Chemometric Analysis of High Molecular Mass Glutenin Fractions and Image Data of Bread Crumb Structure from Croatian Wheat Cultivars

Damir Magdić, Daniela Horvat [1], Zorica[1] Jurković, Rezica[1] Sudar, Želimir[2] Kurtanjek*

Faculty of Food Technology, University "J. J. Strossmayer" in Osijek, F. Kuhača 18, HR-31107 Osijek, Croatia , damirm@ptfos.hr
[1]Agricultural Institute Osijek, Južno predgrađe 17, HR-31000 Osijek, Croatia, daniela.horvat@poljinos.hr
[2] Faculty of Food Technology and Biotechnology, University of Zagreb Pierottijeva 6, HR-10000 Zagreb, Croatia, zkurt@mapbf.pbf.hr

Summary

The aim of this work is to investigate functional relationships among wheat properties, high molecular mass (weight) (HMW) glutenin subunit fractions and bread quality produced from eleven Croatian wheat cultivars by chemometric analysis. HMW glutenin subunits were fractionated by sodium dodecylsulfate polyacrylamid gel electrophoresis (SDS-PAGE) and subsequently analysed by scanning densitometry in order to quantify HMW glutenin fractions. Wheat properties are characterised by four variables: protein content, sedimentation value, wet gluten and gluten index. Bread quality is assessed by the standard measurement of loaf volume, and visual quality of bread slice is quantified by 8 parameters by the use of computer image analysis.

* *Corresponding author*

The data matrix with 21 columns (measured variables) and 11 rows (cultivars) is analysed for determination of number of latent variables. It was found that the first two latent variables account for 92, 85 and 87 % of variance of wheat quality properties, HMW glutenin fractions, and the bread quality parameters respectively. Classification and functional relationships are discussed from the case data (cultivars) and variable projections to the planes of the first two latent variables. Between Glu-D1y proportion and the bread quality parameters (standard parameter loaf volume and bread crumb cell area fraction determined by image analysis) the strongest positive correlations are found, r = 0.651 and r = 0.885 respectively. Between Glu-B1x proportion and the bread quality parameters the strongest negative correlations are found, r = -0.535 and r = -0.841 respectively.

The results are discussed in view of possible development of new and improvement of existing wheat cultivars and optimisation of bread production.

Keywords: chemometrics, wheat, HMW glutenin subunits, bread quality, image analysis

Introduction

Recent development of automatic computer aided systems for gene determination and proliferation of highly effective analytical instrumentation, such as IR, FTIR, NIR, NMR, MS and others, has revolutionised experimental and theoretical methods in biotechnology. Models of functional relationships between varieties genome and protein system are very complex, and in order to cope with immense number of data new modelling methods are being applied. Traditional univariable analysis (one input and one output variable) can not account for multivariable

interactions among gene, proteins and biological effects. Classical multivariate statistical methods deal with huge amount of data and are focused on error analysis for postulated models (structures) among measured variables. Chemometric analysis is also a statistical method, but in contrast with classical statistics, it is focused toward the extraction of functional information among variables (*1*). Collinearity among multivariate data are exploited for reconstruction of few latent variables, which can account for observed complex biological effects. Large data sets are effectively reduced, and only the functionality among latent variables is investigated. After data reduction, redundant information is interpreted as a measurement error, and/or random influence of surroundings on experiment, and the both are usually discarded

Experimental data can be viewed as points in a high dimension space of *n* observed variables (*1-3*). Due to their functional relationship (correlations) observed variables can be projected to a space of low dimension of *r* latent (internal or principal) variables without loss of essential information (*2,3*). The projection can be formally denoted as:

$$(x_1, x_2, x_3, \cdots x_n) \rightarrow (l_1, l_2, \cdots l_r) \qquad r < n \qquad /1/$$

Set of measurement data of *m* samples of *n* variables form a matrix ***X***(*m* x *n*) (usually autoscaled for average and/or standard deviation elimination), which is, by projection from space of dimension *n* into space of dimension *r*, decomposed into a sum of *r* sub-matrices $\boldsymbol{X}_i$ and a residual matrix ***E***.

$$\mathbf{X}(m\,x\,n) = \sum_{i=1}^{r} \mathbf{X}_i (m\,x\,n) + \mathbf{E}(m\,x\,n) \qquad /2/$$

The partial matrices $\boldsymbol{X}_i$ correspond to latent variables, and they "capture" deterministic components of measured data, while measurement errors (stochastic components) are retained in the error matrix $\boldsymbol{E}$. Variances of the sub-matrices are ordered, so that the maximum variance corresponds to the first latent variable:

$$\sigma^2(\mathbf{X}_1) > \sigma^2(\mathbf{X}_2) > \cdots \sigma^2(\mathbf{X}_r) \qquad /3/$$

The sub-matrices are determined as the outer products of score (target) vectors $\boldsymbol{t}_i$ and corresponding latent variable (represented by its principal component vector $\boldsymbol{p}_i$):

$$\mathbf{X}_i = \mathbf{t}_i^T \cdot \mathbf{p}_i \qquad \mathbf{t}_i = \mathbf{X} \cdot \mathbf{p}_i \qquad /4/$$

First principal vector is determined by minimisation of the variance between the data matrix $\boldsymbol{X}$ and the first sub-matrix $\boldsymbol{X}_1$:

$$\mathbf{p}_1 = \min_{\mathbf{p}_1} \sigma^2(\mathbf{X} - \mathbf{X}_1) \qquad /5/$$

Minimisation leads to the eigenvalue problem of the covariance matrix:

$$\mathrm{cov}(\mathbf{X}) \cdot \mathbf{p}_i = \lambda_i \cdot \mathbf{p}_i \qquad /6/$$

$$\mathrm{cov}(\mathbf{X}) = \frac{1}{m-1} \cdot \mathbf{X}^T \cdot \mathbf{X} \qquad /7/$$

Eigenvectors and eigenvalues are calculated by the iterative singular value decomposition procedure (*3*). Linear multivariable models between variable y_k of the output set and score variables of the input set are developed:

$$y_k = \boldsymbol{\beta}_1^T \cdot \mathbf{t}_1 + \cdots \boldsymbol{\beta}_r^T \cdot \mathbf{t}_r + e_k \qquad /8/$$

Model parameters are determined from minimisation of the variance (principal component regression, PCR), or the directions of the principal components can be tied to the minimisation of model variance leading to the partial least squares method (PLS).

Quantity of high molecular mass (HMW) glutenin in wheat is important for bread making quality as well as their composition (4-6). All HMW glutenin subunits are encoded by the genes at three complex loci (Glu-A1, Glu-B1 and Glu-D1). These genes encode HMW glutenin that is classified as *x* or *y* type, according to its higher or lower molecular mass, respectively. In spite of the fact that HMW glutenin accounts for approximately 10 % of the wheat storage proteins, it plays a key role in bread making quality of wheat cultivars (*7*).

Among bread making parameters accessed by flour and dough analysis and baking test, bread volume and crumb quality are certainly very important. Evaluation of bread properties can be partially aided by computer image analysis methods that combine techniques for computing statistics of pixel properties, mostly applied to grey level intensities. The main crumb texture attributes can be evaluated by computer programms based on different algorithms (*8-12*). In evaluation of these visual attributes, image analysis has been confirmed as an objective and highly confident method, and in the last decade it has become almost a standard crumb structure evaluation method (*13-15*).

In this work, the analysis is performed on $\boldsymbol{X}$ data matrix with dimension 11x 21. Measured are 21 variables, which include 4 variables of wheat quality properties, 7 variables are high molecular mass (HMW) glutenin fractions, and 10 bread making quality parameters (8 bread crumb structure variables are determined by computer image analysis, additional quality variable is the standard bread making quality measurement, and the last quality variable is Glu-1 quality score). Rows of the matrix $\boldsymbol{X}$ are samples corresponding to 11 Croatian wheat cultivars. The data matrix $\boldsymbol{X}$ provides the basis of chemometric analysis.

Materials and Methods

Wheat samples

Grain samples (Prebasic seeds) from ten winter wheat cultivars: Žitarka, Super Žitarka, Srpanjka, Barbara, Klara, Golubica, Monika, Kata, Ana and Demetra (selected at Agricultural Institute Osijek, Croatia) and cultivar Divana (Jošt-Sjeme, Križevci, Croatia) as the improver standard, were taken from the harvest of 2000.

