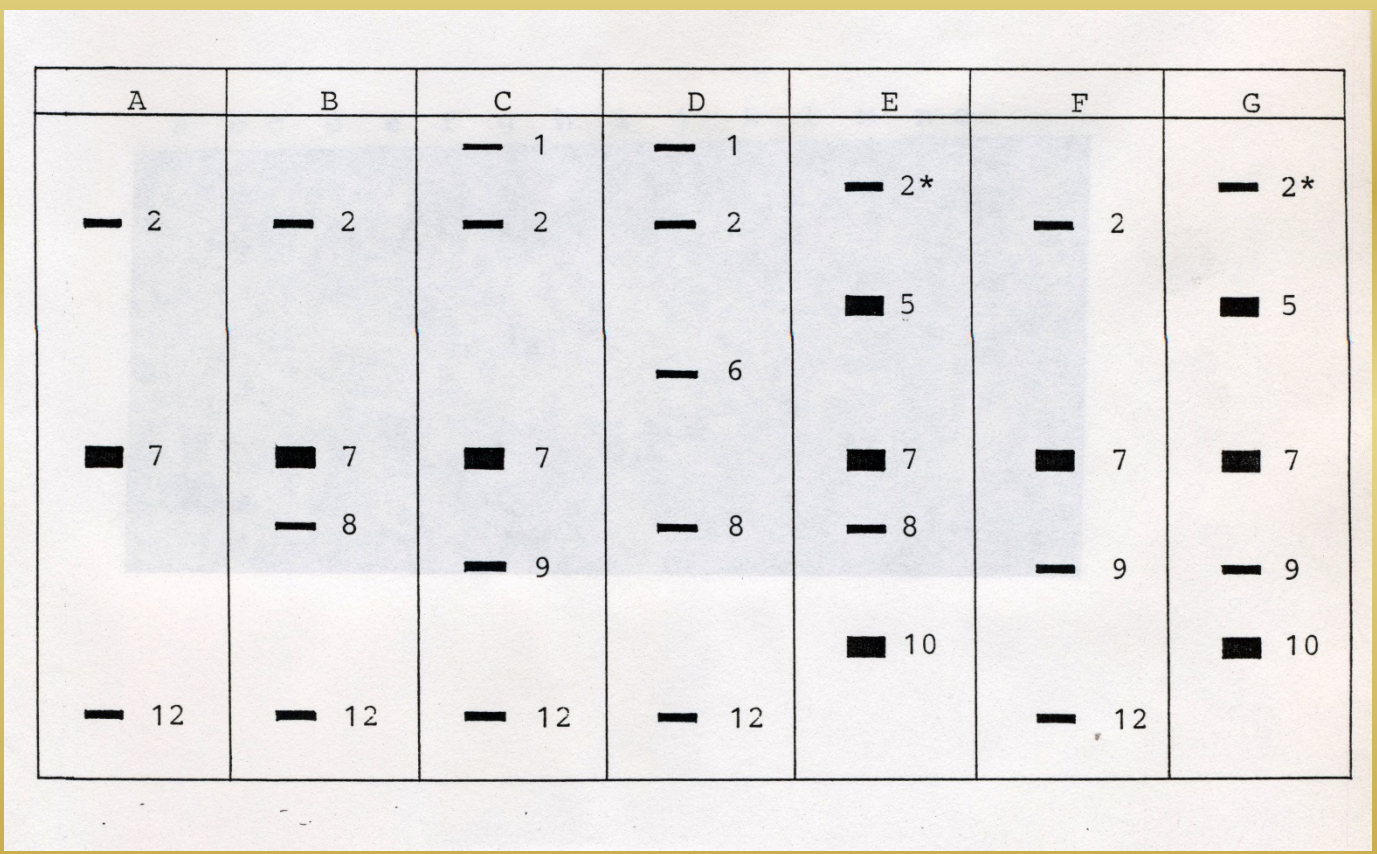
- **Statistical analisis (Student t-test and Analisis of variance F-test) was applied to determine the significance of the mean values differences of particular quality properties of wheat kernel, flours and bread.**



No.	Variety
1	Pelagonia
2	Vardarka
3	Makedonka
4	Orovchanka
5	Prilepchanka
6	Sterna
7	Ohridjanka
8	Babuna
9	Radika
10	Lihnida
11	Nova Skopjanka
12	Partizanka

Fig. 2. ELECTROPHOREGRAMS SDS-PAGE OF MACEDONIAN WHEATS

- **Identification of the HMW glutenin subunits was done in the same way as the identification of the gliadins.**



Sheme of HMW glutenin subunits of invrestigated wheat varieties:

A- ***Pelagonia,*** **B-second biotyip of** ***Skopjanka*****, C- B-second biotyip of** ***Super Zlatna*****, D-first biotyip of** ***Super Zlatna*****, E-***Lihnida*****, F-***Radika*****,** ***Orovcanka*** **and first biotyip of** ***Skopjanka*****, G-***Partizanka*****,** ***Yugoslavia*****,** ***Nova Skopjanka*** **and** ***Babuna***

