*Acta Alimentaria, Vol. 46 (2), pp. 137–144 (2017)*
*DOI: 10.1556/066.2016.0003*

# PREDICTION OF THE GENETIC SIMILARITY OF WHEAT AND WHEAT QUALITY BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND LAB-ON-CHIP METHODS

A. Torbica[a]*, D. Horvat[b], D. Živančev[a], M. Belović[a], G. Šimić[b], D. Magdić[c], N. Đukić[d] and K. Dvojković[b]

[a]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad. Serbia
[b]Agricultural Institute Osijek, Južno Predgrađe 17, 31000 Osijek, Croatia
[c]Faculty of Food Technology, J.J. Strossmayer University of Osijek, Franje Kuhača 18, 31000 Osijek. Croatia
[d]Faculty of Natural Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac. Serbia

(Received: 20 March 2015; accepted: 24 August 2015)

The aim of this study was to compare efficiency of RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) and LOC (Lab-on-Chip) methods for wheat gluten protein quantification regarding 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), as well as amounts of gliadin and glutenin fractions by RP-HPLC and LOC 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 (LOC) was better for grouping of genetically similar wheat cultivars than quantification of proteins separated by their different solubility in specified solvent gradient (RP-HPLC). LOC method showed higher potential in wheat quality prediction.

**Keywords:** wheat quality, RP-HPLC, LOC, 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 (Bietz et al., 1977; Kasarda et al., 1983) and their amino acid compositions (Freedman et al., 1988), whereas glutenins are classified into high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) on the basis of their mobility on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bietz et al., 1975; Cornish et al., 2006). The molecular weights of HMW-GS determined by SDS-PAGE are in the range of 80 to 130 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).

* To whom correspondence should be addressed.
Phone: +381 21 485 3625; fax: +381 21 450 725; e-mail: aleksandra.torbica@fins.uns.ac.rs

Different studies have been conducted to examine the effects of gluten proteins – glutenins (Payne et al., 1984; MacRitchie et al., 1991; Pirozi et al., 2008) and gliadins (Payne et al., 1984; Huebner et al., 1997; Gil-Humanes et al., 2012) on the viscoelastic properties of dough and technological quality of wheat. One of the first studies on this topic 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 Glu D1 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 that 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-Chip (LOC) technique (Živančev et al., 2013; 2015), whereas for accurate determination of their amounts a combined extraction - Reversed-Phase High-Performance Liquid Chromatography (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 studied as 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 significantly reduced the maximum resistance and increased the extensibility of dough (Schropp & Wieser, 1996). Opposite to this study, research of 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 Extensograph (Zhang et al., 2007; Indrani et al., 2011) is still used, whereas for the determination of dough mixing properties Farinograph still represents common rheological method (Rakszegi et al., 2008; Indrani et al., 2011).

The aim of this paper was to compare the efficiency of the two most applied methods for relative quantification of gluten proteins regarding 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. Materials and methods

### *1.1. Materials*

Nine bread wheat (*Triticum aestivum* 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 (ICC, 1996a). 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 method 155 (ICC, 1996b). Rheological quality of flour samples were determined by Farinograph, Extensograph, and Amylograph (C.W. Brabender, Duisburg, Germany) according to Hungarian Standard (1988) and ICC methods (ICC, 1996c, d), respectively. The extractions of gliadin and glutenin subunits for LOC and RP-HPLC methods as well as LOC and 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 calculated in 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-HPLC and LOC 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-HPLC method are less variable since SD of all protein fractions gained by RP-HPLC method are lower than SD gained by LOC method.

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

| Protein fractions | RP-HPLC | | | LOC | | |
|---|---|---|---|---|---|---|
| | 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 |

### *2.2. Technological quality of wheat*

Regarding the wheat end-use quality parameters (Table 2), a large variability of some of them was noticed. The range of WG, FN, dough development time (DDT), dough stability (Stab), dough resistance (R), degree of softening (DS), dough energy (E), and dough extensibility (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, water absorption (WA), dough resistance on 5 min ($R_{5min}$), ratio of dough resistance on 5 min and extensibility ($R_{5min}$/EXT), flour extraction yield (Y), and specific volume of bread (SV) showed lower variation.