Wheat and flour quality assessments

Crude protein content of wholemeal flour (Cyclone Sample Mill, Tecator, 1 mm sieve) was measured by Kjeldahl method (ICC 105/2; N x 5.7 % DM). The following wheat quality parameters of flour (ash content 0.55 %, Brabender Quadrumat Jr. Mill) were determined: wet gluten content and gluten index (ICC No 155); Zeleny sedimentation volume (ICC No 116/1). The baking test was performed according to modified ICC standard method No 131 (280 g flour instead of standard 250 g per loaf because of the bigger mould volume).

Sample preparation and SDS-PAGE

Total proteins were extracted from 50 mg of 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). To avoid considerable variation in the amount of individual HMW glutenins between kernels of a sample, 30 grams of kernels were milled (Retsch Mill, Type ZM1, 1 mm sieve) to produce wholemeal flour. Glutenin extract (1 μL) from each investigated cultivar was applied on two lines over six PhastGel gradients 4-15 (43 x 50 x 0.45 mm) and HMW glutenin molecules were fractionated through SDS-PAGE using Phast System, Pharmacia LKB, under the following conditions: 40 min, 250 V, 10 mA, 15 °C and 120 V h. Gels were stained

using 0.1 % Coomassie Brilliant Blue R. HMW glutenin were identified according to Payne and Lawrence (*16*). Subsequently, gels were analysed by scanning densitometry in order to quantify HMW glutenin fraction bands. The protein content (average values of three scans) of a single band is determined in arbitrary units and represents the integrated numerical value of the area under a peak. (Image Master VDS Software, v. 2.0, Pharmacia LKB) (*17*). The results of the quantification of CBB-stained HMW glutenin fractions were evaluated using STATISTICA (Statistica, v. 6.0, StatSoft Inc.) (*2*).

Image analysis

In preparation for quality assessment by image analysis, bread loaves were sliced in the middle, providing two cross sections. Slices were properly illuminated (700 lux) and images were captured by (Sony CCD-TR427E PAL, 320x240 pixels) camera, digitised (by Hauppauge Win TV-Premio-FM card Modell 719) and saved as 8-bit colour bitmap files. The acquired images represented surfaces with the size of 10 x 8 cm (960 pixels per cm^2). Three loaves were produced from each of eleven wheat cultivars and images of each half of a loaf were recorded as 8-bit colour bitmap files. A slice of the middle area of the original image was cropped, transformed and saved in 8-bit bitmap file with 256 grey levels. The threshold operation was applied on such images with threshold at grey level 128. The threshold value was determined by the "trail and error" method in order to gain the best cell resolution. All analysed images were resized in the same frame size (200 x 150 pixels, still with 960 pixels per cm^2), and the same image processing operations were applied to all records (Fig. 2). After the image pre-processing, the evaluation of crumb texture appearance was performed. Slice texture analysis was determined by calculation of: total area of cells (sum of all spots where more than 5 pixels were connected), number of cells bigger than 5 pixels,

average cell area (slice area inside the frame was involved in calculation, while the background around the slice was excluded), minimum and maximum radius, average radius, and perimeter and roundness of crumbs/cells (measure of roundness is in the range between 0 (not round) and 1 (perfectly circular)). The value is calculated as: roundness = 4 π total area / (perimeter squared) as the main crumb texture attributes. The attributes were evaluated by the use of GLI/2 Scientific Imaging Software (*18*).

Chemometric analysis

Average values of all the variables (wheat properties, HMW glutenin fractions, and quality data) were collected in a matrix with 11 rows and 21 columns (stored as an EXCEL data file). The data file was exported for numerical evaluation and graphical plotting to MATLAB (*19*) and STATISTICA (*2*) software. Numerical algorithms provided by the software from Wise and Gallagher (*3*) were applied.

Results and Discussion

Average values of wheat quality parameters for the eleven Croatian wheat cultivars are presented in Table 1. Protein content (mass fraction) varies in the range 11.17-15.21 %, while sedimentation value is in the range 31-61 cm^3. Cultivars with protein content higher than 12 % and sedimentation value above 40 cm^3 are considered as better quality according to the official Croatian Regulations (*20*)**.** By this criteria, cultivar Divana, with the maximum protein content of 15.21 % and sedimentation value 61 cm^3, is of the highest quality. However, at levels >14 %, higher protein means more gliadins, so breadmaking quality no longer improves with subsequent increases in protein content. Content (mass fraction) of gluten, expressed as wet gluten, varies between 24.98 and 42.75 %. Gluten index, as the measure of

gluten quality, varies between 62 and 99. According to ICC standard method, cultivars with gluten index values in the range 60-90 are recommended as good bread cultivars (*21*). Cultivars Divana, Ana and Demetra (gluten index > 90) are already known as bread quality improvers.

The results of bread making quality, determined by volume yield, are also presented in Table 1. Loaf volume yields vary between 406 and 553 cm^3, with maximum value obtained for Divana cultivar.

In Fig.1. SDS-PAGE electrophoregrams of Croatian wheat cultivars are presented. Results of HMW glutenin identification and quantitative evaluation of HMW subunit fractions are given in Tables 2,3.

According to HMW glutenin composition and the Glu-1 quality scores (*16*) (Table 2) the investigated cultivars can be placed in five classes (N 7+8 2+12; 1 7+8 2+12; N 7+9 2+12; N 7+9 5+10 and 1 7+9 5+10). HMW glutenin Glu-1 quality scores for Croatian cultivars are in the range between 5 and 9, with the score 10 being the theoretical maximum.

Proportion of HMW glutenin at Glu-D1 locus was found to be higher than its proportion at Glu-B1. In general, proportion of HMW glutenins *y*-type (lower molecular mass) is in higher proportion than that of *x*-type (higher molecular mass), given in Table 3.

The results of bread crumb structure evaluated by computer image analysis (Figs. 2,3) are presented in Table 4. It has been reported that textural appearance of bread crumbs depends on the quality of flour protein, amount of gluten, proportion of gluten protein, type of glutenin subunits, and especially on HMW glutenin fractions (*22-26*). Correlation coefficients between HMW glutenin fractions and computer image bread crumb structure parameters are presented in Table 5. Large differences in

bread crumb structure among cultivars are visible. Total crumb cell area ranges from 0.66 up to 5.78 % of total surface area. Cultivars Srpanjka and Divana have the highest porosity, 5.78 and 5.52 % respectively, while the lowest porosity 0.66 % is determined for Žitarka. It is also clearly visible that some cultivars have smaller average cell radius, while other cultivars have larger, although the difference among loaf volumes is not visible. Calculated hole roundness for all cultivars lies between 0.55 and 0.66. This indicates that the shape of the porous part of medium bread parts is independent from loaf volume, more or less, they all have approximately elliptical shape (1:2).

Significant positive and negative correlations between image analysis results and HMW glutenin fractions are found, Table 5. The highest positive correlation r = 0.89 is found between porosity (total crumb cell area) and Glu-D1y, while the most negative correlation r = -0.84 is found with Glu-B1x proportion.

However, due to high correlation between measured variables, the interpretation of the effects of HMW glutenin fractions on bread making quality is obscured. In order to reveal the number of latent variables which can explain functional relationship, the data matrix $\boldsymbol{X}$ is decomposed into principal components /2/. In Fig. 4. the results of relative cumulative contribution of the latent variables on the variances of measured data of wheat quality properties (4 variables), HMW glutenin fractions data (7 variables), and bread quality data (11 variables) are presented. The first two latent variables account for 95 % of wheat properties, 87.1 % of HMW glutenin fractions, and 83.1 % of bread quality data. These results prove very strong functional interdependence of the variables, which is statistically reflected as significant correlation among all the variables. Especially the result for HMW glutenin content is significant, indicating that only two latent variables can account for

genetic expression of the full spectrum HMW glutenins. In Fig. 5A projections of the cultivars on the score plane of the first two latent variables extracted only from the quantitative data for HMW glutenin subunits are presented. The first and second latent variables account for 59.45 and 27.64 % of the total variance. Clustering of the cultivars based on latent variables of HMW glutenin fractions can be observed. Two well-distinguished clusters are formed. First cluster form Klara, Demetra and Ana, while the second consists of Žitarka, Barbara, Super Žitarka and Monika. Cultivars Kata and Golubica could be attributed to the third cluster. Divana has a very singular position with Srpanjka being located in relative proximity. Cultivar Divana is a known bread quality wheat improver. Relationships between the clusters and HMW glutenin fractions can be discerned from the score plot of the variables, Fig. 5B. The major effect of Divana's singular position as bread quality improver is due to its Glu-D1y proportion. Srpanjka is closest with its share of Glu-D1x and Glu-D1y. The first cluster is mostly related to Glu-A1x, while the score positions for the rest of the cultivars are dominated by Glu-B1x and Glu-B1y. The HMW glutenin fractions of *x* and *y* type (Glu1-1x and Glu-1y variables), which are in literature defined as the important variables for the prediction bread making quality (BMQ), actually define the first latent variable, and are projected at the opposite end points of L_1 eigenvector, Fig. 5B.

