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Wheat breadmaking properties in dependance on wheat enzymes status and climate conditions

Running title: Breadmaking quality influenced by wheat enzymes status and climate

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Abstract

The objective of this study was to evaluate albumins profile, proteolytic and amylolytic activity level and baking performance of wheat varieties grown in two production years with different climate conditions (2011 and 2012) in four locations. The results of ANOVA showed that variety, location, production year, and their interactions all had significant effects on all tested wheat quality parameters. The enzymatic activity and specific bread volume were mainly influenced by the variety. The samples from 2012 production year, had the lower values of albumin content, proteolytic and amylolytic activity, and bread specific volume. The correlation analysis, performed for 2011 production year, showed that albumin fraction (15-30 kDa) and proteolytic activity were negatively correlated with bread specific volume indicating the role of this fraction on lowering the crucial bread quality parameter. In 2012 production year, albumin fractions (5-15 kDa; 50-65 kDa) showed the most correlations, especially with parameters of bread quality.

Key words: wheat albumins, climate, enzymes activity, baking performance
Wheat flour represents a complex system whose quality is influenced by many factors. In addition to the major components, starch and protein, wheat flour contains many other components that have direct or indirect impact in terms of processability of the flour and the quality of the final products (Goesaert, Brijs, Veraverbeke, Courtin, Gebruers & Delcour, 2005). The quality and content of these components is strongly interrelated and depends primarily on the genetic background and growing conditions (especially climate and soil). As the level of gene expression is highly dependent on growing conditions, examining the impact of these factors and their interactions on the final quality of wheat flour has been the subject of many studies (Triboi, Abad, Michelena, Lloveras, Ollier & Daniel, 2000; Vázquez et al., 2012).

The protein content and composition are the most important factors determining the quality of the flour. In order to determine the qualitative difference between the proteins of wheat flour, different approaches were used. The most often used method involves determining the correlation between the specific protein and the functional properties of wheat flour (Stojceska & Butler, 2012).

Wheat flour quality has been generally estimated on the basis of the characteristics and content of gluten proteins (Veraverbeke & Delcour, 2002). In contrast to the gluten proteins, there are a few reports concerning flour quality depending on the content of non-gluten proteins (albumins and globulins) (Chiang, Chen & Chang, 2006; Preston, Lukow & Morgan, 1992; Unbehend, Unbehend & Lindhauer, 2003).

The content of albumins and globulins of wheat endosperm represents about 20% of total wheat proteins (Merlino, Leroy, Chambon & Branlard, 2009). In comparison with the gluten
proteins, these proteins are important from nutritional point, due to very good amino acid balance (Gianibelli, Larroque, MacRitchie & Wrigley, 2001). A major part of the non-gluten proteins has the molecular weights (MW) lower than 25 kDa, but subunits between 60 and 70 kDa are also present in a significant proportion (Veraverbeke & Delcour, 2002).

These proteins are mainly enzymes involved in different metabolic functions while some high MW albumins and certain globulins have a storage function. Albumins include α-amylase, α-amylase/protease inhibitors (13 and 16 kDa) as well as enzymes with different physiological functions (62 kDa serine carboxypeptidase) (Singh, Blundell, Tanner & Skerritt, 2001). Some albumins, particularly those belonging to a family of trypsin and α-amylase inhibitors, have been demonstrated (Shewry et al., 1984; Silano et al., 1975).

Dong et al. (2012) established the enzymatic character of these proteins and found that among the identified 89 non-prolamin proteins more than 80% were various enzymes classified into eight functional categories including carbohydrate metabolism (27%), protein metabolism (27%), stress/defense/detoxification (11%), cell metabolism (6%), transcription/translation (4%), nitrogen metabolism (4%), photosynthesis (4%) and signal transduction (1%).

Examination of these groups of proteins in terms of determining of their impact on wheat flour quality dates back to early 1950s (Pence & Elder, 1953). Even though the importance of this group of proteins on functional properties of flour was not evident, Hoseney, Finney, Shogren and Pomeranz (1969) reported that their presence is essential for obtaining a loaf of bread with optimal volume.

However, incomplete knowledge of albumin proteins, related enzymes and the influence of many mentioned factors (growing conditions, variety) on these groups of proteins, complicate our understanding of their role in breadmaking quality. The aim of this research was therefore to evaluate albumins profile, proteolytic and amylolytic activity level and baking performance of wheat varieties from two production years. Additionally, we determined by statistical
methods the existing relations between the mentioned biochemical and breadmaking quality
of wheat.