Table 2. Allelic variants and and HMW glutenin subunit composition

	Variety	*Glu*-1 alleles	HMW glutein subunits *Glu*-A1	*Glu*-B1	*Glu*-D1	*Glu*-1 score
1	Skopjanka	c c a	N	7+9	2+12	5
	Skopjanka	c b a	N	7+8	2+12	6
2	Vardarka	c a a	N	7	2+12	4
3	Pelagonija	c a a	N	7	2+12	4
4	Nova Skopjanka	b c d	2*	7+9	5+10	9
5	Radika	c c a	N	7+9	2+12	5
6	Lihnida	b b d	2*	7+8	5+10	10
7	Orovčanka	c c a	N	7+9	2+12	5
8	Babuna	b c d	2*	7+9	5+10	9
	Standards cv.					
1	*Chinese Spring*	c b a	N	7'+8	2+12	
2	*Cajeme*	b c d	2*	7+9	5+10	
3	*Yecora Rojo*	a i d	1	17+18	5+10	
4	*Stephens*	b a a	2*	7'	2+12	

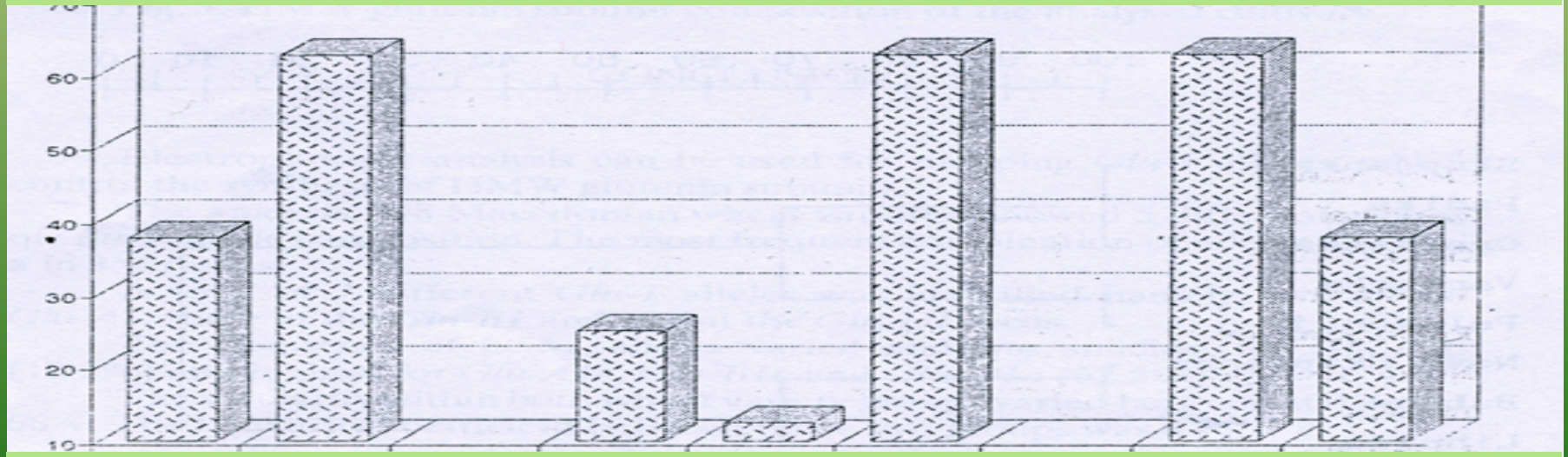


Fig. 3. Frequency of HNM glutenin alleles of Macedonian wheat varieties

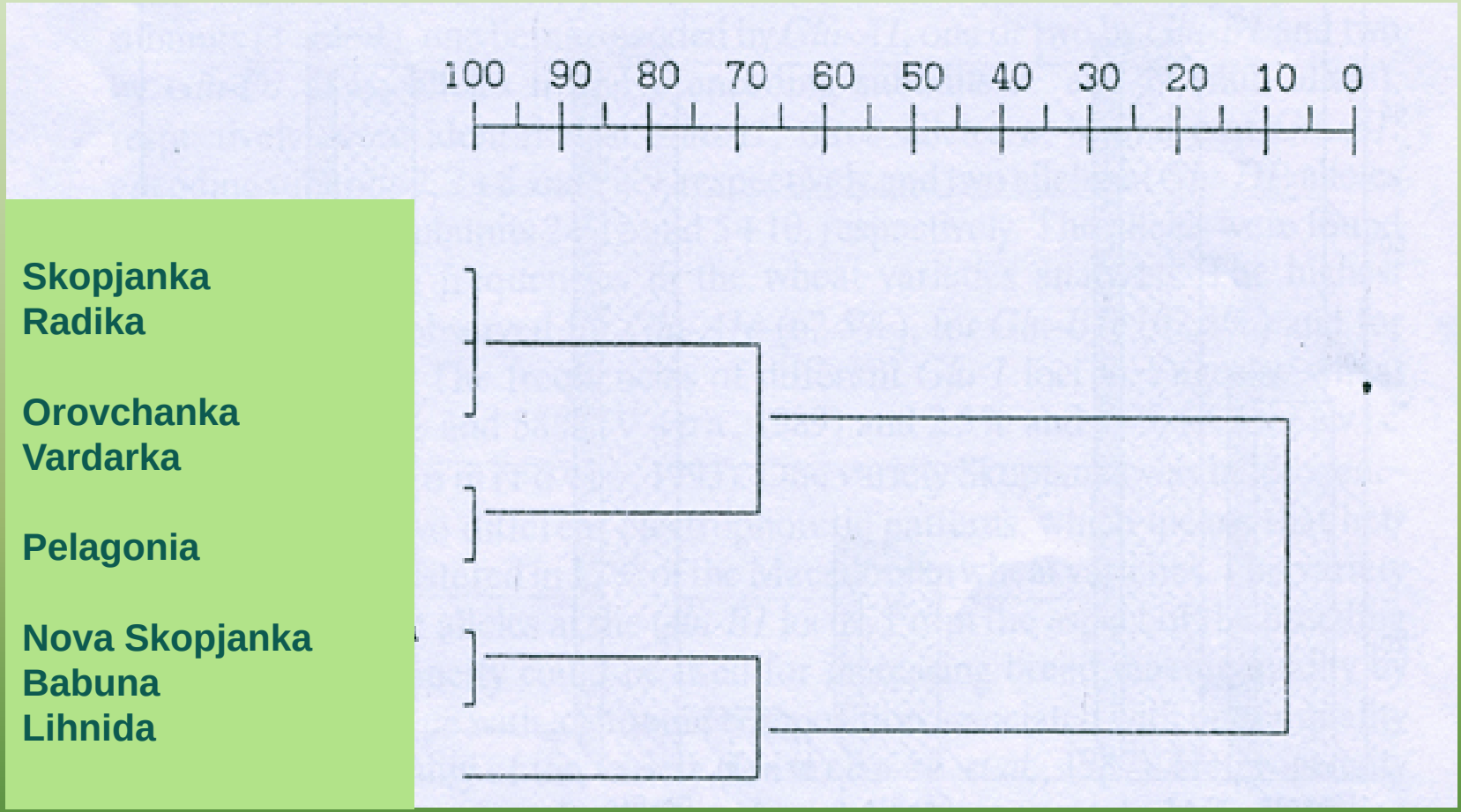


Fig. 4. UPGMA dendogram of Macedonian wheat varieties obtained on the basis of the similarity coefficient of *Glu-1* alleles

Source: *Knezevic and Menkovska, 1994*)

RESULTS AND DISCUSSION

I. Wheat kernel quality

Table 4. Physical –chemical quality data of wheat kernel

Variety	Endosperm structure (%)	1000 Kernel weight (g,db,)	Test Weight (kg/m3) (13%mb)	Protein Content (%, db)	Sediment. Value (ml) (14%mb)
Partizanka	80.0	35.3	842	14.0	50.0
Yugoslavia	75.5	39.1	823	13.9	41.0
N. Skopjanka	80.5	40.1	845	14.6	38.5
Radika	89.0	36.4	835	15.2	44.0
Lihnida	80.7	44.6	844	13.9	31.5
Orovchanka	56.5	42.2	797	14.2	55.5
Babuna	77.0	32.6	791	14.9	49
S.Zlatna	33.0	30.2	788	14.4	32.5
