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Prediction of the genetic similarity of wheat and wheat quality by reversed-phase High-Performance liquid Chromatography and Lab-on-Chip methods

Short running title: RP-HPLC and LoaC gluten proteins quantification

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The aim of this study was to compare efficiency of RP-HPLC (High-Performance Liquid Chromatography) and LoaC (Lab-on-a-Chip) methods for wheat gluten protein quantification regard clustering of wheat cultivars according to the genetic similarity (HMW-GS combinations), as well as to explore relations of these two methods to wheat quality parameters.For that purpose, wheat quality parameters (protein content, falling number, wet gluten content, gluten index, Farinograph, Extensograph, and Amylograph) and amounts of gliadin and glutenin fractions by RP-HPLC and LoaC methods were determined in two different sets of wheat cultivars (Croatian and Serbian). The percentages of gluten proteins and the values of quality parameters were used to characterize the samples by principal component analysis (PCA). Gluten protein quantification performed by method based on the protein fraction separation by molecular weights (LoaC) was better in grouping of genetically similar wheat cultivars than quantification of proteins separated by their different solubility in specified solvent gradient (RP-HPLC). LoaC method showed higher potential in wheat quality prediction.

Key words: wheat quality, RP-HPLC, LoaC, gluten proteins, genetic similarity

Wheat flour possesses numerous different utilizations in food industry that depend upon the type of desired product. When it is mixed with water it forms dough that possesses unique viscoelastic properties due to the formation of gluten. Gluten consists of two different types of proteins: monomeric gliadins and polymeric glutenin. These complex compounds are most responsible for the viscoelastic properties of dough and baking quality of wheat (PANOZZO et al., 2001).Gliadins are classified into α-gliadins, β-gliadins and ω-gliadins on the basis of NH_2-terminal amino acid sequences (KASARDA et al., 1983; BIETZ et al., 1977), whereas gluteninsare classified into high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) on the basis their mobility on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (CORNISH et al., 2006; BIETZ et al., 1975). The molecular weights of HMW-GS determined by SDS-PAGE are in the rangeof 80 to130 kDa (BUNCE et al., 1985), whereas the molecular weights of LMW-GS determined by SDS-PAGE are in the range of 30 to 50 kDa (GRAS et al., 2001).

Different studies have been conducted to examine the effects of gluten proteins - glutenins (MACRITCHIE et al., 1991; PAYNE et al., 1984; PIROZI et al., 2008) and gliadins (GIL HUMANES et al., 2012; HUEBNER et al., 1997; PAYNE et al., 1984) on the viscoelastic properties of dough and technological quality of wheat.One of the firststudiesonthis topicwas conducted by PAYNE and co-workers (1984) showed that the glutenins are responsible for the elasticity of the dough. It is well-known fact that a couple of subunits HMW-GS from D1 loci 5+10 formed stronger dough than a couple HMW-GS from D1 loci 2+12, because of an extra cysteine residue on repetitive amino acid sequence of the subunit 5 (LAFIANDRA et al., 1993). Also, ANDERSON and BÉKÉS (2011) showed that the addition of individual glutenin subunits from GluD1 loci HMW-GS of x-type (2 and 5) in five different dough samples has a greater impact on the

technological dough parameters than addition of HMW-GS of y-type (10 and 12). Furthermore, specific composition of HMW-GS in wheat cultivars is one of the most important genetic factors which influence the rheological properties of dough (Payne et al., 1987). It has been recently demonstrated that composition and quantity of HMW-GS could be determined by Lab-on-a-Chip (LoaC) technique (Živančev et al., 2013; 2015), whereas for accurate determination of their amounts a combined extraction - Reversed-Phase High-Performance Liquid Chromatography (RP-RP-HPLC) procedure developed by Wieser and co-workers (1998) is still used.

