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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

1. Introduction

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

79 proteins, these proteins are important from nutritional point, due to very good amino acid
80 balance (Gianibelli, Larroque, MacRitchie & Wrigley, 2001). A major part of the non-gluten
81 proteins has the molecular weights (MW) lower than 25 kDa, but subunits between 60 and 70
82 kDa are also present in a significant proportion (Veraverbeke & Delcour, 2002).
83 These proteins are mainly enzymes involved in different metabolic functions while some high
84 MW albumins and certain globulins have a storage function. Albumins include α-amylase, α-
85 amylase/protease inhibitors (13 and 16 kDa) as well as enzymes with different physiological
86 functions (62 kDa serine carboxypeptidase) (Singh, Blundell, Tanner & Skerritt, 2001). Some
87 albumins, particularly those belonging to a family of trypsin and α-amylase inhibitors, have
88 been demonstrated (Shewry et al., 1984; Silano et al., 1975).
89 Dong et al. (2012) established the enzymatic character of these proteins and found that among
90 the identified 89 non-prolamin proteins more than 80% were various enzymes classified into
91 eight functional categories including carbohydrate metabolism (27%), protein metabolism
92 (27%), stress/defense/detoxification (11%), cell metabolism (6%), transcription/translation
93 (4%), nitrogen metabolism (4%), photosynthesis (4%) and signal transduction (1%).
94 Examination of these groups of proteins in terms of determining of their impact on wheat
95 flour quality dates back to early 1950s (Pence & Elder, 1953). Even though the importance of
96 this group of proteins on functional properties of flour was not evident, Hoseney, Finney,
97 Shogren and Pomeranz (1969) reported that their presence is essential for obtaining a loaf of
98 bread with optimal volume.
99 However, incomplete knowledge of albumin proteins, related enzymes and the influence of
100 many mentioned factors (growing conditions, variety) on these groups of proteins, complicate
101 our understanding of their role in breadmaking quality. The aim of this research was therefore
102 to evaluate albumins profile, proteolytic and amylolytic activity level and baking performance
103 of wheat varieties from two production years. Additionally, we determined by statistical

104 methods the existing relations between the mentioned biochemical and breadmaking quality
105 of wheat.
106
107 **2. Materials and methods**
108
109 2.1. Samples
110 Four wheat varieties of *Triticum aestivum* Pobeda (Pob), Zvezdana (Zve), Gordana (Gord)
111 and Apache (Ap) grown in two production years (2011 and 2012) in four locations Bačka
112 Topola (BT), Sremska Mitrovica (SM), Sombor (SO) and Vršac (VR) in Northern Serbia
113 were selected for the study. Pobeda (wheat standard in Serbia), Zvezdana and Gordana
114 (Serbian varieties from last decade) were bred by the Institute of Field and Vegetable Crops,
115 Novi Sad, Serbia, whereas Apache (the most cultivated variety in French and wide spread in
116 Serbia) was bred by Limagrain, Chappes, France. All measurements were taken at wheat
117 samples harvested from one experimental plot for each variety at each location. Conventional
118 cultural practices were applied in all the test plots.The wheat samples were cleaned, tempered
119 and milled using a Bühler MLU 202 (Bühler, Uzwil, Switzerland) according to AACC
120 methods (1999).
121
122 2.2 Wheat flour characteristics
123 The rheological properties of wheat dough were determined using the Brabender Farinograph
124 according to ICC 115/1, the Brabender Extensograph according to ICC 114/1, the Brabender
125 Amylograph according to ICC 126/1, the Chopin Alveograph according to ICC 121 (ICC,
126 1992), and the Chopin Mixolab according to ICC 173 (ICC, 2011).
127 Gluten index (GI) was measured in two different ways: according to the ICC standard method
128 155 (ICC, 1994) and after incubation of dough ball at 37 °C for 90 min (Torbica, Antov,
129 Mastilović & Knežević, 2007).

130

131 2.3. Protein extraction and Lab-on-a-Chip electrophoresis

132 The extraction of albumins was performed following the sequential Osborne extraction
133 procedure (Osborne, 1907) with modifications. The wheat albumins were extracted (30 mg)
134 with 300 μL of deionized water during 24 h at room temperature and then centrifuged at 14
135 000 rpm for 20 min. The supernatant was collected as the albumin fraction and evaporated in
136 a Reacti-Therm I (Thermo Fisher Scientific Bellefonte, PA. U.S.A.) to dryness at room
137 temperature.