Skopjanka	80.5	43.5	833	13.6	43.5
Pelagonia	00.0	28.0	787	13.5	14.5

II. Rheologycal quality of wheat

- The difference obtained in the flour extraction rate, ash content, protein as well as the content of other compnents from kernel of the investigated wheat varieties, had influence on the rheological properties of the milled flours.

- They were compared with the quality properties of two wheat standards.

- Among the farinograms of the the ivestigated wheat varieties there were similarities and differences.

Table 5. Average rheologycal properties values of milled flours at the total level of flour extraction of investigated wheat varieties

Q P		P	J	O	S	B	R	L	NS	SZ	Pe
WGC	Aver.	37.5	39.5	33.9	36.5	39.2	37.9	38.2	41.4	35.0	36.1
	SD	9.9	11.6	6.1	14.8	6.7	10.9	16.2	17.3	3.7	7.2
WA	Aver.	63.5	64.8	65.5	68.6	65.0	65.9	69.4	68.0	56.3	56.1
	SD	3.6	4.5	4.2	4.8	4.0	4.4	4.0	6.0	4.4	3.8
DDT	Aver.	4.3	3.0	5.5	2.2	2.9	2.8	2.2	2.6	1.6	0.6
	SD	2.2	1.5	3.5	1.2	1.5	1.1	0.6	1.2	1.0	0.6
DST	Aver.	2.9	1.3	3.1	0.6	3.1	1.1	0.6	1.0	0.9	0.5
	SD	3.1	0.4	2.1	0.4	2.3	0.8	0.2	0.6	0.3	0.3
DSO	Aver.	35.0	88.7	35.0	110.0	55.0	80.0	127.5	80.0	81.2	130.0
	SD	33.2	44.8	23.8	57.1	26.4	59.9	55.0	55.8	23.9	49.5
QN	Aver.	68.7	59.4	80.1	46.1	71.0	55.4	40.4	53.9	53.9	31.9
	SD	12.2	17.4	16.0	21.5	16.5	21.1	15.9	19.0	4.6	17.2

WGC-wet gluten content, WA-water absorption, DDT-dough develop.time, DST-dough stability, QN-quality number