*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 ($cm^3g^{-1}$) | 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 min; EXT (mm): dough extensibility; $R_{5min}$/EXT: ratio of dough resistance on 5 min and extensibility; Y (%): flour extraction yield; SV ($cm^3g^{-1}$): specific volume of bread

### *2.3. Wheat cultivars HMW-GS composition*

Allelic variation at *Glu-1* loci of examined wheat cultivars obtained by LOC 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 HMW-GS combinations of Serbian cultivars were 2* 7+9 5+10 and 7+9 2+12 (33.33% for both combinations).

*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 |

N: HMW-GS not detected

### *2.4. Principal component analysis of wheat genetic similarity and wheat quality prediction*

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

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 that 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 with one 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 $R_{5min}$, and negatively correlated ($P<0.05$) with WG.

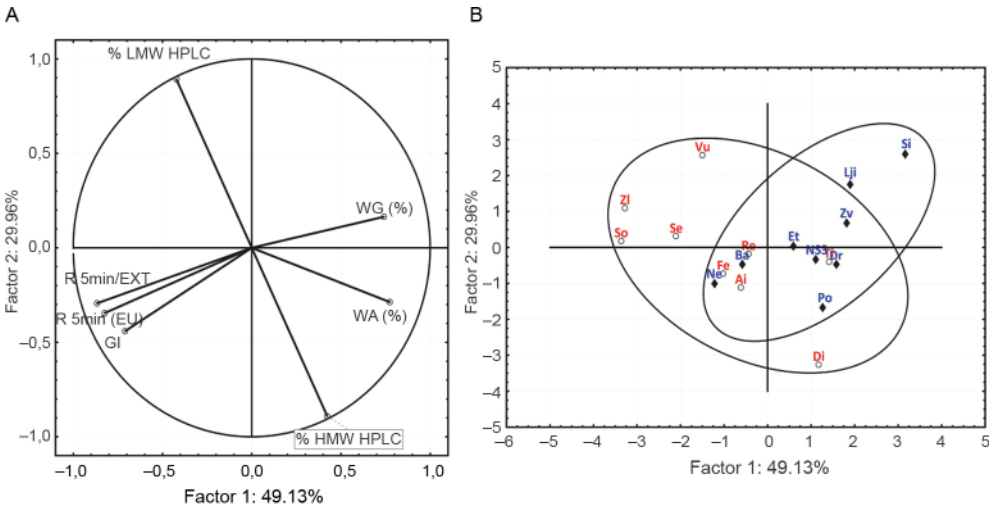


*Fig. 1.* PCA plot of relationship between the glutenin subunits' percents determined by RP-HPLC method and quality parameters (A) and differentiation between the cultivars (B) after the reduction of variables
(A) WG (%): wet gluten content; GI: gluten index; WA (%): water absorption; $R_{5min}$ (EU): dough resistance on 5 minutes; $R_{5min}$/EXT: ratio of dough resistance on 5 minute and extensibility; % HMW HPLC: percentage of high molecular weight glutenin subunits; % LMW HPLC: percentage of low molecular weight glutenin subunits.
(B) Di: Divana, Ai: Aida, Fe: Felix, Se: Seka, Re a: Renata, So: Soissons, Zl: Zlata, Vu: Vulkan and Ti: Tihana grown in Croatia (○; red letters in online version); Dr: Dragana, Lji: Ljiljana, Po: Pobeda, Ba: Bastijana, Ne: Nevesinjka, Si:Simonida, Et: Etida, Zv: Zvezdana, and NS 3:NS3-5299/2 grown in Serbia (♦ ; blue letters in online version)

The score plot (Fig. 1B) shows 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 LOC 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) shows that cultivars were not 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 the majority of Serbian cultivars.