2. Materials and methods

2.1. Samples

Four wheat varieties of *Triticum aestivum* Pobeda (Pob), Zvezdana (Zve), Gordana (Gord) and Apache (Ap) grown in two production years (2011 and 2012) in four locations Bačka Topola (BT), Sremska Mitrovica (SM), Sombor (SO) and Vršac (VR) in Northern Serbia were selected for the study. Pobeda (wheat standard in Serbia), Zvezdana and Gordana (Serbian varieties from last decade) were bred by the Institute of Field and Vegetable Crops, Novi Sad, Serbia, whereas Apache (the most cultivated variety in French and wide spread in Serbia) was bred by Limagrain, Chappes, France. All measurements were taken at wheat samples harvested from one experimental plot for each variety at each location. Conventional cultural practices were applied in all the test plots. The wheat samples were cleaned, tempered and milled using a Bühler MLU 202 (Bühler, Uzwil, Switzerland) according to AACC methods (1999).

2.2 Wheat flour characteristics

The rheological properties of wheat dough were determined using the Brabender Farinograph according to ICC 115/1, the Brabender Extensograph according to ICC 114/1, the Brabender Amylograph according to ICC 126/1, the Chopin Alveograph according to ICC 121 (ICC, 1992), and the Chopin Mixolab according to ICC 173 (ICC, 2011).

Gluten index (GI) was measured in two different ways: according to the ICC standard method 155 (ICC, 1994) and after incubation of dough ball at 37 °C for 90 min (Torbica, Antov, Mastilović & Knežević, 2007).
2.3. Protein extraction and Lab-on-a-Chip electrophoresis

The extraction of albumins was performed following the sequential Osborne extraction procedure (Osborne, 1907) with modifications. The wheat albumins were extracted (30 mg) with 300 µL of deionized water during 24 h at room temperature and then centrifuged at 14000 rpm for 20 min. The supernatant was collected as the albumin fraction and evaporated in a Reacti-Therm I (Thermo Fisher Scientific Bellefonte, PA, U.S.A.) to dryness at room temperature. The obtained extract were diluted with 2x treatment buffer (0.125 M tris-Cl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and water (1 v/v treatment buffer and 1 v/v water) and heated at 100 °C for 5 min. The chip-based separations were performed on the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer’s protocol (Protein 80 LabChip kit). The results were analyzed using Protein 80 software assay on Agilent 2100 Expert Software. For each investigated sample, analysis was conducted in two independent replications.

2.4. Measurement of α-amylase activity

α-amylase activity (CUg−1) of flour was measured using the Ceralpha method (Megazyme International, Wicklow, Ireland) for the measurement of plant and microbial alpha-amylases. At least three replicates were performed for each analysis.

2.5. Measurement of proteolytic activity

Proteolytic activity of wheat flour was determined as described by Calucci et al. (2004) and Strelec, Ugarčić-Hardi, Balkić and Šimunić (2007) with some modifications. Flour (2.5 g) was suspended in 5 ml sodium acetate buffer (50 mM, pH 5.0). As a substrate, 1% (w/v) hemoglobin (Hb) dissolved in sodium acetate buffer (0.1 M, pH 4.0) was used. The reaction
was initiated by adding flour extract (600 µL) in 2.7 mL of Hb and after incubation at 45 °C for 1 h terminated by adding 25% (w/v) trichloroacetic acid (TCA). After the centrifugation (10 min at 15000 g), 0.5 mL of the supernatant was utilized to determine the TCA-soluble products by the Lowry method (Lowry, Rosenbrough, Fair & Randall, 1951). At least three replicates were performed for each analysis.

2.6. Microstructure of dough and bread samples
The microstructure of the samples was analyzed by SEM technique, using a Jeol, JSM-6460LV SEM (Oxford Instruments, Abingdon, UK), operated at 25 kV. The pieces of the dough and bread samples were cut into sizes of about 1x1x1cm. The samples were prepared according to Ribotta, Pérez, León & Añón (2004). Dehydrated samples were dried using a critical point dryer 030 (BAL-TEC, Germany) and coated with gold using BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). The obtained micrographs were taken at two different magnifications: 1000x for dough samples and 2000x for bread samples.