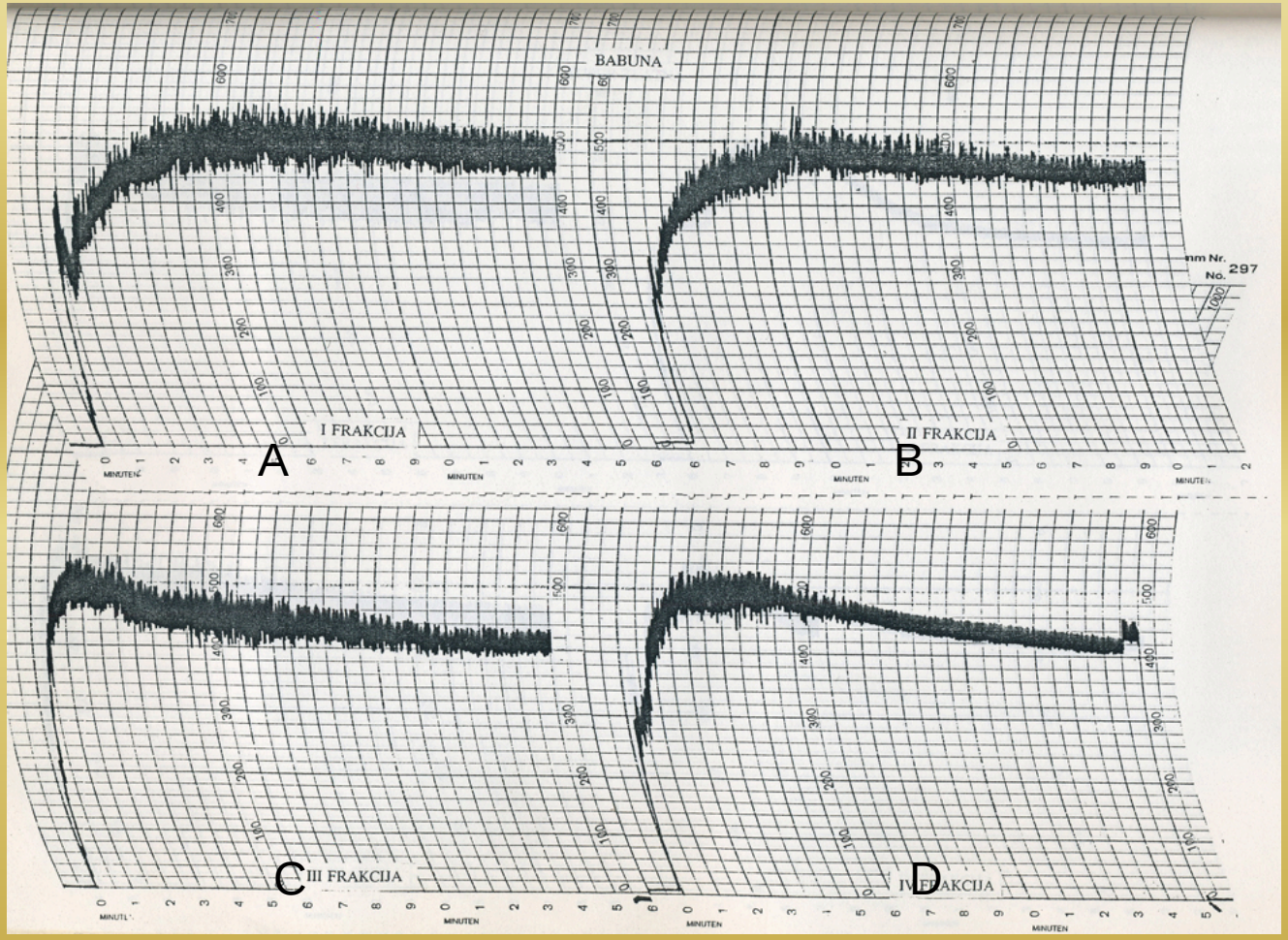


Fig. 5. Farinograms of milled flours of wheat varity *Babuna*

A-fraction I, B-fraction II, C-fraction III, D-fracrion IV

Rheologycal quality of wheat continue

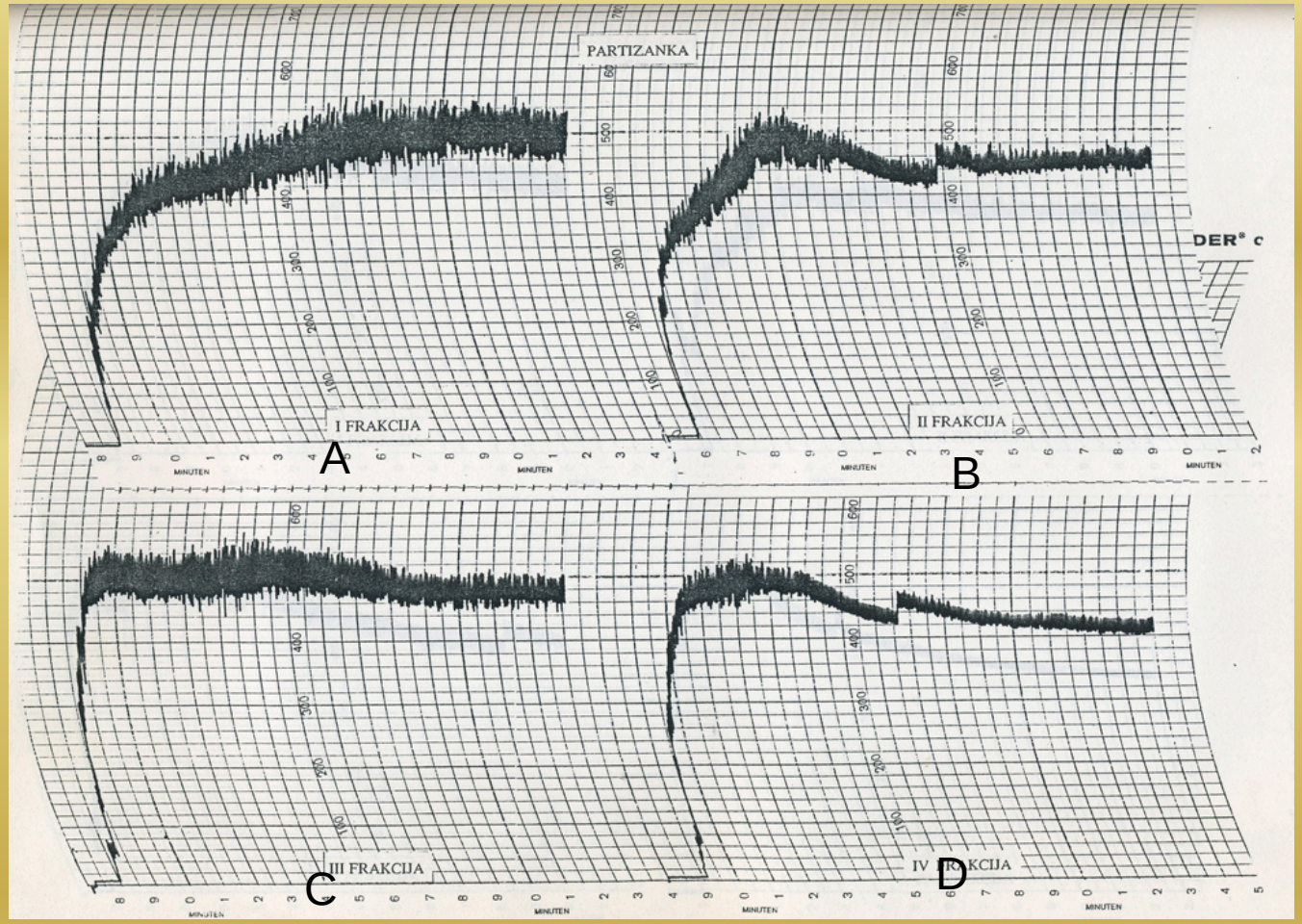


Fig. 6. Farinograms of milled flours of standard wheat *Partizanka*

A-fraction I, B-fraction II, C-fraction III, D-fracrion IV