Functional relationship between HMW glutenin fractions and wheat properties, and then followed by bread quality parameters, have been determined by projections onto the score planes defined by the corresponding joint data sets. Results are presented in Figs. 6 and 7. The first two latent variables account for (48.44 and 23.12 %) and (61.08 and 16.16 %) of the total variances, respectively. Again this indicates very strong functional relationship between the variables, and only the first

two latent variables can explain variance in each joint data set almost up to the level of error measurements (estimated to 7 %).

In Fig. 6A classification of the cultivars from the data set with wheat quality properties is given. Results are similar to the classification obtained from HMW glutenin fractions, Fig 5A (there is only an irrelevant difference in the sign of the first eigenvector). Due to its wheat properties, cultivar Srpanjka now belongs to the same first cluster as previously defined for Klara, Demetra, and Ana. Divana now has even more pronounced singular position, which confirms its selection as the principal bread quality improver cultivar. All the other cultivars have not significantly changed their score co-ordinates, which clearly indicates that wheat quality properties are significantly correlated with HMW glutenin fractions. From Fig. 7A it can be inferred that gluten index has the same effect as the key Glu-1y glutenin subunit, while wet gluten acts approximately like the Glu-1x glutenin subunit. Protein content is projected on the second eigenvector L_2 and can not be explained by HMW glutenin fractions.

Functional relationship between HMW glutenin fractions and bread quality parameters can be inferred from the projections presented in Figs. 7B and 8. The bread quality variables include 9 parameters from image analysis, loaf volume and gluten quality score. The degree of collinearity between variables is here even more pronounced. All image parameters are projected on the first latent variable L_1 along Glu-D1y. The first latent vector based on HMW glutenin proportion, defined by the projections of Glu-1x and Glu-1y glutenin subunit, is now rotated clockwise for about 30^0, which is the result of the weight from large number of image parameters in the data set. However, relative positions of HMW glutenin projections are unchanged. The variables of loaf volume and Glu-1 quality score are closely projected toward

each other, along the rotated vector defined by the HMW *x* and *y* type glutenin fractions. hence, loaf volume is determined prevalently by Glu-1y glutenin subunit, and/or Glu-1 quality score. Due to the rotation of the latent vectors, classification of the cultivars is also rotated, less clustered, but still Divana stands out as the singular cultivar.

Predictive value of HMW glutenin fractions on bread quality is illustrated by the linear models presented in Figs. 8 and 9. Dependent variables are physical parameter (loaf volume) and an image analysis parameter (total area of holes). Independent variables are Glu-B1x and Glu-D1y glutenin subunits, which define a clock-wise tiled line for the angle of 15^0 from the first latent variable (Fig. 5B). Due to the proximity of this line to L_1, these predictions can be interpreted as a main influence of the first latent variable, *i.e.* Glu-1x and Glu-1y glutenin subunits. From the results it is obvious that the independent variables have the opposite effects on the quality parameters. Linear relationships are obtained by the classical least squares method. The correlation coefficients for the quality parameter, total area, determined by computer image analysis, are r = -0.841 and r = 0.887 for Glu-B1x and Glu-D1y respectively, while for the standard physical quality parameters, loaf volume, the correlations are lower with values r = -0.535 and r = 0.651.

In order to verify proposed models and conclusion a validation test is performed. Accuracy and robustness of model predictions is determined dominantly by the number of experimental data. Although the number of cultivars in this study is relatively low, 11 cultivars are included, due to 12 replicate measurements for each cultivar, the total number of experimental data is relatively large, *i.e.* 11 (cultivars) x 12 (parallel experiments) with 21 measured variables, which amounts to the total of 2772 data.

Model accuracy and robustness are validated by a test in which one cultivar is removed from the data set for model development. For this purpose cultivar Golubica was selected by random choice as a test sample. The test cultivar is now treated as an "unknown" cultivar. Principal component analysis is re-evaluated with the reduced set of 10 remaining cultivars, and projections are obtained with the new components.

The unknown (test) cultivar Golubica is projected with principal components derived from the set of patterns (HMW glutenin fractions) not included in the model development. Results of the evaluation test are presented in Fig. 10. From the results relative errors for the first and second projection for the unknown cultivar (test Golubica) of 8 and 4 % are estimated. Errors of projections for the complete set of cultivars are within variance captured by the first two principal components.

From the performed validation test it can be inferred that derived model and conclusions are statistically significant. Although relatively small number of cultivars is included in this research, it is compensated by large number of biochemical, chemical, physical and qualitative data, which enable induction of true latent variables.

Conclusions

Chemometric analysis of the eleven Croatian wheat cultivars has revealed very strong functional relationship between HMW fractions, wheat quality properties and bread quality. The first two latent variables can effectively explain relationships within 7 variables for HMW glutenin fractions, 4 variables for wheat quality properties, and 11 variables of bread quality properties (9 variables are parameters of computer image analysis). Projections of the HMW glutenin fractions clearly show that the first latent variable is defined by Glu-1x and Glu-1y type glutenin subunits,

which dominantly determines wheat and bread quality properties of the Croatian cultivars.

The first two latent variables of the HMW glutenin fractions can explain 87.1 % of the total variance of the data matrix with estimated average 7 % measurement error level. The first latent variable accounts for 59.45 % of variance and is clearly defined by Glu-1y and Glu-1x glutenin subunits.

Cultivars have been classified into clusters based on their scores. The first cluster includes Klara, Ana, and Demetra, and is mostly characterised by the presence of Glu-A1x subunits. The second, broader cluster, encompasses Kata, Golubica, Žitarka, Barbara, Super Žitarka and Monika, and is characterised by Glu-B1x and Glu-B1y subunits. Cultivar Divana, which is a known bread quality improver, stands out at the singular position due to its Glu-D1y proportion. In view of only HMW glutenin fractions, cultivar Srpanjka is the most similar to Divana, but has more significant Glu-D1x proportion.

The projections of cultivars on the first two latent variables revealed from wheat quality properties and HMW glutenin fractions account for 71.66 % of variance. The first latent variable is unchanged, and the cultivar classification into clusters is essentially the same, and dominated by HMW glutenin fractions, mostly from Glu-1x and Glu-1y.

Chemometric analysis of bread quality parameters gave the same conclusions. All of the 9 computer image bread parameters are projected in the same score region defined by Glu-1y glutenin subunit. However, due to numerous image parameters, the first latent variable defined by *x* and *y* type of glutenin fractions is tilted clockwise for an angle of 15^0. The cultivar classification based on computer image parameters is less efficient than on HMW gluten fractions. The scores of the variables of loaf

volume and glutenin score are closely projected, revealing their close functional relationship.

From economic and technological viewpoints, the results obtained by chemometric analysis can be utilised for development of PLS and PCR mathematical models for wheat compositions. Such models can be applied for optimisation of bread quality at the least price for wheat production.

Developed models of relation between physical properties (quality) and HMW glutenin fractions may provide an experimental short-cut method in cultivar selection. Furthermore, the models can be applied in Linear Programming optimisation of cultivars for production of a whole spectrum of products with specific properties and qualities.

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Cultivar	*w* (protein) / %	Sedimentation value / cm^3	*w* (wet gluten) / %	Gluten index	Volume / cm^3
Žitarka	12.76	43	40.92	68	495
Srpanjka	11.55	39	24.98	99	471
Super Žitarka	13.00	50	39.96	79	484
Barbara	12.23	49	37.30	84	502
Klara	11.75	46	30.76	95	549
Golubica	13.90	53	42.75	87	415
Kata	13.04	35	41.23	62	418
Monika	11.17	31	27.42	94	406
Ana	12.19	51	30.38	98	444
Demetra	12.12	53	29.89	98	448
Divana	15.21	61	40.45	96	553

Table 1. Quality parameters of wheat cultivars

Cultivar	Glu-A1x	Glu-B1	Glu-D1	Glu-1 quality score
		x + y	x + y	
Žitarka	N	7 + 8	2 + 12	6
Srpanjka	N	7 + 8	2 + 12	6
Super Žitarka	N	7 + 8	2 + 12	6
Barbara	N	7 + 8	2 + 12	6
Klara	1	7 + 8	2 + 12	8
Golubica	N	7 + 9	2 + 12	5
Kata	N	7 + 9	2 + 12	5
Monika	N	7 + 9	2 + 12	5
Ana	1	7 + 9	5 + 10	9
Demetra	1	7 + 9	5 + 10	9
Divana	N	7 + 9	5 + 10	7

Table 2**.** HMW glutenin subunits and Glu-1 quality scores of Croatian wheat cultivars.