The influence of LMW-GS composition on dough properties has not been studiedas much as the influence of HMW-GS composition. The study of Luo and co-workers (2001) showed that the specific composition of LMW-GS has a greater impact on the dough extensibility than on the dough strength, and these properties were directly related to the HMW-GS.

It is believed that the monomeric gliadins act as plasticizers of the polymeric gluten system and thus provide plasticity/viscosity to wheat dough (Khatkar et al.,1995). Addition of the gliadin fractions to the wheat flours ignificantly reduced the maximum resistance and increases the extensibility of dough (Schropp & Wieser, 1996). In opposite to this study research of the Gil-Humanes and co-workers (2012) showed that the content of γ-gliadin was positively correlated with the dough development as measured by Mixolab, which contributes to the dough strength.

For the determination of dough extensibility Extensigraph (Indrani et al., 2011; Zhang et al, 2007) is still used, whereas for the determination dough mixing properties Farinograph still represents common rheological method (Indrani et al., 2011; Rakszegi et al., 2008).

The aim of this paper was to compare efficiency of two most applied methods for relative quantification of gluten proteins regard clustering of wheat cultivars according to the genetic

similarity (HMW-GS combinations),as well as to explore relations of these two methods to wheat quality parameters.

1. Material and methods

1.1. Materials

Nine bread wheat (*Triticumaestivum*L.) cultivars ("Divana", "Aida", "Felix", "Seka", "Renata", "Soissons", "Olimpija", "Vulkan", and "Tihana") grown in Croatia at the Agricultural Institute in Osijek and nine bread wheat cultivars ("Dragana", "Ljiljana", "Pobeda", "Bastijana", "Nevesinjka", "Simonida", "Etida", "Zvezdana", and NS3-5299/2) grown in Serbia at the Institute of Field and Vegetable Crops in Novi Sad harvested in season 2009 were investigated in the present study.

1.2. Samples preparation and analytical procedure

Protein content (P) was determined on wheat kernels by FOSS Infratec 1241 Grain Analyzer (FOSS ANALYTICAL AB, Hillerød, Denmark), whereas Falling number (FN) was determined by Falling Number 1600 (PERTEN INSTRUMENTS, Huddinge, Sweden) according to ICC standard method 107/1.Wheat samples were milled by MLU – 202 (Bühler, Uzwil, Switzerland) and obtained flours were used for further rheological analyses. Wet gluten content (WG) and gluten index (GI) were determined by Glutomatic 2100 (PERTEN INSTRUMENTS, Huddinge, Sweden) according to ICC standard method155. Rheological quality of flour samples were determined by Farinograph, Extensograph and Amylograph (C.W. BRABENDER, Duisburg, Germany) according to (MSZ6369/6-1988, ICC114/1, ICC126/1 respectively). The extractions of gliadin and glutenin subunits for LoaC and reversed-phase high-performance liquid chromatography (RP-RP-HPLC) methods as well as

LoaC and RP-RP-HPLC analyses were performed according to ŽIVANČEV and co-workers (2015).

1.3. Data analysis

The data were statistically analysed by STATISTICA 12.0 software (StatSoft Inc., USA, 2013). Descriptive statistics was used to explore the percentage amounts of gluten proteins as well as rheological parameters and for that purpose, mean values, ranges and coefficients of variation (CV) were calculated. The percentages of gluten proteins and the values of quality parameters were used to characterize the samples by principal component analysis (PCA). The PCA was performed on the symmetric correlation matrix. Pearson correlation coefficients were calculatedin order to further discuss the relationships between examined variables.

2. Results and discussion

2.1 Quantification of protein fractions

The quantitative results of gluten protein fractions obtained by RP-RP-HPLC and LoaC methods were summarized in Table 1. In general, the results of gliadin subunits show much better agreement between these two examined methods than results of glutenin subunits. Also, the results obtained by RP-RP-HPLC method are less variable since SD of all protein fractions gained by RP-RP-HPLC method are lower than SD gained by LoaC method.