Rheologycal quality of wheat continue

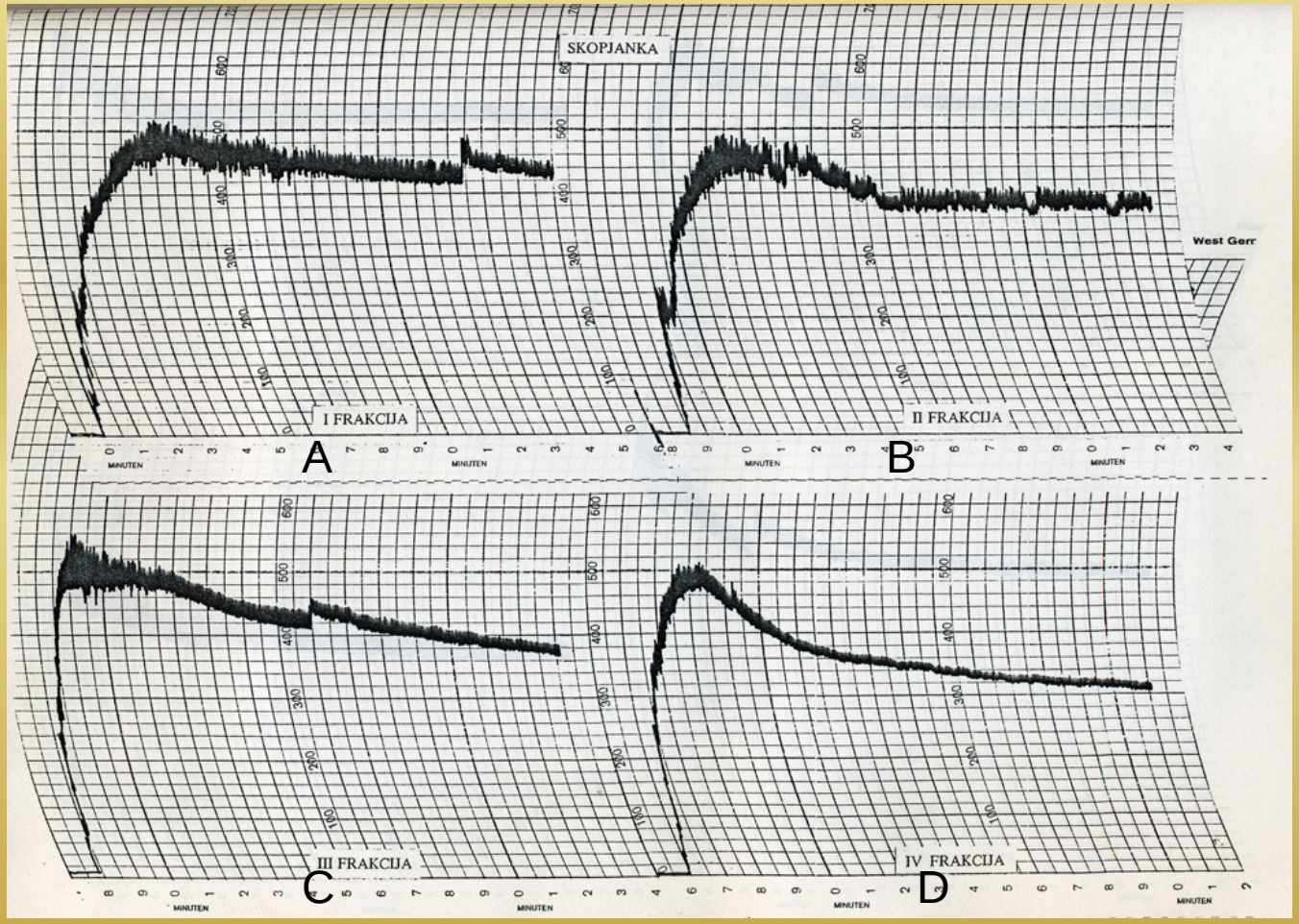


Fig. 7. Farinograms of milled flours of wheat variety *Skopjanka*

A-fraction I, B-fraction II, C-fraction III, D-fracrion IV

III. Bread-making quality

- **There were also differences in the bread loaf quality of the investigated wheat varieties**