138 The obtained extract were diluted with 2x treatment buffer (0,125 M tris-Cl pH 6.8, 4% SDS,
139 20% glycerol, 10% 2-mercaptoethanol) and water (1 v/v treatment buffer and 1 v/v water) and
140 heated at 100 °C for 5 min. The chip-based separations were performed on the Agilent 2100
141 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to manufacturer's
142 protocol (Protein 80 LabChip kit). The results were analyzed using Protein 80 software assay
143 on Agilent 2100 Expert Software. For each investigated sample, analysis was conducted in
144 two independent replications.

145

146 2.4. Measurement of α-amylase activity

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

150

151 2.5. Measurement of proteolytic activity

152 Proteolytic activity of wheat flour was determined as described by Calucci et al. (2004) and
153 Strelec, Ugarčić-Hardi, Balkić and Šimunić (2007) with some modifications. Flour (2.5 g)
154 was suspended in 5 ml sodium acetate buffer (50 mM, pH 5.0). As a substrate, 1% (w/v)
155 hemoglobin (Hb) dissolved in sodium acetate buffer (0.1 M, pH 4.0) was used. The reaction

156 was initiated by adding flour extract (600 µL) in 2.7 mL of Hb and after incubation at 45 °C
157 for 1 h terminated by adding 25% (w/v) trichloroacetic acid (TCA). After the centrifugation
158 (10 min at 15000 g), 0.5 mL of the supernatant was utilized to determine the TCA-soluble
159 products by the Lowry method (Lowry, Rosenbrough, Fair & Randall, 1951). At least three
160 replicates were performed for each analysis.
161
162 2.6. Microstructure of dough and bread samples
163 The microstructure of the samples was analyzed by SEM technique, using a Jeol, JSM-
164 6460LV SEM (Oxford Instruments, Abingdon, UK), operated at 25 kV. The pieces of the
165 dough and bread samples were cut into sizes of about 1x1x1cm. The samples were prepared
166 according to Ribotta, Pérez, León & Añón (2004). Dehydrated samples were dried using a
167 critical point dryer 030 (BAL-TEC, Germany) and coated with gold using BAL-TEC SCD
168 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). The obtained micrographs were
169 taken at two different magnifications: 1000x for dough samples and 2000x for bread samples.
170
171 2.7. Breadmaking Procedure
172 Baking trials were conducted under laboratory conditions. For 300 g bread making method,
173 the test baking formula was: 300 g of flour (14 g/100 g moisture basis), 2% fresh yeast (flour
174 basis) and 2% salt (flour basis). The total volume of water required for dough consistency of
175 400 BU is calculated on the basis of farinograph data: water absorption and the degree of
176 softening according to Serbian official methods (1988). All ingredients were mixed in a high-
177 speed Diosna mixer (Dierks&Söhne, Maschinenfabrik, Osnabrück, Germany) for 5 min. After
178 mixing, dough was fermented for 120 min at 30 °C and 75% relative humidity (RH), with
179 punches after 60 and 90 min. The fermented dough was divided into 130-g portions, hand-
180 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

232 In order to illustrate the variability of chosen sample set, descriptive statistic was performed
233 and results are given in Supplementary Table 1. Among the many parameters of empirical
234 rheological methods, we focused on a few of them which are commonly used to estimate the
235 wheat flour quality for a particular purpose.
236 Wide variation was evident for all measured quality parameters and this variability was
237 conditioned by the all three investigated factors (production year, variety and location). The
238 range was broader among varieties than among growing locations. These results were in
239 disagreement with the findings of Vázquez et al. (2012), who reported that variability, in the
240 case of Alveograph parameters, was more dependent on environments. In general, the
241 unfavourable climatic conditions in 2012 deteriorated all technological parameters, but in
242 different extent.
243 Degree of softening ranged between 5 and 105 BU, and majority of the cultivars showed
244 degree of softening between 40 and 60 BU. Gord variety expressed the lowest values of
245 softening degree indicating stable cohesiveness of dough during kneading.
246 The extensographs parameters, extensibility (Ex) and resistance (R), as indicators of dough
247 processing characteristics, were characterized by wide ranges of values indicating variable
248 technological quality of wheat flour. It is also clearly visible effect of all three factors on these
249 parameters, especially the influence of production year on extensibility. Obtained values for
250 extensibility were lower for 2012 production year. In terms of the location influence, variety
251 Gord was more stable compared to the others varieties.
252 The energy values (E) were dominantly influenced by production year. These values were
253 significantly higher for 2011 compared to 2012 production year.
254 Regarding the alveographic energy, the influence of different climatic conditions was
255 expressed in a much lesser extent. Variety Ap was evaluated as weaker flour with a lower
256 alveographic energy (W), while other varieties had almost the same level of W average values