2.2 Technological quality of wheat

Regarding the wheat end-use quality parameters (Table 2), a large variability of some analyzed parameters was noticed. The range of WG, FN, DDT, Stab, R, DS, E and EXT varied between weak or medium to excellent, which indicate significant differences among dough rheology of examined wheat cultivars. In opposition to them the range of P, GI, WA, R_{5min}, R_{5min}/EXT, Y and SV showed lower variation.

2.3 Wheat cultivars HMW-GS composition

Allelic variation at *Glu-1* loci of examined wheat cultivars obtained by LoaC method is shown in Table 3. The most frequent HMW-GS combinations of Croatian cultivars were 2* 7+8 5+10 and 2* 7+9 5+10 (22.22%, for both combinations), whereas the most frequent combinations HMW-GS of Serbian cultivars were 2* 7+9 5+10 and 7+9 2+12 (33.33%, for both combinations).

2.4 Principal component analysis of wheat genetic similarity and wheat quality prediction

For the purpose of statistical analysis, whole data set was divided into two parts; quantification results from electrophoregrams (LoaC) and quantification results from chromatograms (RP-HPLC). Both data sets were correlated with technological quality parameters of wheat flour (data not shown).

Principal Component Analysis (PCA) was applied in order to reduce the initial complex data set to smaller number of independent variables which represent the linear combination of all examined quality parameters. Therefore all variables than did not contribute significantly to explanation of data set variability were excluded from further considerations.

The first two principal components (Fig. 1a – loading plot) explained 79.09 % of reduced data set total variability, which incorporated the results of gluten fractions quantification obtained by RP-HPLC method. Variation of HMW and LMW percentages (which are negatively correlated one with another) caused change of WA, WG, and GI values, as well as extensogram parameters R_{5min}/EXT and R_{5min}. On the basis of vector position it could be seen that WA was in significant positive correlation ($p<0.05$) with percentage of HMW subunits and in significant negative correlation ($p<0.05$) with LMW percentages. On the other hand, GI was positively correlated ($p<0.05$) with R5min, and negatively correlated ($p<0.05$) with WG.

The score plot (Fig. 1b) showed that the cultivars were mostly grouped by WG, LMW and HMW percentages, but Croatian and Serbian assortments were not completely separated.

The first two PCs (Fig. 2a–loading plot) explained 75.58% of reduced data set total variability, which included the results of gluten fractions quantification obtained by LoaC method. In this case, variation of all gluten fractions percentages (both gliadins and glutenins) caused change in GI value and extensogram parameters E, R_{5min}/EXT and R_{5min}. The above mentioned parameters GI and E were in significant positive correlation ($p<0.05$) with R_{5min}.

The score plot (Fig. 2b) did not show that cultivars were grouped by amount of particular protein fraction, but Croatian and Serbian assortments were completely separated. The exception was cultivar "Tihana", which was the only Croatian cultivar with 2+12 subunit combination, possessed by majority of Serbian cultivars.

Correlation coefficients between the percentage share of protein fractions and tested wheat technological quality parameters were higher when LoaC method was applied. However, these dependencies were not statistically significant.

3. Conclusions

The aforementioned results indicated that gluten protein quantification performed by method based on the protein fraction separation by molecular weights (LoaC) was better in grouping of genetically similar wheat cultivars than quantification of proteins separated by their different solubility in specified solvent gradient (RP-HPLC). LoaC method showed higher potential in wheat quality prediction.