2.7. Breadmaking Procedure
Baking trials were conducted under laboratory conditions. For 300 g bread making method, the test baking formula was: 300 g of flour (14 g/100 g moisture basis), 2% fresh yeast (flour basis) and 2% salt (flour basis). The total volume of water required for dough consistency of 400 BU is calculated on the basis of farinograph data: water absorption and the degree of softening according to Serbian official methods (1988). All ingredients were mixed in a high-speed Diosna mixer (Dierks&Söhne, Maschinenfabrik, Osnabrück, Germany) for 5 min. After mixing, dough was fermented for 120 min at 30 °C and 75% relative humidity (RH), with punches after 60 and 90 min. The fermented dough was divided into 130-g portions, hand-moulded and placed into lightly greased pans for final proofing for another 70 min at 30 °C.
and 75% RH. Doughs were then baked for 15 min at 220 °C. After cooling at room temperature for 1h, the loaves were kept in a climate chamber for 23h in controlled conditions of temperature (22 ± 0.7 °C) and humidity (75 ± 0.5%).

2.8. Characterization of bread

2.8.1 Specific volume

The breads were weighed after the cooling and their volume (cm3) was determined by millet displacement method. The specific volume (cm3/g) was calculated as loaf volume/bread weight.

2.8.2. Texture Measurements

The analysis of texture was carried out 24h after final baking using the TA XT2 Texture Analyser (Stable Micro Systems, UK) with a 30-kg load cell. Texture profile analysis TPA (texture profile analysis in a double compression cycle) was conducted using a P/75 (75-mm diameter) aluminium compression platen. Samples from the centre of the crumb slices were cut into cylinders (35 mm diameter, 12.5 mm thick) and compressed. The TPA method was conducted under these conditions: pre test speed: 1 mm/s; test and post-test speed, 5 mm/s; deformation, 75%; and wait time between first and second compression cycles, 5 s. The measured parameters were firmness, cohesiveness, springiness, chewiness and resilience. Each experimental point was the mean of three samples.

2.9. Statistical analysis

The experimental data collected was analyzed using the analysis of variance (ANOVA). The comparison among means was done by the Fisher’s LSD test regarded significant at p<0.05. The correlations between the certain biochemical and technological characteristics and
breadmaking quality of wheat were evaluated using Pearson’s correlation (significant level at 5%). In order to illustrate the variability of a chosen sample set, descriptive statistic was performed. Statistical methods were performed using the Statistica 12.0 software (Statsoft, Tulsa, OK).

3. Results and discussion

3.1. Meteorological data

Meteorological data for period from May to July (from anthesis to harvest maturity) were collected by automatic hydrological stations (provided by the Agricultural advisory services). The 2011 and 2012 growing seasons provided a wide range of growing conditions across the study locations, which contributed to the diversity of wheat qualitative characteristics. The 2011 production year was characterized by lower temperatures, drier conditions with an average growing season temperature across the locations from 16.6 °C (for May) to 23.3 °C (for July). Maximum daily temperatures, for the May and July, were above 28 °C and 35 °C, respectively. The number of days with maximum temperatures above 30 °C for the tested locations ranged from 12 to 26. The precipitation was quite variable across the locations and ranged from 40 to 120 mm. The 2012 production year was much warmer, with an average growing season temperature range across the locations of 17 (for May) to 27 °C (for July). June and July were characterized by deficient precipitation with extremely high maximum temperatures. Maximum temperatures were above 35 °C and the number of days with maximum temperatures above 30 °C was markedly higher than in the 2011 production year (from 31 to 41).

3.2. Descriptive statistics of selected parameters of wheat flour technological quality
In order to illustrate the variability of chosen sample set, descriptive statistic was performed and results are given in Supplementary Table 1. Among the many parameters of empirical rheological methods, we focused on a few of them which are commonly used to estimate the wheat flour quality for a particular purpose.

Wide variation was evident for all measured quality parameters and this variability was conditioned by the all three investigated factors (production year, variety and location). The range was broader among varieties than among growing locations. These results were in disagreement with the findings of Vázquez et al. (2012), who reported that variability, in the case of Alveograph parameters, was more dependent on environments. In general, the unfavourable climatic conditions in 2012 deteriorated all technological parameters, but in different extent.