(above 200) 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

282 despite low level of PV values the tested wheat flour samples had relatively good
283 breadmaking potential.
284
285 3.3. Albumin profile
286 Albumin content in the flour, determined by LoaC electrophoresis, was affected by all three
287 investigated factors (production year, location and variety) and significant interactions were
288 observed (Figure 1). For 2011, the content of albumins ranged from 0.29 to 2.39 g/100 g flour
289 (dry weight basis). Similar results were obtained by Chiang, Chen & Chang (2006) who found
290 that content of albumin and globulin fractions was in the range of 1.1 to 2.1% of flour.
291 The albumin content for 2012 was significantly lower ($p<0.05$) compared to 2011 production
292 year and ranged from 0.41 to 1.27% of flour. Evaluation of the protein fractions content of
293 wheat flour, including albumins, has been conditioned by numerous factors and primarily
294 depends on extraction method, wheat variety and growing conditions. HMW-albumins tend to
295 form polymers between themselves and certain HMW-albumins can form disulfide bonds
296 with LMW-glutenins (Gianibelli *et al.*, 2001) which additionally complicated separation and
297 quantification of albumins (Kuktaite *et al.*, 2003). Moreover, numerous studies, based on
298 different techniques of protein fractionation, isolation and identification, indicate that non-
299 gluten polymers represent a non-negligible part of the total wheat polymers (MacRitchie,
300 1987; Gupta et al., 1992). All mentioned factors influenced wide variability of obtained
301 results. The greatest difference in the albumin content showed variety Pob, as evidenced by
302 the appearance of gel image of albumin fractions, where those differences are manifested
303 through the differences in the number of protein bands, as well as the intensity of their
304 coloration in the whole range of molecular weights (Supplementary figure 1).
305 The lowest content and the greatest variability of albumin subunits for all tested varieties were
306 recorded in the range of molecular weight from 50 to 65 kDa (data not shown). The obtained
307 results were in agreement with the findings of Balázs et al. (2012) who reported that

308 significant differences in the albumins content for different varieties were found in the region
309 of high molecular weight albumins.
310
311 3.4. Proteolytic and amylolitic activity
312 Figure 2a shows proteolytic activity depending on production year, varieties and locations.
313 The proteolytic activity of tested samples for both production years was in the range of 1.45-
314 3.75 U/g. The obtained values of the total proteolytic activity were influenced by all three
315 factors and by their interactions, but it could be noted that the variability was the most
316 conditioned by variety effects. Generally, the total proteolytic activity was significantly higher
317 ($p<0.05$) for 2011 compared to 2012 production year. Compared to the other varieties, Ap
318 variety had significantly ($p<0.05$) higher protease activity and this activity was almost at the
319 same level regardless of the locations from which it originated.
320 Amylolytic activity varied widely among wheat varieties; it is an intrinsic characteristic and
321 was also influenced by the other two factors (climate factor and location). The amylolytic
322 activity of tested samples for both production years ranged from 0.06 to 0.13 U/g (Figure 2b).
323 In the study by Johansson (2002), it was reported that amylolytic activity was influenced by
324 both genotype and environment. The obtained results showed that the highest activity was
325 seen in Ap variety, especially for 2012 production year. Generally, in comparison to 2011, the
326 2012 production year was characterized by lower values of amylolytic activity (minimum and
327 maximum values were 0.06 and 0.09 U/ g of flour, respectively) and by minor differences in
328 terms of locations and varieties.
329
330 3.6. Microstructure of the dough samples
331 The microstructure of dough samples from two production years with maximum and
332 minimum values of bread specific volume is shown in Figure 3.

333 The samples from the 2011 production year had the higher number of the large starch
334 granules. In these samples, the formed gluten matrix was in the form of lamellas and fibrils,
335 and structure of the dough seemed more scattered, meaning that the dough contained more
336 free water in contrast to the samples from the 2012 production year. The samples from the
337 2012 production year had the higher values of water absorption, so the water was probably
338 mostly bounded within swollen starch granules appearing as tightly embedded in a continuous
339 protein matrix.
340 Samples with maximum values of bread specific volumes of both years differed exclusively
341 by values of Amylograph peak viscosity (PV), which could be the consequence of the way of
342 packing starch granules and their size but not the consequence of differences in amylolytic
343 activities.
344
345 3.7. Baking performance
346 Results of the specific volume of breads as the most important quality parameter are shown in
347 Figure 4.
348 In general, differences in bread specific volume brought by locations and production years
349 were much smaller than those seen between varieties. Namely, it could be noticed that variety
350 Ap differed from other varieties in relation to the lowest specific volume (Figure 4).
351 The samples from 2012 had the significantly lower values of this parameter compared to 2011
352 production year. This difference may be caused by the differences in the values of high
353 temperatures during the growing season. The 2012 production year was characterized by
354 higher temperatures which could cause changes in the protein composition. According to
355 lower values of enzymes activity in the 2012 production year, it could be presumed that the
356 levels of these activities for that year were under optimum which negatively affected the
357 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