Cultivar	Glu-A1x	Glu-B1x	Glu-B1y	Glu-D1x	Glu-D1y	Glu-1x	Glu-1y
Žitarka	-	21.68	24.62	27.33	26.37	49.01	50.99
Srpanjka	-	17.71	21.34	31.04	29.92	48.75	51.26
S. Žitarka	-	20.34	23.82	28.95	26.89	49.29	50.71
Barbara	-	20.22	25.43	27.45	26.89	47.67	52.32
Klara	15.19	20.29	22.96	14.06	29.60	49.54	52.56
Golubica	-	22.92	23.55	28.82	24.71	51.74	48.26
Kata	-	23.34	25.44	27.88	23.16	51.22	48.60
Monika	-	20.62	22.95	29.67	26.76	50.29	49.71
Ana	9.96	19.73	24.47	16.19	29.66	45.88	54.13
Demetra	11.39	19.90	23.41	16.52	28.78	47.81	52.19
Divana	-	17.69	20.94	28.26	33.11	45.95	54.05

Table 3. HMW glutenin subunits (%) of Croatian wheat cultivars. Presented data are average values obtained from 12 samples for each cultivar.

Cultivar	Total area / %	Number of cells	Area / pixel	Radius / pixel	Peri-meter / pixel	Min. radius / pixel	Max. radius / pixel	Round-ness (0-1)
Žitarka	0.66	55	13.34	2.73	17.65	0.33	4.53	0.58
Srpanjka	5.78	50	25.57	3.24	22.25	0.45	5.43	0.60
S. Žitarka	2.30	58	12.77	2.72	17.25	0.24	4.67	0.58
Barbara	1.81	73	11.99	2.63	16.34	0.30	4.42	0.58
Klara	5.63	46	16.69	2.88	18.46	0.39	4.76	0.57
Golubica	1.07	41	11.51	2.61	15.17	0.29	4.33	0.64
Kata	0.81	55	11.21	2.57	14.90	0.32	4.26	0.66
Monika	3.04	41	18.78	2.85	18.02	0.39	4.78	0.62
Ana	4.84	63	22.50	3.06	21.43	0.45	5.07	0.55
Demetra	3.68	60	21.18	3.15	20.88	0.49	5.20	0.59
Divana	5.52	62	20.84	3.01	21.45	0.52	5.04	0.55

Table 4**.** Results of computer image analysis of bread crumb cell structure. Presented data are average values obtained from three loaves prepared from each cultivar. Each image is composed of 200 x 150 pixels.

	Total area / %	Number of cells	Area / pixel	Radius / pixel	Perimeter / pixel	Minimal radius / pixel	Maximum radius / pixel	Roudness (0-1)
Glu-A1x	0,50	0,01	0,34	0,42	0,36	0,41	0,35	-0,39
Glu-B1x	**-0,84**	-0,31	**-0,80**	**-0,81**	**-0,88**	**-0,69**	**-0,85**	**0,70**
Glu-B1y	**-0,74**	0,33	**-0,68**	**-0,67**	**-0,66**	**-0,63**	**-0,69**	0,27
Glu-D1x	-0,40	-0,14	-0,26	-0,34	-0,31	-0,38	-0,26	0,43
Glu-D1y	**0,89**	0,25	**0,77**	**0,78**	**0,87**	**0,79**	**0,79**	**-0,81**
Glu-1x	-0,57	**-0,72**	-0,58	-0,59	**-0,74**	**-0,63**	-0,60	**0,87**
Glu-1y	**0,70**	**0,60**	0,57	0,60	**0,74**	**0,64**	0,60	**-0,93**

Table 5. Correlation coefficients between HMW glutenin fractions and computer image analysis of bread crumb structure. Significant coefficients ($p < 0.05$) are printed in bold.

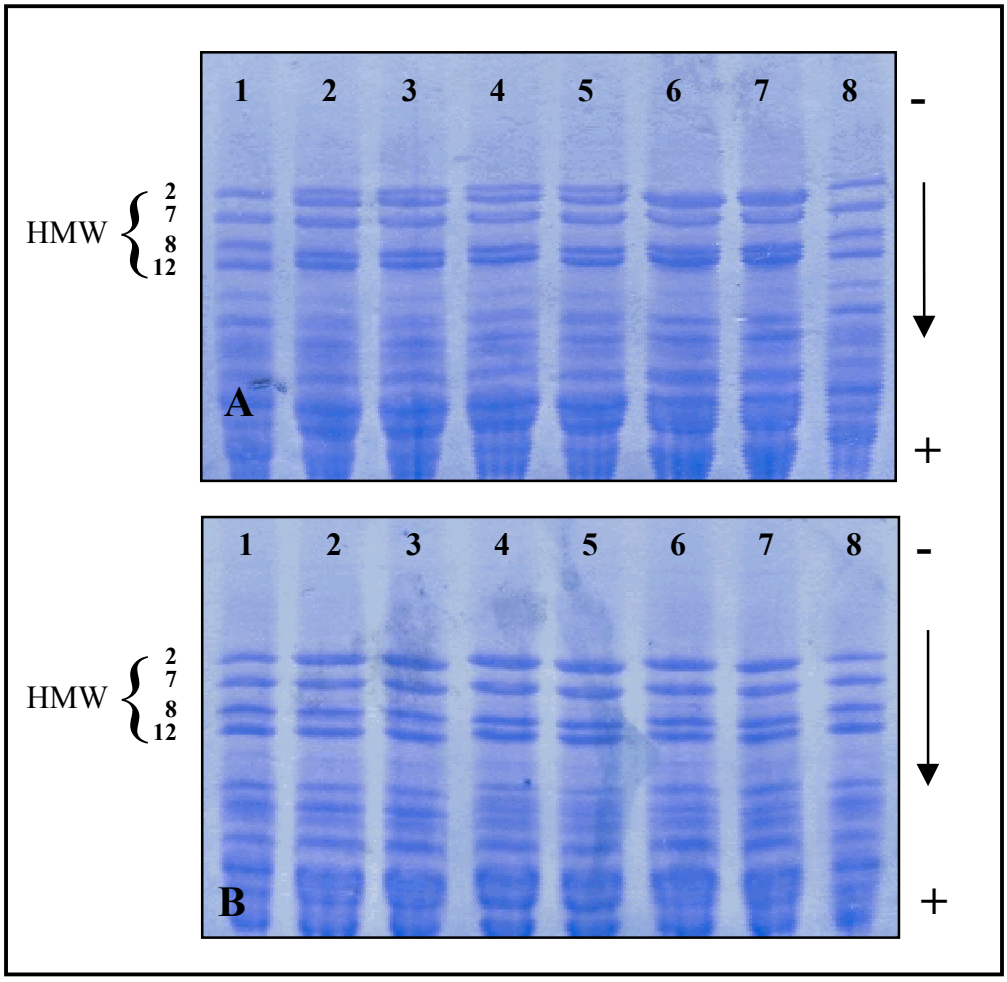

Fig. 1A. SDS-PAGE electrophoresis diagrams of Croatian wheat cultivars. On gels A and B the lines 1 and 8 correspond to the standard wheat sample Chinese Spring with HMW composition (N 7+8 2 +12). On gel A, lines 2 and 3 correspond to cultivar Ana (1 7+9 5+10), lines 4 and 5 to Demetra (1 7+9 5+10), and lines 6 and 7 correspond to Divana (N 7+9 5+10). On gel B, lines 2 and 3 correspond to cultivar Žitarka (N 7+8 2+12), lines 4 and 5 to Golubica (N 7+9 2+12), and lines 6 and 7 correspond to Kata (N 7+9 2+12). The arrow denotes the direction of electrophoresis.