Table 6. Average bread-making properties values of milled flours at the total level of flour extraction of investigated wheat varieties

QP		P	J	O	S	B	R	L	NS	SZ	Pe
LV	MV	366. 5	384.0	361.2	368.5	390.0	358.0	363.5	366.5	288.0	326.7
	SD	40.8	50.2	74.4	37.1	66.0	39.3	28.5	23.4	8.6	28.8
LW	MV	135.8	138.0	137.8	139.2	136.3	139.5	138.4	139.6	131.9	132.6
	SD	3.4	5.8	3.4	4.0	2.5	4.2	4.7	4.3	5.1	7.7
QNC	MV	3.6	3.2	3.2	3.7	4.4	3.7	3.7	3.5	1.4	1.5
	SD	2.5	3.2	2.7	2.1	2.0	2.6	2.1	2.3	1.2	0.0
BY	MV	140.5	142.4	139.0	142.6	142.0	141.2	142.9	143.7	135.2	134.2
	SD	3.0	4.9	6.1	3.8	2.3	4.1	4.1	4.5	4.7	3.4
VY	MV	379.4	396.7	365.9	377.4	406.0	362.1	375.5	371.5	293.0	330.8
	SD	43.9	54.3	70.8	37.7	67.1	35.8	29.8	24.3	10.4	29.4

Legend:LV-loaf vol., LW-loaf weight, QNC-qual. number of crumb, BY-bread yield, VY-volume yield QP-Quality properties