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

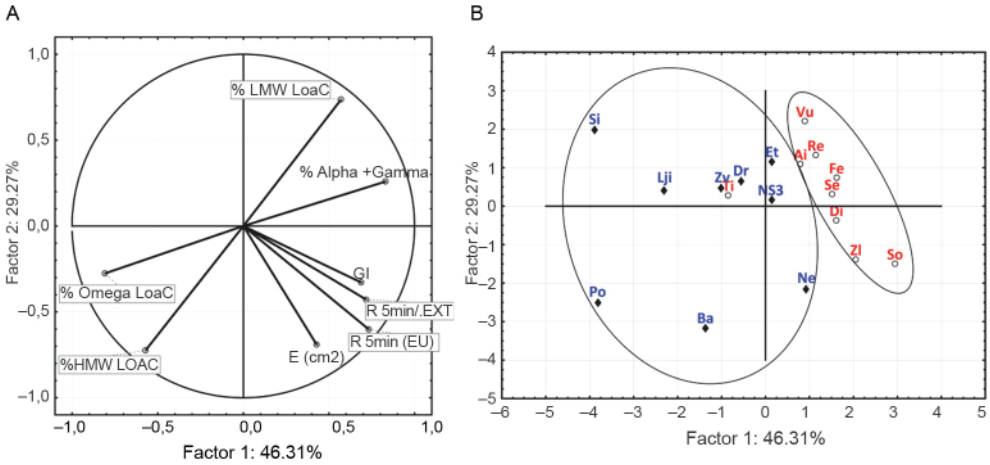


*Fig. 2.* PCA plot of relationship between the glutenin subunits' percents determined by LOC method and quality parameters (A) and differentiation between the cultivars (B) after the reduction of variables
(A) GI: gluten index; $R_{5min}$ (EU): dough resistance on 5 minutes; $R_{5min}$/EXT: ratio of dough resistance on 5 minute and extensibility; E ($cm^2$): dough energy; % HMW LOC: percentage of high molecular weight glutenin subunits; % LMW LOC: percentage of low molecular weight glutenin subunits; % Omega LOC: percentage of omega gliadin subunits; % Alpha + Gamma LOC: total percentage of alpha and gamma gliadin subunits.
(B) Legend: the same as in Figure 1.

## 3. Conclusions

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

*

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia within the Project of technological development No. TR 31007 and bilateral cooperation between Serbia and Croatia — Genetic polymorphism of gluten proteins and its relationship to bread-making quality of wheat (*Triticum aestivum* L.). Dr. Nikola Hristov from Institute of Field and Vegetable Crops (Novi Sad, Serbia) is also acknowledged for providing one set of examined cultivars.

## References

Anderson, O.D. & Békés, F. (2011): Incorporation of high-molecular-weight glutenin subunits into doughs using 2 gram mixograph and extensigraphs. *J. Cereal Sci., 54,* 288–295.

Bietz, J.A., Huebner, F.R., Sanderson, J.E. & Wall, J.S. (1977): Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. *Cereal Chem., 54,* 1070–1083.

Bietz, J.A., Shepherd, K.W. & Wall, J.S. (1975): Single kernel analysis of glutenin: Use in wheat genetics and breeding. *Cereal Chem.*, *52,* 513–532.

Bunce, N.A.C., White, R.P. & Shewry, P.R. (1985): Variation in estimates of molecular weights of cereal prolamins by SDS–PAGE. *J. Cereal Sci., 3,* 131–142.

Cornish, G.B., Békés, F., Eagles, H.A. & Payne, P.I. (2006): Gliadin and Glutenin. -in: Wrigley, C. & Bushuk, W. (Eds), *The unique balance of wheat quality*, AACC International, St. Paul, MN, USA, pp. 243–280.

Freedman, A.R., Wieser, H., Ellis, H.J. & Ciclitira, P.J. (1988): Immunoblotting of gliadins separated by reversed-phase high-performance liquid chromatography: Detection with monoclonal antibodies. *J. Cereal Sci., 8,* 231–238.

Gil-Humanes, J., Pistón, F., Rosell, C.M. & Barro, F. (2012): Significant down-regulation of γ–gliadins has minor effect on gluten and starch properties of bread wheat. *J. Cereal Sci., 56,* 161–170.

Gras, P.W., Anderssen, R.S., Keentock, M., Békés, F. & Appels, R. (2001): Gluten protein functionality in wheat flour processing: a review. *Aust. J. Agr. Res., 52,* 1311–1323.

Huebner, F.R., Nelsen, T.C., Chung, O.K. & Bietz, J.A. (1997): Protein distributions among hard red winter wheat varieties as related to environment and baking quality. *Cereal Chem.*, *74,* 123–128.

Hungarian Standard (1988): *Lisztvizsgálati módszerek. Vízfelvevő képesség és sütőipari érték* (Flour testing methods. Determination of water absorption capacity and baking quality). MSZ 6369/6

ICC Standard (1996a): *Determination of the "Falling Number" according to Hagberg*. International Association for Cereal Science and Technology. No. 107/1. pp.1–22.