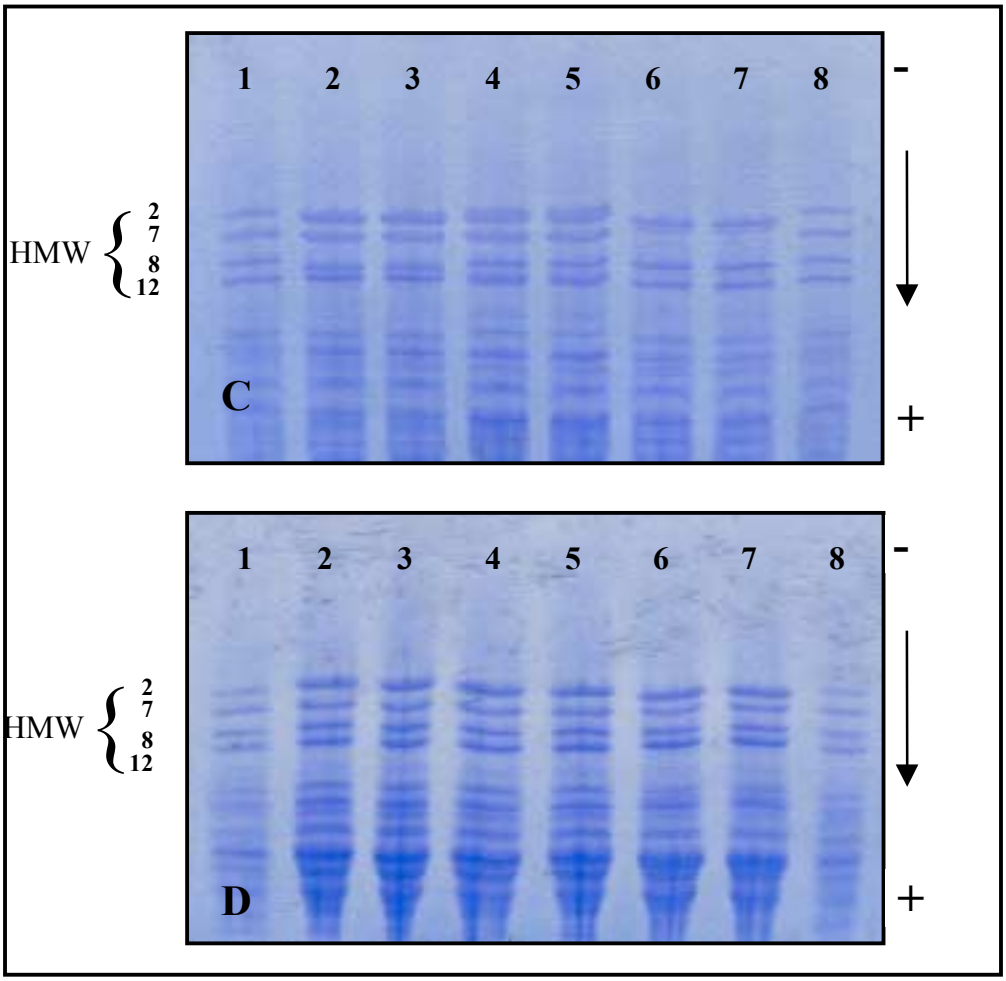

Fig. 1B. **SDS-PAGE electrophoresis diagrams of Croatian wheat cultivars.** On gels C and D the lines 1 and 8 correspond to the standard wheat sample Chinese Spring with HMW composition (N 7+8 2 +12). On gel C, lines 2 and 3 correspond to cultivar Monika (N 7+9 2+12), lines 4 and 5 to Klara (1 7+8 2+12), and lines 6 and 7 correspond to Sana (not included in study) (N 6+8 2+12). On gel D, lines 2 and 3 correspond to cultivar Barbara (N 7+8 2+12), lines 4 and 5 to Super Žitarka (N 7+8 2+12), and lines 6 and 7 correspond to Srpanjka (N 7+8 2+12). The arrow denotes the direction of electrophoresis.

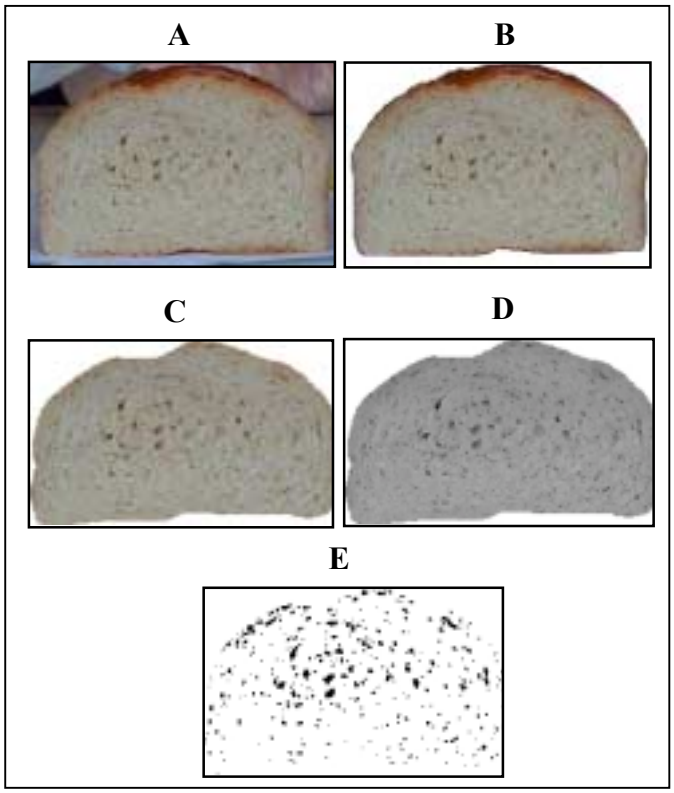

Fig. 2. Image transformations in the process of computer analysis of bread crumb structure: A) original image, B) elimination of background, C) a slice cut from the medium part, D) transformation to 8-bit BMP format with 256 grey levels, E) transformation to a black and white image by use of 128 grey level threshold.

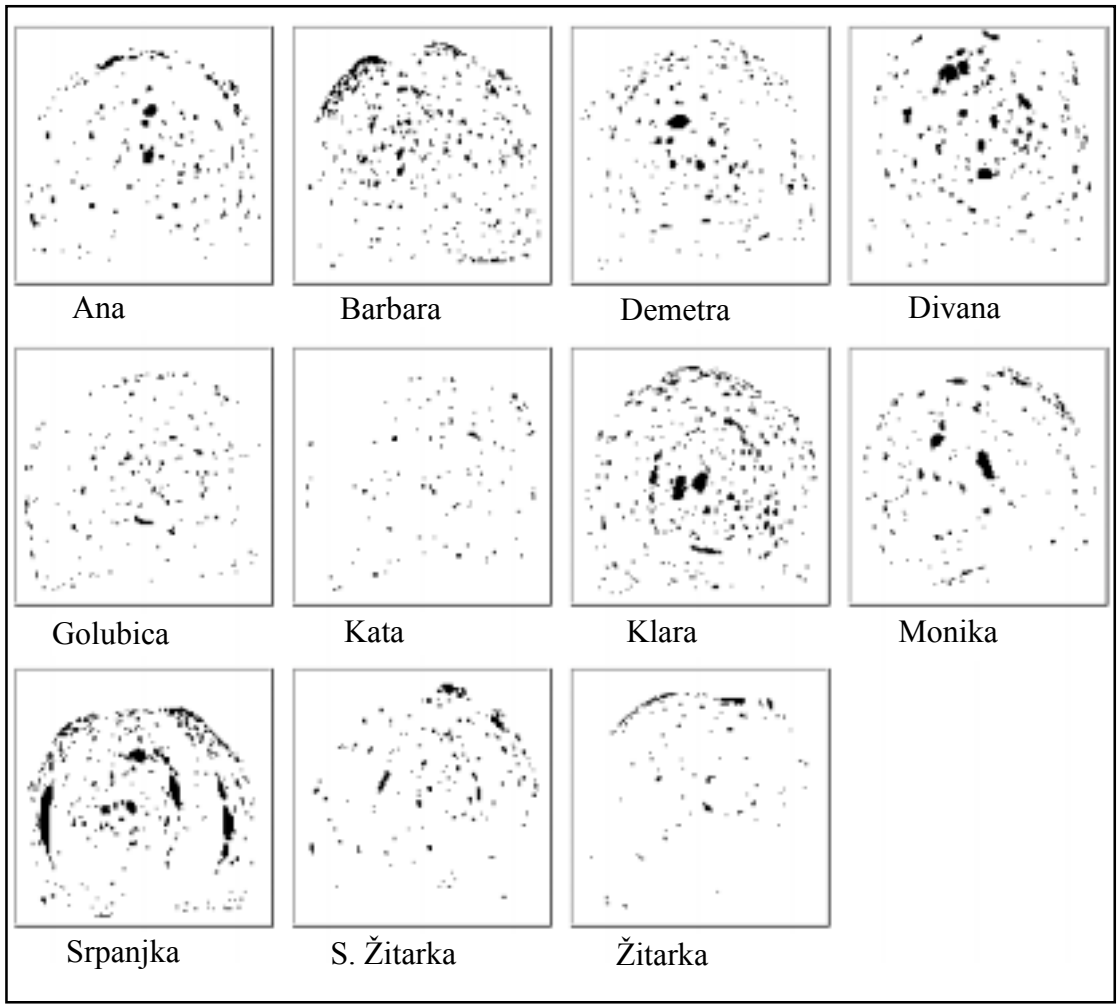

Fig. 3. **Images of bread crumb structures of medium parts of bread loaves produced from pure Croatian cultivars.** The images are produced by transformation to black and white pictures by use of 128 grey level threshold.