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Table 1. Quantification of protein fractions performed by RP-RP-HPLC and LoaC methods

Protein fractions	RP-RP-HPLC			LoaC		
	Mean	**Range**	**SD**	**Mean**	**Range**	**SD**
% α + γ subunit	7.32	4.40–15.73	2.31	7.34	3.10–17.32	3.59
% ω subunit	92.72	84.26–95.60	2.31	92.55	82.68–96.90	3.61
% HMW-GS	27.60	20.75–37.50	3.52	13.26	4.66–29.57	5.50
% LMW-GS	72.39	62.43–79.25	3.52	86.84	70.43–95.34	5.49
% HMW/LMW	38.45	26.18–60.07	6.97	15.75	4.89–41.98	7.83

Table 2. Wheat end-use quality parameters

Parameter	Mean	Range	SD
P (% d.w.)	13.6	12.1–16.4	0.9
WG (%)	29.7	22.7–40.0	4.6
GI (%)	96.4	81.0–99.8	5.0
FN (s)	302.9	111.0–436.0	74.0
WA (%)	61.9	56.9–67.0	3.0
DDT (min)	3.1	1.5–10.3	2.0
Stab (min)	2.3	0.1–7.0	2.2
R (min)	6.5	1.6–30.0	6.5
DS (FU)	55.5	2.4–110.0	29.3
E (cm^2)	85.8	47.0–128.0	27.3
R_{5min} (EU)	235.8	140.0–350.0	54.1
EXT (mm)	170.7	132.0–209.0	21.2
R_{5min}/EXT	1.4	0.7–2.1	0.4
Y (%)	72.1	67.0–76.0	2.2
SV (cm^3/g)	3.4	3.0–3.8	0.2

List of abbreviations: *P (% d.w.) – protein content per dry weight,WG (%) – wet gluten content, GI – gluten index, FN (s) – falling number, WA (%) – water absorption, DDT (min) – dough development time, Stab (min) – dough stability, R (min) – dough resistance, DS (FU) – degree of softening, E (cm^2) – dough energy, R_{5min} (EU) – dough resistance on 5 minutes, EXT (mm) – dough extensibility, R_{5min}/EXT – ratio of dough resistance on 5 minute and extensibility, Y (%) – flour extraction yield, SV (cm^3/g) – specific volume of bread.*

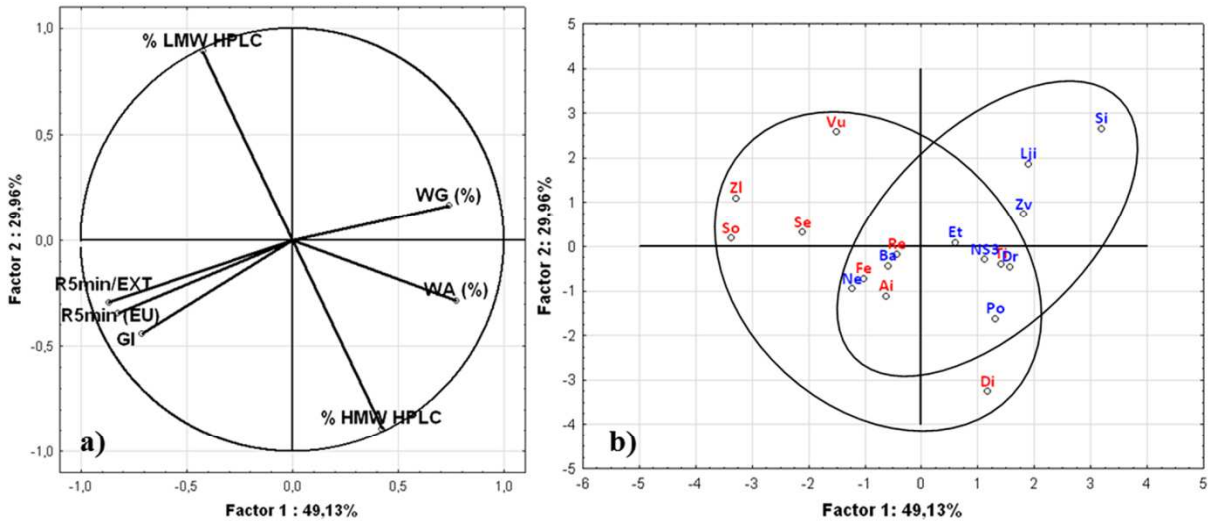
Table 3. Composition of HMW-GS in wheat cultivars