Degree of softening ranged between 5 and 105 BU, and majority of the cultivars showed degree of softening between 40 and 60 BU. Gord variety expressed the lowest values of softening degree indicating stable cohesiveness of dough during kneading.

The extensographs parameters, extensibility (Ex) and resistance (R), as indicators of dough processing characteristics, were characterized by wide ranges of values indicating variable technological quality of wheat flour. It is also clearly visible effect of all three factors on these parameters, especially the influence of production year on extensibility. Obtained values for extensibility were lower for 2012 production year. In terms of the location influence, variety Gord was more stable compared to the others varieties.

The energy values (E) were dominantly influenced by production year. These values were significantly higher for 2011 compared to 2012 production year.

Regarding the alveographic energy, the influence of different climatic conditions was expressed in a much lesser extent. Variety Ap was evaluated as weaker flour with a lower alveographic energy (W), while other varieties had almost the same level of W average values.
indicating a good technological quality of tested flour samples. For 2011 production year, variety Ap had inconsistent values of W among the locations with range from 86 to 227 \(10^{-4}\) J, and these values were the much lesser than its genetic potential (Schäfer & Ferret, 2005).

Almost all tested wheat flour samples had the markedly high level of Amylograph peak viscosity (PV) regardless of the production year, which were above the optimum suitable for processing in baking industry (>650 BU). The exceptions were varieties Gord and Pob from 2011 production year. These varieties had the same level of Amylograph peak viscosity (PV) which classifies them as wheat suitable for processing in baking industry (Đaković, 1997). In relation to the other varieties, these two varieties were distinguished by better technological quality. In relation to the other varieties, these two varieties were distinguished by better technological quality, which was not a surprise for Pob variety considering that it belongs to a group of excellent bread varieties and improvers (Denčić, Kobiljski, Mladenović, & Kovačević, 2011). Compared to 2011 production year, samples from 2012 production year were characterized by significantly higher PV values, i.e. 69% of flour samples had PV value above 1000 BU. The high values of Amylograph peak viscosity could be the consequence of unfavourable climatic conditions in 2012 production year which caused the changes in the synthesis of enzymes, primarily amylases (Johansson et al., 2013). In conditions of heat stress, changed properties of the starch-amylose complex as a result of reduced proteins and starch biosynthesis might have happened as well. Based on these findings it can be assumed that high PV values could be the consequence of the way of packing starch granules and their size, not exclusively the consequence of amylolytic activity. Since the other quality parameters in our study showed relatively good processing quality of the wheat flour samples, the redefining the importance of common technological parameters becomes necessary. These observations were supported by the findings of Ichinose et al. (2001) who reported that
despite low level of PV values the tested wheat flour samples had relatively good breadmaking potential.

3.3. Albumin profile

Albumin content in the flour, determined by LoaC electrophoresis, was affected by all three investigated factors (production year, location and variety) and significant interactions were observed (Figure 1). For 2011, the content of albumins ranged from 0.29 to 2.39 g/100 g flour (dry weight basis). Similar results were obtained by Chiang, Chen & Chang (2006) who found that content of albumin and globulin fractions was in the range of 1.1 to 2.1% of flour.

The albumin content for 2012 was significantly lower (p<0.05) compared to 2011 production year and ranged from 0.41 to 1.27% of flour. Evaluation of the protein fractions content of wheat flour, including albumins, has been conditioned by numerous factors and primarily depends on extraction method, wheat variety and growing conditions. HMW-albumins tend to form polymers between themselves and certain HMW-albumins can form disulfide bonds with LMW-glutenins (Gianibelli et al., 2001) which additionally complicated separation and quantification of albumins (Kuktaite et al., 2003). Moreover, numerous studies, based on different techniques of protein fractionation, isolation and identification, indicate that non-gluten polymers represent a non-negligible part of the total wheat polymers (MacRitchie, 1987; Gupta et al., 1992). All mentioned factors influenced wide variability of obtained results. The greatest difference in the albumin content showed variety Pob, as evidenced by the appearance of gel image of albumin fractions, where those differences are manifested through the differences in the number of protein bands, as well as the intensity of their coloration in the whole range of molecular weights (Supplementary figure 1).

The lowest content and the greatest variability of albumin subunits for all tested varieties were recorded in the range of molecular weight from 50 to 65 kDa (data not shown). The obtained results were in agreement with the findings of Balázs et al. (2012) who reported that
significant differences in the albumins content for different varieties were found in the region of high molecular weight albumins.