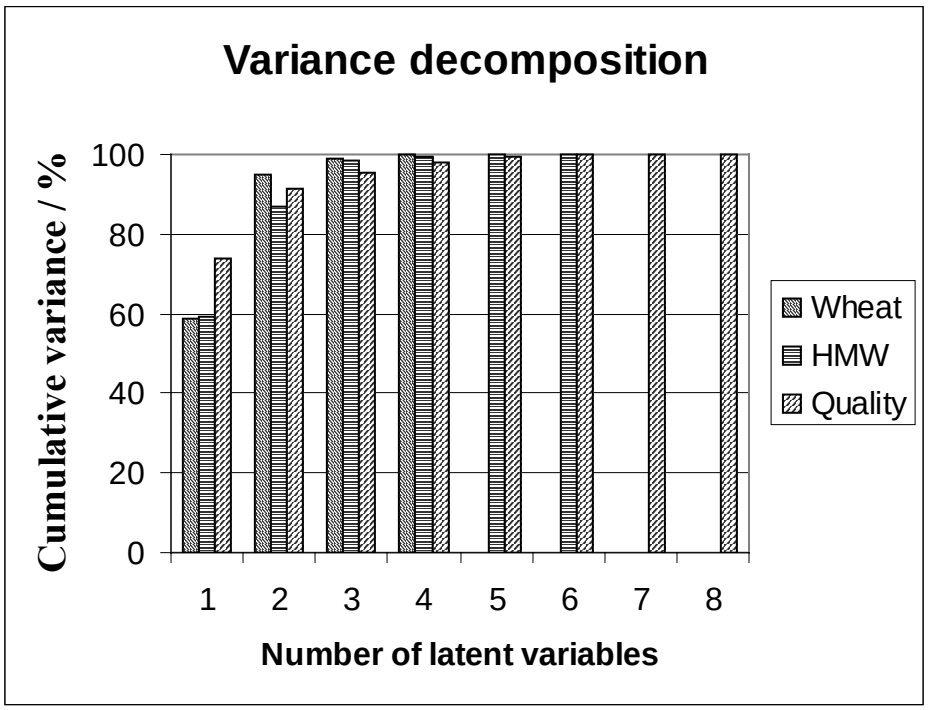

Fig. 4. Decomposition of variance of data sets based on number of latent variables for: a) wheat analysis; b) HMW glutenin fractions; c) quality data from computer image analysis and loaf volume measurement. The first two latent variables account for 95 % of wheat properties data, 87.1 % for HMW glutenin fractions, and 83.1 % of BMQ quality data.

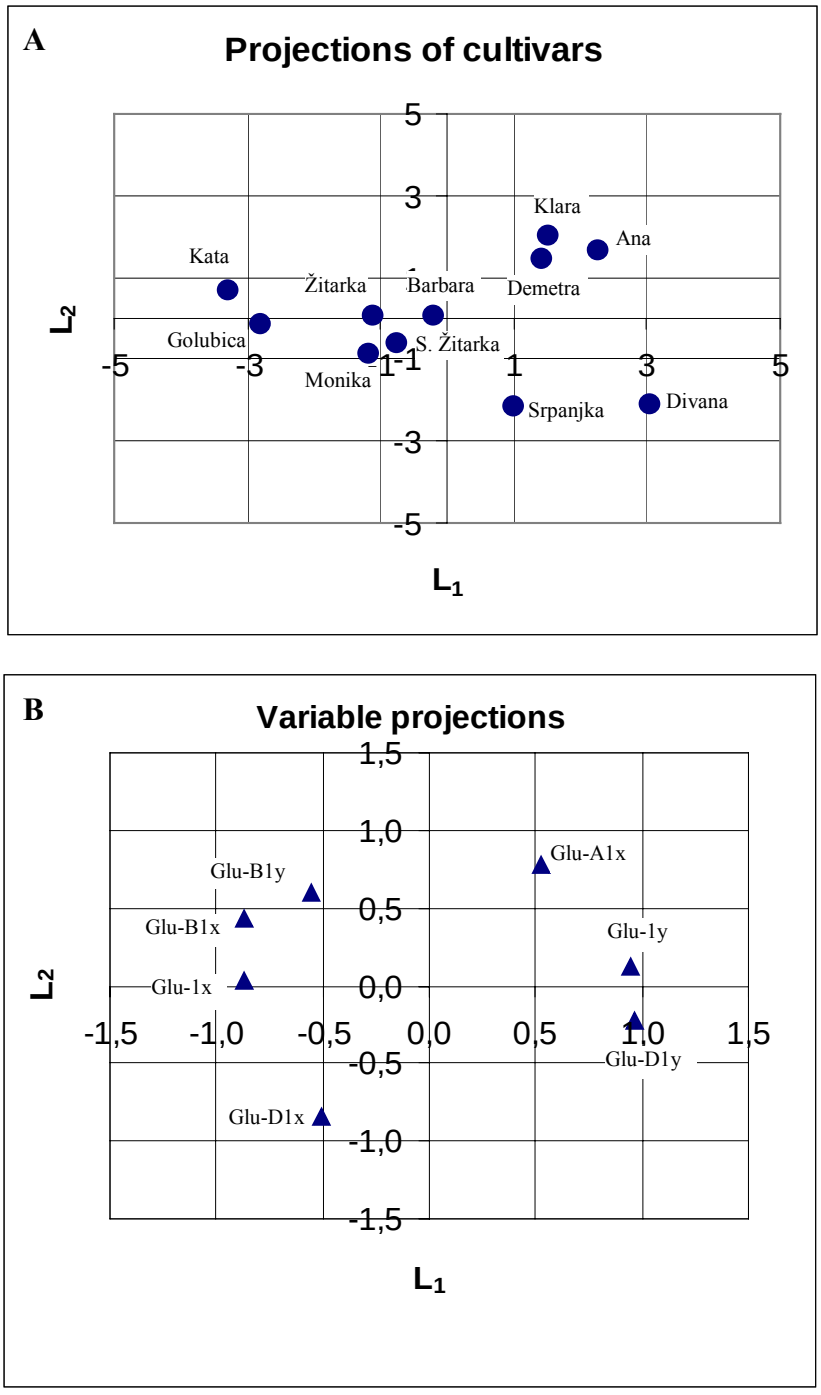

Fig. 5. **Classification of Croatian cultivars based on the projections onto the latent variables of HMW glutenin fractions.** The first latent variable L_1 accounts for 59.45 and the second L_2 for 27.64 % of the total variance. In the projections (A) clustering of wheat clutivars based on HMW glutenin is presented. The projection (B) of variables explains the impact of HMW glutenin fractions on cluster attributes.