Baking quality, contunued

Fig. . Bread crumb of standard wheat *Partizanka*

A-fraction I **B-fractionII**

C- fraction III **D-fraction IV**

Baking quality, contunued

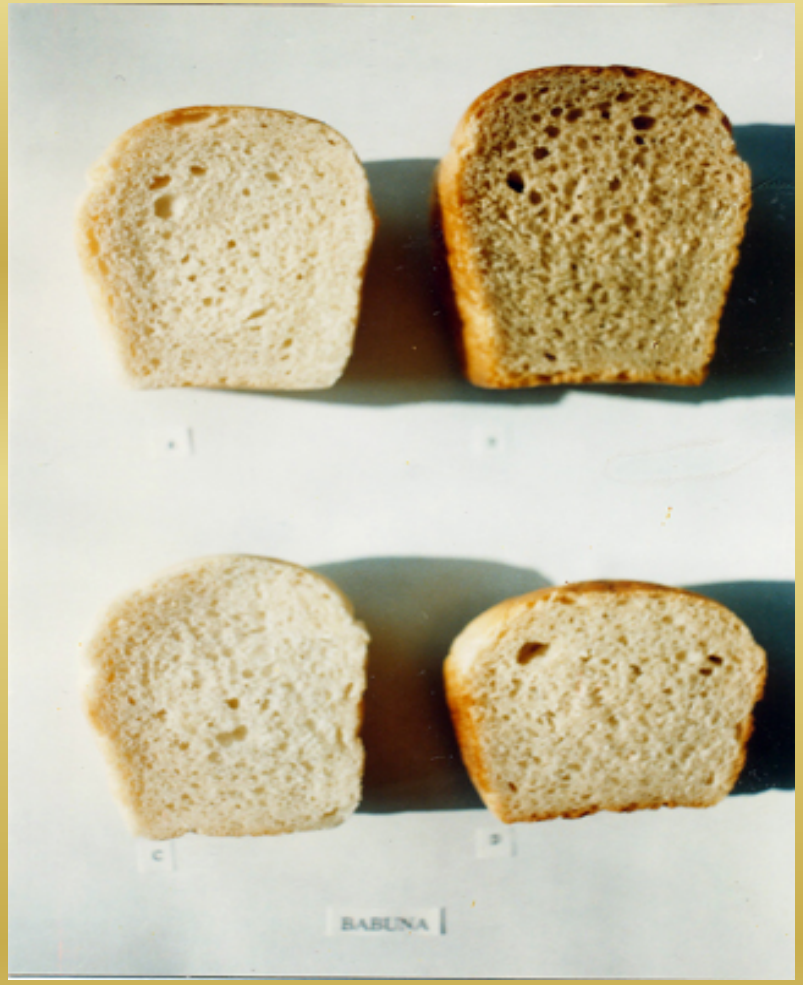


Fig. . Bread crumb of wheat variety *Babuna*

A-fraction I **B-fraction II**

C- fraction III **D-fraction IV**

Baking quality, contunued

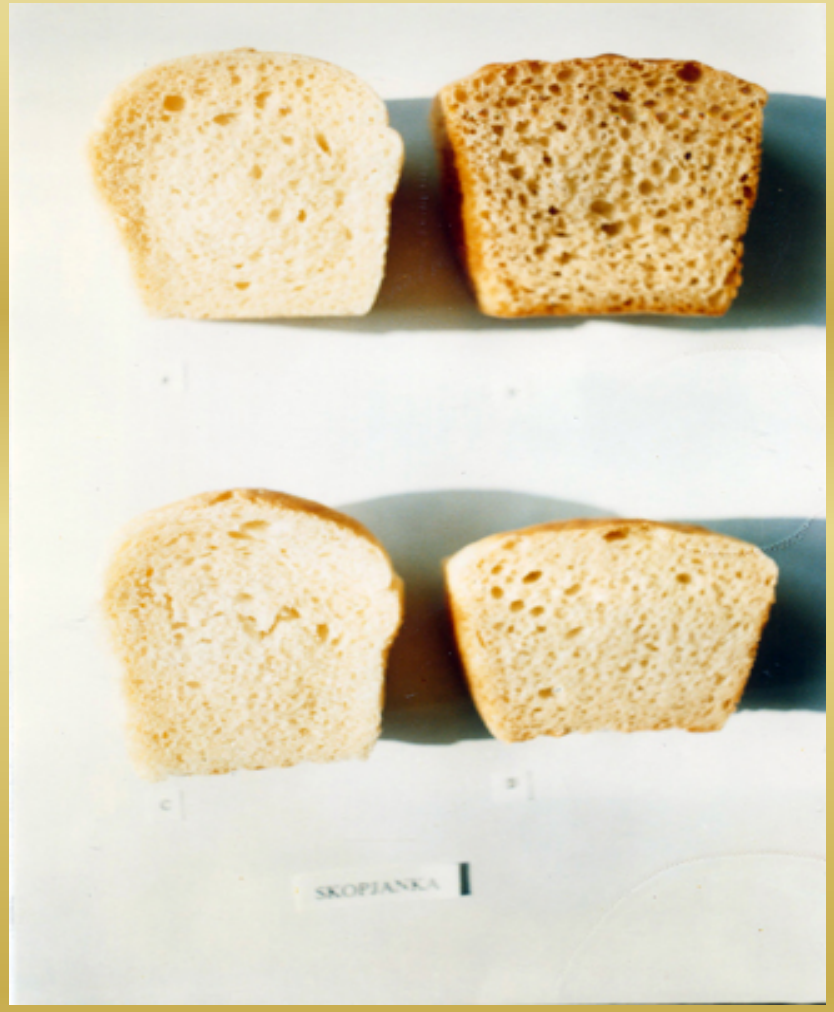


Fig. . Bread crumb of wheat variety *Skopjanka*

A-fraction I **B-fractionII**

C- fraction III **D-fraction IV**

Table 7. Composition of gliadin variants of investigated wheat varieties

Variety	1A	1B	1D	6A	6B	6D
P	11	1	1	11	21	1
J	11	3	2	2	1	6
NS	11+4	3	2	6+1	11?	4(15)
R	4	3	1	6+2	1?	1
L	4	3	2	1	5	7?
O	4	1	1	1	2	4
B	4+11	1	1	1+11?	21	1
SZ	4	4	12	11	30+25	18
S	4	3	2	6+2	1	4
Pe	5	16	1	2	?	4

Table 8. Composition of HMW glutenin subunits of investigated wheat varieties

Variety	1A	1B	1C	*Glu-1* score
P	2*	7+9	5+10	9
J	2*	7+9	5+10	9
NS	2*	7+9	5+10	9
R	0	7+9	2+12	5
L	2*	7+8	5+10	10
O	0	7+9	2+12	5
B	2*	7+9	5+10	9
SZ	1	6+8	2+12	6
	1	7+9	2+12	7
S	0	7+9	2+12	5
	0	7+8	2+12	6
Pe	0	7	2+12	4

- **Gliadin components from each block of each wheat vareity are presented in the form of genetic formula.**
- **At each gliadin encoded loci different numbers of alleles were present.**
- **The alleles are distinquished among themselves according to the electrophoretic mobility and molecular weight of the gliadin components from the block composition.**
- **Different numbers of alleles at each analysed glutenin encoded loci.**

Correlation between protein composition and wheat quality properties

- **The existence of significant relationship was determined between the presence of the particular gliadin block and HMW glutenin subunits from the protein composition of the investigated wheat varieties and particular technological quality characteristics of kernel, milled flours and bread loafs (crumb and crust).**

Table 9. Correlation between presence of particular allelic variants of gliadins and kernel quality

Alleles	Protein content (%)	SE (ml)	1000 kernel weight (g)
1A4	0.78	-1.06	0.85
1A11	-0.15	2.51*	1.68
1B1	0.40	9.19***	-0.43
1B3	0.08	-1.15	5.33***
1D1	0.60	3.48**	-3.12*
1D2	-0.77	-2.15	5.91***
6A1	0.31	3.78**	2.25
6A6	0.69	-0.06	2.49*
6D1	1.45	5.73***	-2.21
6D4	0.86	-2.55*	1.33

Legend:

I- flours from 1st, 2nd and 3rd break

II- flours from 4th, 5th, and 6th break

III-flours from 1st, 2nd and 3rd reduction

IV-floursfrom 4th, 5th, and 6th reduction

t-Student t-test

***-significant for P lower of 0.001

** - significant for P lower of 0.01

* - significant for P lower of 0.05

Table 10. Correlation between presence of particular allelic variants of HMW glutenins and kernel quality

Alleles	Protein content (%)	SE (ml)	1000 kernel weight (g)
1A2 (*)	0.17	2.71*	1.50
1A0	-0.33	-1.49	0.34
1B (7+8)	-1.06	-2.96*	5.33***
1B (7+9)	0.81	9.38***	2.61*
1D (2+12) 1D (5+10)	-0.17 0.17	-2.71* 2.71*	-1.50 1.50

Legend:
I- flours from 1st, 2nd and 3rd break
II- flours from 4th, 5th, and 6th break
III-flours from 1st, 2nd and 3rd reduction
IV-floursfrom 4th, 5th, and 6th reduction

t-Student t-test
***-significant for P lower of 0.001
** - significant for P lower of 0.01
* - significant for P lower of 0.05