3.4. Proteolytic and amylolitic activity

Figure 2a shows proteolytic activity depending on production year, varieties and locations. The proteolytic activity of tested samples for both production years was in the range of 1.45-3.75 U/g. The obtained values of the total proteolytic activity were influenced by all three factors and by their interactions, but it could be noted that the variability was the most conditioned by variety effects. Generally, the total proteolytic activity was significantly higher (p<0.05) for 2011 compared to 2012 production year. Compared to the other varieties, Ap variety had significantly (p<0.05) higher protease activity and this activity was almost at the same level regardless of the locations from which it originated.

Amylolytic activity varied widely among wheat varieties; it is an intrinsic characteristic and was also influenced by the other two factors (climate factor and location). The amylolytic activity of tested samples for both production years ranged from 0.06 to 0.13 U/g (Figure 2b). In the study by Johansson (2002), it was reported that amylolytic activity was influenced by both genotype and environment. The obtained results showed that the highest activity was seen in Ap variety, especially for 2012 production year. Generally, in comparison to 2011, the 2012 production year was characterized by lower values of amylolytic activity (minimum and maximum values were 0.06 and 0.09 U/g of flour, respectively) and by minor differences in terms of locations and varieties.

3.6. Microstructure of the dough samples

The microstructure of dough samples from two production years with maximum and minimum values of bread specific volume is shown in Figure 3.
The samples from the 2011 production year had the higher number of the large starch granules. In these samples, the formed gluten matrix was in the form of lamellas and fibrils, and structure of the dough seemed more scattered, meaning that the dough contained more free water in contrast to the samples from the 2012 production year. The samples from the 2012 production year had the higher values of water absorption, so the water was probably mostly bounded within swollen starch granules appearing as tightly embedded in a continuous protein matrix.

Samples with maximum values of bread specific volumes of both years differed exclusively by values of Amylograph peak viscosity (PV), which could be the consequence of the way of packing starch granules and their size but not the consequence of differences in amylolytic activities.

3.7. Baking performance

Results of the specific volume of breads as the most important quality parameter are shown in Figure 4. In general, differences in bread specific volume brought by locations and production years were much smaller than those seen between varieties. Namely, it could be noticed that variety Ap differed from other varieties in relation to the lowest specific volume (Figure 4).

The samples from 2012 had the significantly lower values of this parameter compared to 2011 production year. This difference may be caused by the differences in the values of high temperatures during the growing season. The 2012 production year was characterized by higher temperatures which could cause changes in the protein composition. According to lower values of enzymes activity in the 2012 production year, it could be presumed that the levels of these activities for that year were under optimum which negatively affected the bread specific volume and that changes in the protein composition were not the consequence...
of enzymes. Exceptions were the results for the SO location. The specific volumes for this location were higher in 2012 production year for all investigated varieties. To illustrate the impact of production year on the baking performance, the four bread samples were chosen on the basis of the maximum and minimum values of the bread specific volume in both years. The values of certain parameters of wheat dough (Supplementary Figure 2a) clearly showed that the differences in volumes of wheat samples from the 2011 production year originating from the protein and starch complex. On the other hand, for the 2012 production year, these differences could be attributed exclusively to the protein component because the values of the maximum viscosity were practically indistinguishable.

The Supplementary Figure 2b shows the microstructure of bread samples from two production years with maximum and minimum values of bread specific volume. The differences in the structure of cell walls are clearly visible for selected samples from 2012 production year, which was not the case for samples from 2011. In the samples with maximum values of specific volume the partially gelatinised starch granules were glued together and appeared remarkably embedded in a network of protein and gelatinised starch. In the case of samples with minimum specific volume, the cell walls were composed of partly swollen starch granules which were not enveloped completely in the network of denatured gluten and gelatinised starch. Moreover, on the surface of swollen starch granules the fibrils of proteins were present. Differences in the structure of cell walls could be attributed to the differences in the characteristics of proteins (measured by Alveogram).