Cultivar	GLU-A1	GLU-B1	GLU-D1
Soissons	2*	7 + 8	5 + 10
Felix	2*	7 + 8	5 + 10
Divana	2*	7 + 9	5 + 10
Zlata	2*	7 + 9	5 + 10
Seka	1	7 + 9	5 + 10
Vulkan	N	7 + 8	5 + 10
Renata	1	7 + 8	5 + 10
Aida	2*	17 + 18	5 + 10
Tihana	1	7 + 9	2 + 12
Etida	N	7 + 9	5 + 10
Ljiljana	N	7 + 9	5 + 10
Pobeda	2*	7 + 9	5 + 10
Bastijana	2*	7 + 9	5 + 10
NS3-5299	2*	7 + 9	5 + 10
Simonida	N	7 + 9	2 + 12
Dragana	N	7 + 9	2 + 12
Zvezdana	N	7 + 9	2 + 12
Nevesinjka	2*	7 + 8	5 + 10 & 2 + 12

Figure captions

Figure 1. PCA plot of relationship between the glutenin subunits' percents determined by RP-HPLC method and quality parameters (a) and differentiation between the cultivars (b) after the reduction of variables

Figure 2. PCA plot of relationship between the glutenin subunits' percents determined by LoaC method and quality parameters (a) and differentiation between the cultivars (b) after the reduction of variables


% LMW HPLC
WG (%)
WA (%)
R5min/EXT
R5min (EU)
GI
% HMW HPLC
a)
Factor 2 : 29,96%
Factor 1 : 49,13%
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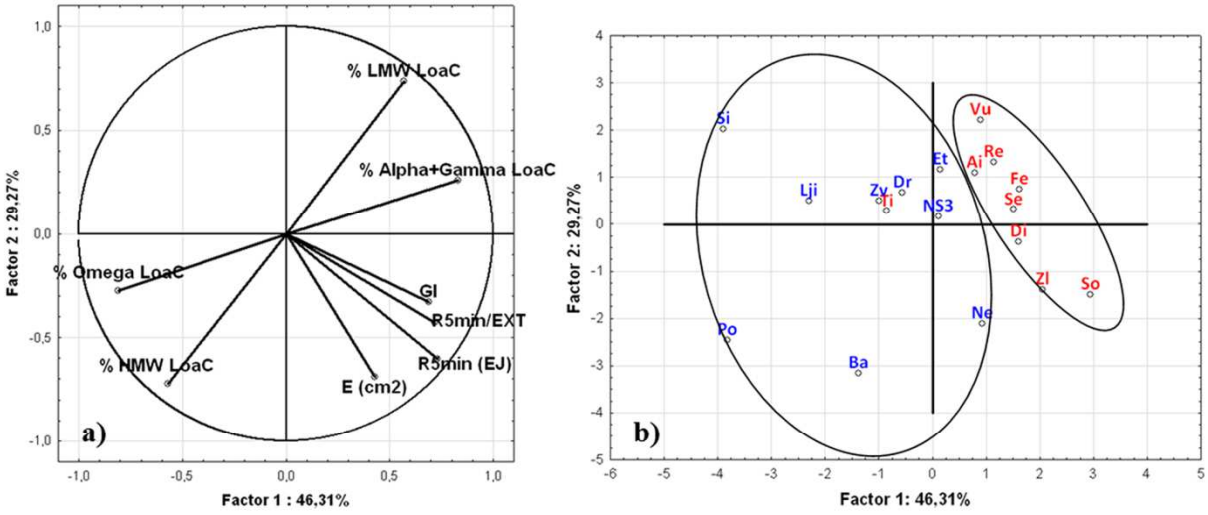
Factor 2: 29,96%
Factor 1: 49,13%

Figure 1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Factor 2 : 29,27%
Factor 1 : 46,31%
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Figure 2