ICC Standard (1996b): *Determination of wet gluten quantity and quality (Gluten Index ac. to Perten) of whole wheat meal and wheat flour* (Triticum aestivum). International Association for Cereal Science and Technology. No. 155. pp. 1–8.

ICC Standard (1996c): *Method for using the Brabender Extensograph*. International Association for Cereal Science and Technology. No. 114/1. pp. 1–15.

ICC Standard (1996d): *Method for using the Brabender Amylograph*. International Association for Cereal Science and Technology. No. 126/1. pp. 1–13.

Indrani, D., Swetha, P., Soumya, C., Rajiv, J. & Venkateswara Rao, G. (2011): Effect of multigrains on rheological, microstructural and quality characteristics of north Indian parotta – An Indian flat bread. *LWT – Food Sci. Technol., 44,* 719–724.

Kasarda, D.D., Autran, J.-C., Lew, E. J.-L., Nimmo, C.C. & Shewry, P.R. (1983): N-terminal amino acid sequences of ω-gliadins and ω-secalins: implications for the evolution of prolamin genes. *Biochim. Biophys. Acta*, *747,* 138–150.

Khatkar, B.S., Bell, A.E. & Schofield, J.D. (1995): The dynamic rheological properties of gluten and gluten sub–fractions from wheats of good and poor bread making quality. *J. Cereal Sci., 22,* 29–44.

Lafiandra, D., D'Ovidio, R., Porceddu, E., Margiotta, B. & Colaprico, G. (1993): New data supporting high molecular glutenin subunit 5 as the determinant of quality differences among the pairs 5+10 vs. 2+12. *J. Cereal Sci.*, *18,* 197–205.

Luo, C., Griffin, W.B., Branlard, G. & McNeil, D.L. (2001): Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality. *Theor. Appl. Genet., 102,* 1088–1098.

MacRitchie, F., Kasarda, D.D. & Kuzmick, D.D. (1991): Characterization of wheat protein fractions differing in contributions to breadmaking quality. *Cereal Chem.*, *68,* 122–130.

Panozzo, J.F., Eagles, H.A. & Wootton. M. (2001): Changes in protein composition during grain development in wheat. *Aust. J. Agr. Res., 52,* 485–493.

Payne, P.I., Holt, L.M., Jackson, E.A., Law, C.N. & Damania, A.B. (1984): Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding [and discussion]. *Philos. T. Roy. Soc. B, 304,* 359–371.

Payne, P.I., Nightingale, M.A., Krattiger, A.F. & Holt, L.M. (1987): The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agr., 40,* 51–65.

Pirozi, M.R., Margiotta, B., Lafiandra, D. & MacRitchie, F. (2008): Composition of polymeric proteins and bread-making quality of wheat lines with allelic HMW–GS differing in number of cysteines. *J. Cereal Sci., 48,* 117–122.

Rakszegi, M., Pastori, G., Jones, H.D., Békés, F., Butow, B., Láng, L., Bedő, Z. & Shewry, P.R. (2008): Technological quality of field grown transgenic lines of commercial wheat cultivars expressing the 1Ax1 HMW glutenin subunit gene. *J. Cereal Sci., 47,* 310–321.

Schropp, P. & Wieser, H. (1996): Effects of high molecular weight subunits of glutenin on the rheological properties of wheat gluten. *Cereal Chem., 73,* 410–413.

Wieser, H., Antes, S. & Seilmeier, W. (1998): Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. *Cereal Chem., 75,* 644–650.

Zhang, P., He, Z., Chen, D., Zhang, Y., Larroque, O.R. & Xia, X. (2007): Contribution of common wheat protein fractions to dough properties and quality of Northern-style Chinese steamed bread. *J. Cereal Sci., 46,* 1–10.

Živančev, D.R., Nikolovski, B.G., Torbica, A.M., Mastilović, J.S. & Đukić, N.H. (2013): Lab-on-a-chip method uncertainties in determination of high-molecular-weight glutenin subunits. *Chem. Ind. Chem. Eng. Q.*, *19,* 553–561.

Živančev, D., Horvat, D., Torbica, A., Belović, M., Šimić, G., Magdić, D. & Đukić, N. (2015): Benefits and limitations of lab-on-a-chip method over reversed-phase high-performance liquid chromatography method in gluten proteins evaluation. *J. Chem.*, *2015,* Article ID 430328.