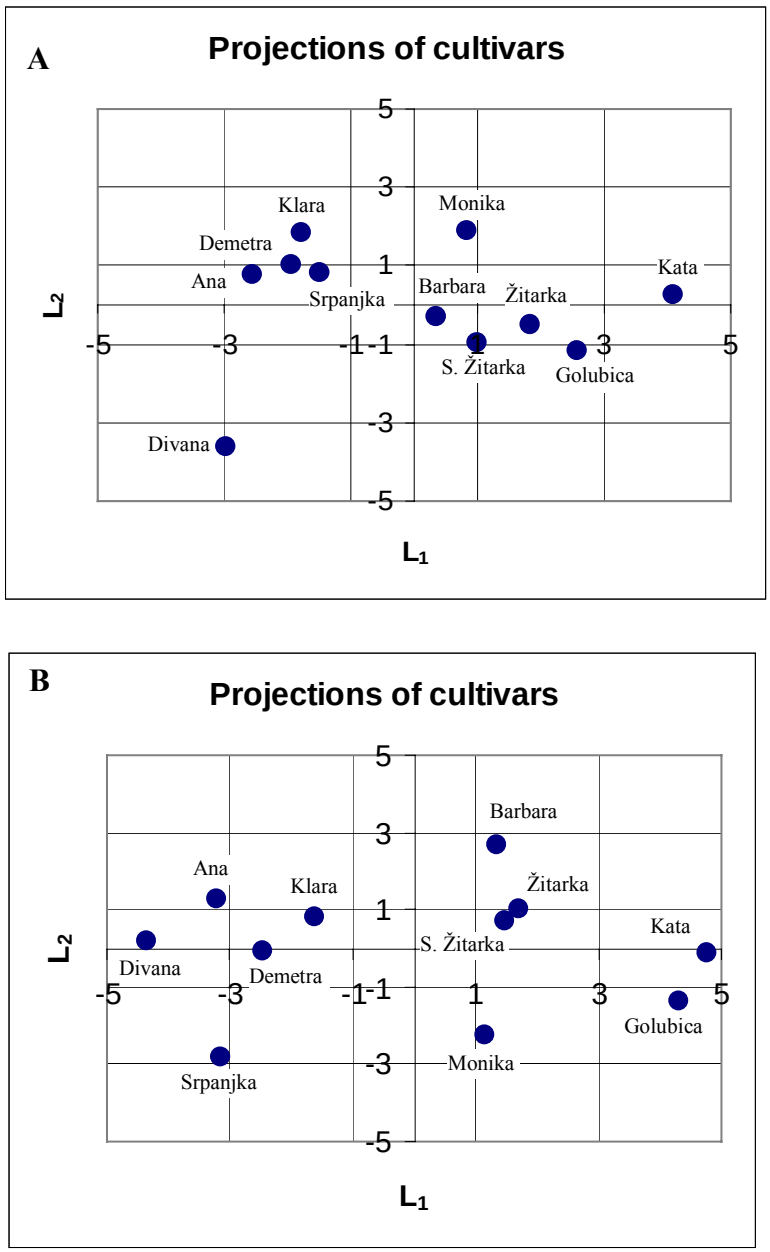

Fig. 6. **Projections of the cultivars on the planes defined by the first two latent variables L_1 and L_2** . The plane A is determined on the basis of projections of joint data sets of wheat properties, and HMW glutenin fractions. The first L_1 and the second L_2 latent variable account for 48.44 and 23.12 % of total variance, respectively. The plane B is determined on the basis of the joint data sets of computer image analysis, loaf volume and HMW glutenin fractions. The first L_1 and the second L_2 latent variables account for 61.08 and 16.16 % of total variance, respectively.

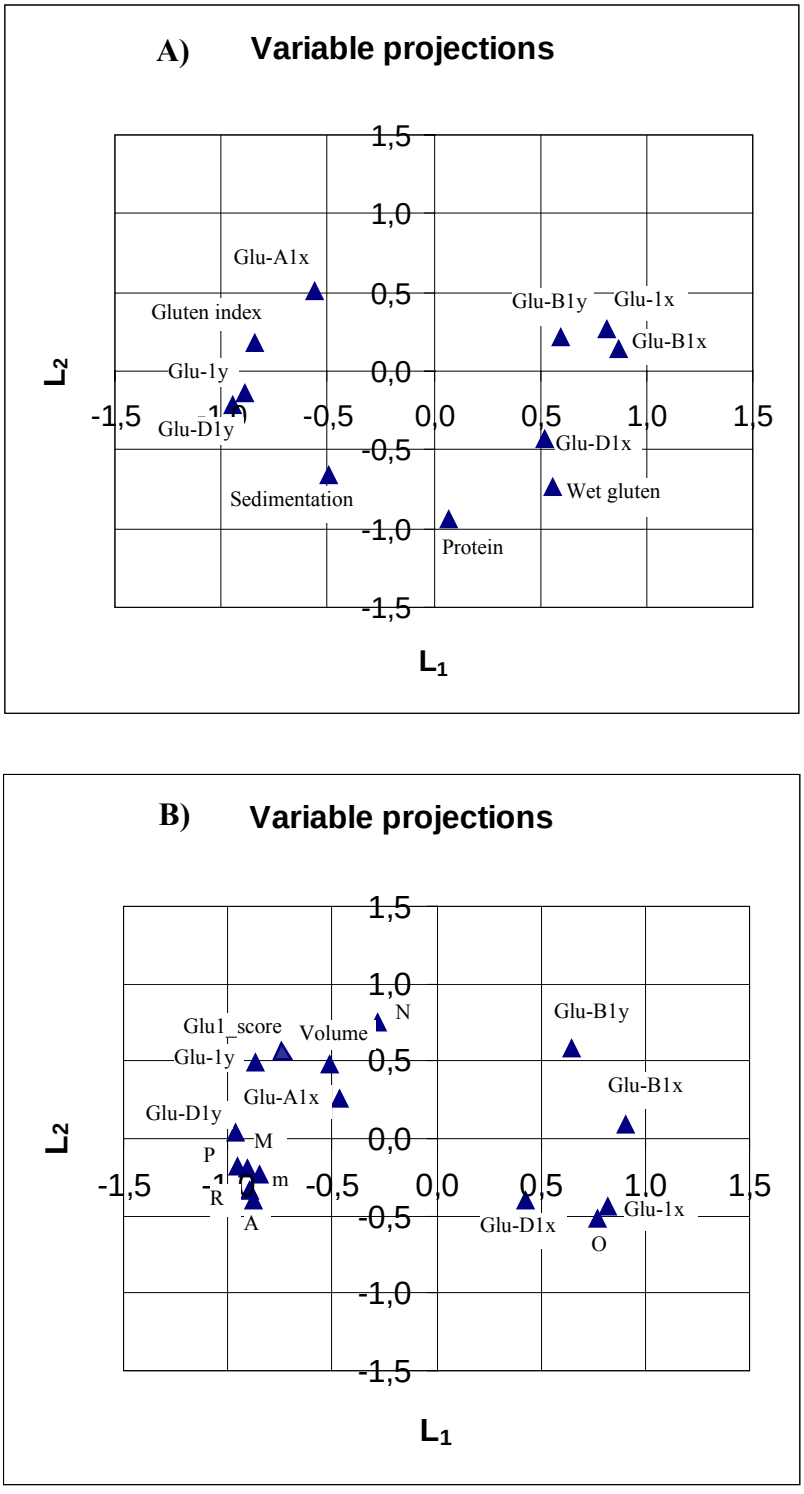

Fig. 7. Projections of the measured variables onto the planes defined by the first L_1 and second latent L_2 variables. Plane A represents projections of the variables of wheat properties and HMW gluten fractions. The first two latent

variables account for 48.44 and 23.12 % of total variance. In plane B presented are projections of computer image variables (A - area fraction of crumb cells, N - number of cells, m - minimal cell radius, M -maximal cell radius, P - average cell perimeter, O - average cell roundness, R - average cell radius, T - cell total area), loaf volume V, and HMW fractions. The first L_1 and second L_2 latent variables account for 61.08 and 16.16 % of total variance, respectively.