3.8. Correlation analysis

The correlation analysis was performed separately for the each production year in order to show different relation between the tested parameters which directly indicates the significant influence of production year. To determine the influence of individual albumin fractions on
the rheological properties of dough and breadmaking quality of wheat, relative amount of albumins in each sample were classified into four intervals according to their molecular weights (5–15 kDa; 15–30 kDa; 30–50 kDa; 50–65 kDa). The intervals were selected on the basis of the electrophoregram patterns (position and frequency of specific molecular weights) and their quantities in the most samples. Relative protein content in each group was determined by the ratio of the peaks area in each group over the peaks area in the overall four groups (Tomić et al., 2015). Traits showing nonsignificant associations with selected biochemical characteristics of wheat (albumin fractions, proteolytic and amylolytic activity) are not presented.

The correlations among variables showed many significant, but modest, values (Tables 1, 2). In 2011 production year, only albumin fraction of 15-30 kDa showed the highest number of correlations with selected wheat quality parameters (Table 1). In our previous paper (Tomić et al., 2015) the obtained results showed significant correlations between this albumin fraction and some rheological parameters which are related to the protein component of flour, such us: water absorption (WA, WAmix, r=-0.52 and r=-0.55, p<0.05, respectively), resistance to extension of dough which was measured uniaxial (R and R/Ex, r=-0.59 and r=-0.65, p<0.05, respectively) and biaxial (P and P/L, r=-0.68 and r=-0.64, p<0.05, respectively). The albumin fraction 15-30 kDa showed a significantly strong correlation with proteolytic activity (r=0.75, p<0.05).

On the other hand, the correlations among the end-product quality parameters (bread specific volume and cohesiveness) and albumin fraction were negative indicating the role of this fraction on lowering the crucial bread quality parameter. The same pattern was observed for proteolytic activity. Weegels, Orsel, van de Pijpekamp, Lichtendonk, Hamer & Schofield, (1995) reported that low Mr wheat proteins had inconsistent influence on bread volume and
primarily was depended on variety. In the case of weaker variety this influence was not expressed or was negative for that parameter.

A positive and significant correlation of proteolytic activity values was found with bread parameter - hardness. The possible explanation for this correlation was that the level of proteolytic activity was not high enough to cause weakening the gluten and reducing the bread firmness. The significant correlations were obtained between amylolytic activity and alveograph parameters related to dough extensibility (L and G). The existence of only these correlations implied that the level of amylolytic enzymes was very low, and the certain activity that would positively influence the handling properties of the dough was desirable.

The quite opposite situation was obtained for 2012 production year where concerning the albumins, albumin fractions of 5-15 kDa and 50-65 kDa showed the most correlations (Table 2). Among the few correlations with $r > 0.5$, the most interesting were significant correlations between albumin fractions of 5-15 kDa and 50-65 kDa and parameters of bread quality. Parameters derived from dough rheological tests did not show such high correlations with these albumin fractions as those derived from end product-bread, probably because these rheological parameters were influenced by other factors.

Amylolytic activity exhibited a significant correlation with the alveograph parameters P, L, G and W ($r=-0.87; r=0.67; r=0.63$ and $r=-0.72$, respectively). Many studies established the different correlations between falling number as indirect mesure of $\alpha$-amylase activity and certain Mixolab parameters related to the starch component of flour (Codină, Mironeasa, Bordei & Leahu, 2010; Collar, Bollain & Rosell, 2007; Szafrańska, 2014). In our study, the obtained correlation analysis showed significant correlation of amylolytic activity with Mixolab parameters such as starch gelatinisation (C3, $r=0.67$), amylolytic activity (C4, $r=0.62$) and starch retrogradation (C5, $r=0.60$). Taking into account the fact that the lower is the amylolytic activity, the higher is the stability of the hot-formed gel (C4), the correlation of
amylolytic activity with C4 was unexpected. This result confirmed previous claim that the level of amylolytic activity was very low and insufficient to cause any major disruption of gel consistency which could be negatively reflected on bread quality (Collar et al., 2007; Szafranska, 2014).

Despite the low level of amylolytic activity, the significant correlation were obtained with texture parameters of bread quality such as springiness, cohesiveness and resilience (r=-0.83; r=-0.74; and r=-0.72, respectively). This implies that the varieties with higher values of amylolytic activity also had lower values of these bread quality parameters.