Table 11. Correlation between presence of particular allelic variants of gliadins and wet gluten content of milled flours

Alleles	I	II	III	IV	Total
1A4	0.22	-1.86	1.23	-0.32	-0.46
1A11	2.43*	3.37**	-1.20	0.08	2.09*
1B1	1.65	-2.30	1.65	2.69*	0.96
1B3	5.12***	6.78***	-0.59	3.93**	3.08**
1D1	0.64	-3.24*	2.81*	2.67*	0.36
1D2	3.30*	6.63	-1.77	-3.93	2.15
6A1	2.43*	-0.42	1.71	0.71	1.49
6A6	3.02*	3.70**	0.10	-2.59	2.02
6D1	3.77**	-0.52	2.46*	-1.97	2.46*
6D4	-1.43	0.49	-0.55	-1.50	-1.01

Legend:

I- flours from 1st, 2nd and 3rd break

II- flours from 4th, 5th, and 6th break

III-flours from 1st, 2nd and 3rd reduction

IV-floursfrom 4th, 5th, and 6th reduction

t-Student t-test

***-significant for P lower of 0.001

** - significant for P lower of 0.01

* - significant for P lower of 0.05

Table 12. Correlation between presence of particular allelic variants of HMW glutenins and wet gluten content of milled flours

Alleles	I	II	III	IV	Total
1A2 (*)	5.30***	4.35**	0.06	0.24	3.76***
1A0	-0.9	-1.9	0.73	-1.06	-1.29
1B (7+8)	1.52	2.46*	-1.45	-5.41***	0.2
1B (7+9)	1.56	-0.24	0.73	1.5	1.23
1D (2+12)	-5.30***	-4.35**	-0.06	-0.24	-3.76***
1D (5+10)	5.30***	4.35**	0.06	0.24	3.76***

Legend:

I- flours from 1st, 2nd and 3rd break

II- flours from 4th, 5th, and 6th break

III-flours from 1st, 2nd and 3rd reduction

IV-floursfrom 4th, 5th, and 6th reduction

t-Student t-test

***-significant for P lower of 0.001

** - significant for P lower of 0.01

* - significant for P lower of 0.05

Table 13. Correlation between presence of particular allelic variants of gliadins and the loaf volume of milled flours

Alleles	I	II	III	IV	Total
1A4	-3.59**	4.39**	-1.85	-7.80***	-2.72**
1A11	13.05***	2.46*	7.02***	4.80**	9.10***
1B1	5.81***	16.71***	-1.48	-3.42**	9.23***
1B3	10.46***	-3.62**	16.11**	5.27***	10.10***
1D1	-1.49	11.02***	-3.08*	-2.22	2.91**
1D2	9.80***	2.38**	11.98***	7.80***	10.15***
6A1	2.23	14.08***	2.02	-0.96	8.60***
6A6	3.38**	-0.46	6.35***	1.47	4.25***
6D1	8.11	6.19***	3.53**	-0.37	8.53***
6D4	6.45	5.71	-3.31*	1.24	-1.15

Legend:

I- flours from 1st, 2nd and 3rd break

II- flours from 4th, 5th, and 6th break

III-flours from 1st, 2nd and 3rd reduction

IV-floursfrom 4th, 5th, and 6th reduction

t-Student t-test

***-significant for P lower of 0.001

** - significant for P lower of 0.01

* - significant for P lower of 0.05

Table 14. Correlation between presence of particular allelic variants of HMW glutenins and the loaf volume of milled flours

Alleles	I	II	III	IV	Total
1A2 (*)	16.26***	3.01*	11.19***	9.11***	15.82***
1A0	-6.65***	5.34***	-2.85	-3.32*	-2.73**
1B (7+8)	2.67*	1.61	5.81***	4.05**	4.00***
1B (7+9)	18.75**	15.83***	9.19***	-0.85	19.59***
1D (2+12) 1D (5+10)	-16.26*** 16.26***	-3.01* 3.01*	-11.19*** 11.19***	-9.11*** 9.11***	-15.82*** 15.82***

Legend:

I- flours from 1st, 2nd and 3rd break

II- flours from 4th, 5th, and 6th break

III-flours from 1st, 2nd and 3rd reduction

IV-floursfrom 4th, 5th, and 6th reduction

t-Student t-test

***-significant for P lower 0.001

** - significant for P lower of 0.01

* - significant for P lower of 0.05

C O N C L U S I O N S

- The wheat varieties investigated have shown different quality of the kernel and milled flours.
- The composition of their gluten proteins: gliadins and HMW glutenins was determined using PAGE and SDS-PAGE.
- The variability of the alleles from the particular *Gli* and *Glu loci* was determined. 25 different alleles from the all six *Gli* and nine alleles from three *Glu* loci were identified.

Conclusions continued

- **A significant relationship was determined between the presence of particular gliadin block and HMW glutenin subunits from the wheat protein composition and particular technological quality properties of the kernel, milled flours and bread loafs.**

- **The *Glu*-1 quality score for the investigated wheat varieties was also determined varying from 3-9 beeing in agreement with the technologycal quality of wheats.**

- **Using the applied biochemical methods on gluten proteins, the technological quality of wheat can be predicted.**

- **These findings are important besides for the wheat breeding, also for the producers, consumers and trade of the bread wheats, for satisfying their needs for wheats and their products with desired technolocal quality.**

THANK YOU FOR YOUR ATTENTION

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