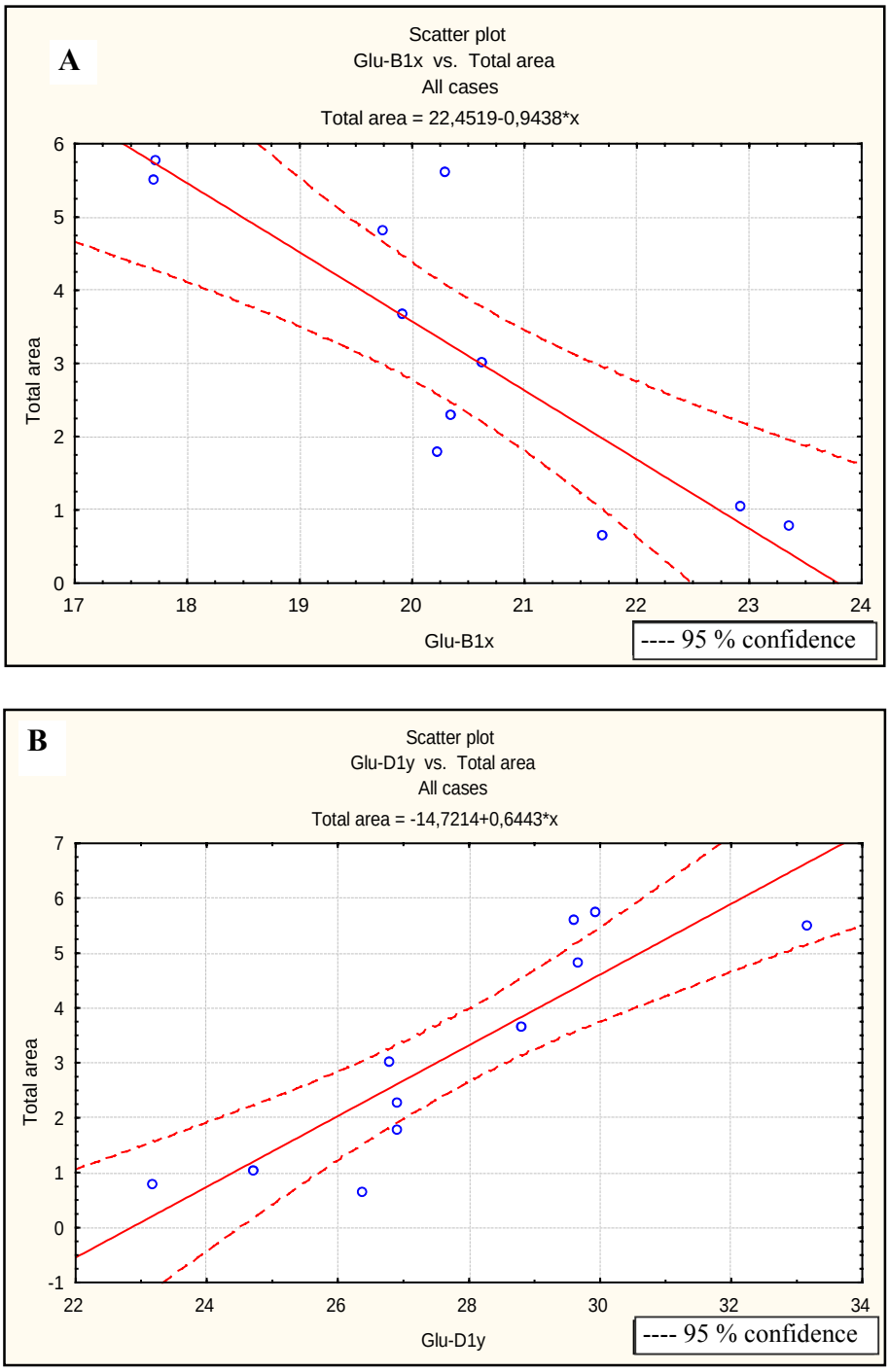

Fig. 8. Linear functional dependence of the fraction of cell total area obtained from computer image analysis on glutenin fractions: (A) Glu-B1x; (B) Glu-D1y. The lines are determined by the ordinary least square method with the correlation coefficients $r = -0.841$ and $r = 0.887$ respectively.

Experimental data are depicted by (o), linear model by full line (–), and 95 % confidence interval by the dotted line (--).

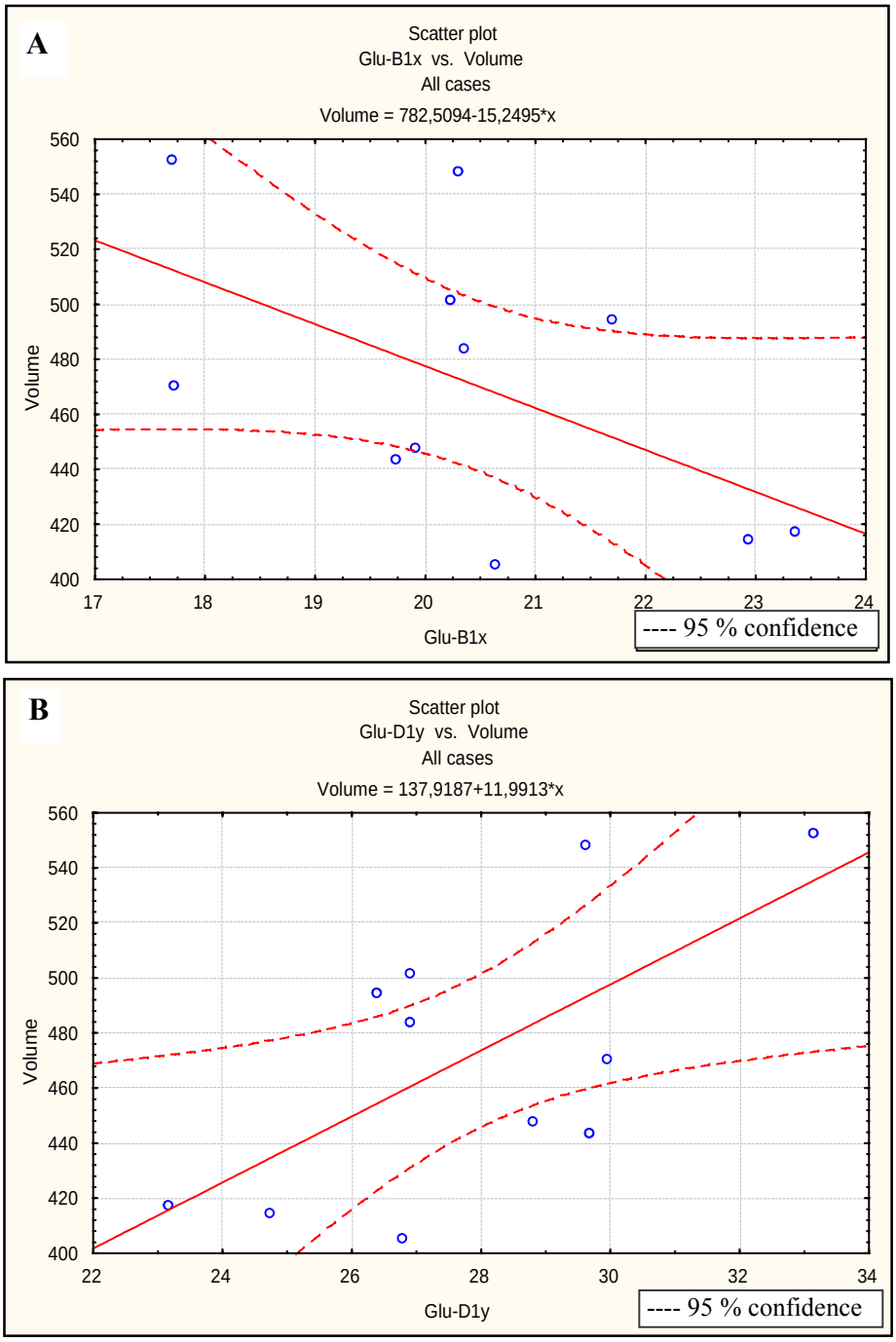

Fig. 9. Linear functional dependence of bread loaf volume on glutenin fractions: (A) Glu-B1x; (B) Glu-D1y. The lines are determined by the ordinary least square method with the correlation coefficients $r = -0.535$ and $r = 0.651$ respectively. Experimental data are depicted by (o), linear model by full line (–), and 95 % confidence interval by the dotted line (--).

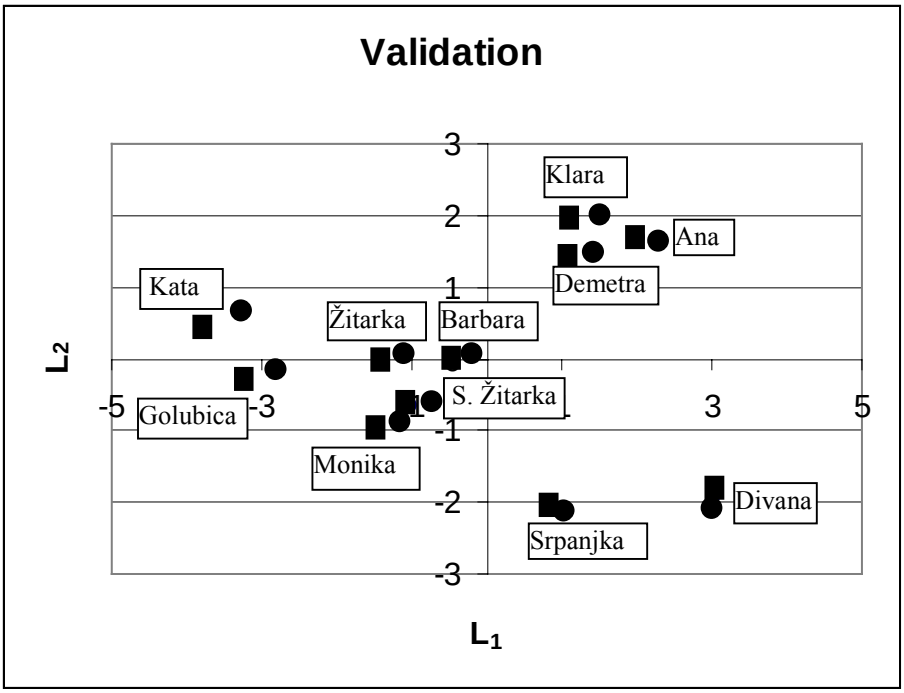

Fig. 10. Validation of cultivar projections by the first two principal components of the HMW glutenin fractions. The original projections of the 11 cultivars are denoted by (●). Projections by the first two principal components of the test set (10 cultivars with Golubica removed) are denoted by (■). The test projection for Golubica is determined by the principal components of the test set. Estimated relative errors for the projections of test cultivar Golubica for the first and second principal component are 8 and 4 %, respectively.