4. Conclusions

The albumin content, proteolytic and amylolytic activity, and bread specific volume values for 2012 production year were significantly lower compared to 2011. By comparing the results of rheological methods, enzyme activities and bread specific volume it could be concluded that level of enzyme activities were low to cause any quality deterioration. In fact, the obtained results implied that the level of proteolytic and amylolytic activity was under optimum and increase of these activities could be beneficial for bread final quality. Heat stress had the dominant effect on enzyme activities in both production years and more intensive heat stress in 2012 caused the inferior rheological and bredmaking wheat quality compared to samples from 2011 production year.

The correlations among the end-product quality parameters (bread specific volume and cohesiveness) and 15-30 kDa albumin fraction were negative indicating the role of this fraction on lowering the crucial bread quality. The same pattern was observed for proteolytic activity.
In 2012 production year albumin fractions of 5-15 kDa and 50-65 kDa showed the most correlations, especially with parameters of bread quality. The amylolytic activity for both production years had the significant positive correlations with alveograph parameters related to dough extensibility (L and G).

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Figure 1. Effects of production year, location and variety on the albumins content. Measured values are the mean ± 0.95 LSD intervals.

Figure 2 (a,b). Effects of production year, location and variety on the proteolytic activity (a) and amylolytic activity (b). Measured values are the mean ± 0.95 LSD intervals.

Figure 3. Scanning electron micrographs of the dough from wheat flour samples selected on the basis of minimum and maximum bread specific volumes in two production year.

Figure 4. Effects of production year, location and variety on the bread specific volume.

Measured values are the mean ± 0.95 LSD intervals;

Supplementary Figure 1. Lab-on-a-Chip gel images of albumins obtained for Pob variety in two production years

Supplementary Figure 2 (a,b). Cut loaves of bread produced from wheat flour samples with minimum and maximum bread specific volumes in two production year and corresponding results of selected technological quality parameters and enzymes activity (a); Scanning electron micrographs of bread crumbs from wheat flour samples selected on the basis of minimum and maximum bread specific volumes in two production year (b).
Figure 3.

Figure 4.
### Table 1

Correlations between biochemical and wheat quality parameters of wheat flour samples from 2011 production year

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WA, Farinograph water absorption (%); R, Extensograph resistance to stretching (BU); Ex, Extensograph extensibility (mm); R/Ex, Ratio of resistance to extensibility; P, Resistance to extension; L, Alveograph extensibility (mm); G, Swelling index; W, Alveograph deformation energy \((x10^4 J)\); P/L, Ratio of gluten elasticity and extensibility; WAMix, Mixolab water absorption (%); DevMix, Mixolab development time (min); C5, Maximum torque of starch pasting (Nm); C3-C4, Breakdown torque (Nm); C5-C4, Setback torque (Nm); \(\beta\), Gelatinization rate (Nm/min); \(\gamma\), Cooking stability rate (Nm/min); Vsp, bread specific volume; Hardness (Hard), Springiness (Spring), Cohesiveness (Cohes), Chewiness (Chew), texture parameters; ALB 1, ALB 2, ALB 3, ALB 4, albumin fractions with molecular weight intervals of 5–15 kDa, 15–30 kDa, 30–50 kDa and 50–65 kDa, respectively; PA, Proteolytic activity (U/g flour); AMYL, \(\alpha\)-amylase activity (U/g flour).
Table 2
Correlations between biochemical and wheat quality parameters of wheat flour samples from 2012 production year

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WA, Farinograph water absorption (%); P, Resistance to extension; L, Alveograph extensibility (mm); G, Swelling index; W, Alveograph deformation energy (x10^-4 J); P/L, Ratio of gluten elasticity and extensibility; WAMix, Mixolab water absorption (%); DevMix, Mixolab development time (min); StabMix, Mixolab stability (min); C3, Mixolab torque in point of maximal torsion (Nm); C4, Minimum torque of starch pasting (Nm); C5, Maximum torque of starch pasting (Nm); C5-C4, Setback torque (Nm); α, Protein network weakening rate (Nm/min); γ, Cooking stability rate (Nm/min); GI, Gluten index; Vsp, bread specific volume; Hardness (Hard), Springiness (Spring), Cohesivenes (Cohes), Resilience (Resil), texture parameters; ALB 1, ALB 2, ALB 3, ALB 4, albumin fractions with molecular weight intervals of 5–15 kDa, 15–30 kDa, 30–50 kDa and 50–65 kDa, respectively; PA, Proteolytic activity (U/g flour); AMYL, α- amylase activity (U/g flour).