383 the rheological properties of dough and breadmaking quality of wheat, relative amount of
384 albumins in each sample were classified into four intervals according to their molecular
385 weights (5–15 kDa; 15–30 kDa; 30–50 kDa; 50–65 kDa). The intervals were selected on the
386 basis of the electrophoregram patterns (position and frequency of specific molecular weights)
387 and their quantities in the most samples. Relative protein content in each group was
388 determined by the ratio of the peaks area in each group over the peaks area in the overall four
389 groups (Tomić et al., 2015). Traits showing nonsignificant associations with selected
390 biochemical characteristics of wheat (albumin fractions, proteolytic and amylolytic activity)
391 are not presented.
392 The correlations among variables showed many significant, but modest, values (Tables 1, 2).
393 In 2011 production year, only albumin fraction of 15-30 kDa showed the highest number of
394 correlations with selected wheat quality parameters (Table 1). In our previous paper (Tomić et
395 al., 2015) the obtained results showed significant correlations between this albumin fraction
396 and some rheological parameters which are related to the protein component of flour, such us:
397 water absorption (WA, WAmix, $r=-0.52$ and $r=-0.55$, $p<0.05$, respectively), resistance to
398 extension of dough which was measured uniaxial (R and R/Ex, $r=-0.59$ and $r=-0.65$, $p<0.05$,
399 respectively) and biaxial (P and P/L, $r=-0.68$ and $r=-0.64$, $p<0.05$, respectively). The albumin
400 fraction 15-30 kDa showed a significantly strong correlation with proteolytic activity ($r=0.75$,
401 $p<0.05$).
402 On the other hand, the correlations among the end-product quality parameters (bread specific
403 volume and cohesiveness) and albumin fraction were negative indicating the role of this
404 fraction on lowering the crucial bread quality parameter. The same pattern was observed for
405 proteolytic activity. Weegels, Orsel, van de Pijpekamp, Lichtendonk, Hamer & Schofield,
406 (1995) reported that low Mr wheat proteins had inconsistent influence on bread volume and

407 primarily was depended on variety. In the case of weaker variety this influence was not
408 expressed or was negative for that parameter.
409 A positive and significant correlation of proteolytic activity values was found with bread
410 parameter - hardness. The possible explanation for this correlation was that the level of
411 proteolytic activity was not high enough to cause weakening the gluten and reducing the
412 bread firmness. The significant correlations were obtained between amylolytic activity and
413 alveograph parameters related to dough extensibility (L and G). The existence of only these
414 correlations implied that the level of amylolytic enzymes was very low, and the certain
415 activity that would positively influence the handling properties of the dough was desirable.
416 The quite opposite situation was obtained for 2012 production year where concerning the
417 albumins, albumin fractions of 5-15 kDa and 50-65 kDa showed the most correlations (Table
418 2). Among the few correlations with $r > 0.5$, the most interesting were significant correlations
419 between albumin fractions of 5-15 kDa and 50-65 kDa and parameters of bread quality.
420 Parameters derived from dough rheological tests did not show such high correlations with
421 these albumin fractions as those derived from end product-bread, probably because these
422 rheological parameters were influenced by other factors.
423 Amylolytic activity exhibited a significant correlation with the alveograph parameters P, L, G
424 and W ($r=-0.87$; $r=0.67$; $r=0.63$ and $r=-0.72$, respectively). Many studies established the
425 different correlations between falling number as indirect mesure of α-amylase activity and
426 certain Mixolab parameters related to the starch component of flour (Codină, Mironeasa,
427 Bordei & Leahu, 2010; Collar, Bollaín & Rosell, 2007; Szafrańska, 2014). In our study, the
428 obtained correlation analysis showed significant correlation of amylolytic activity with
429 Mixolab parameters such as starch gelatinisation (C3, $r=0.67$), amylolytic activity (C4,
430 $r=0.62$) and starch retrogradation (C5, $r=0.60$). Taking into account the fact that the lower is
431 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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578 **Figure 1.** Effects of production year, location and variety on the albumins content. Measured
579 values are the mean ± 0.95 LSD intervals.
580 **Figure 2 (a,b).** Effects of production year, location and variety on the proteolytic activity **(a)**
581 and amylolytic activity **(b)**. Measured values are the mean ± 0.95 LSD intervals.
582 **Figure 3.** Scanning electron micrographs of the dough from wheat flour samples selected on
583 the basis of minimum and maximum bread specific volumes in two production year.
584 **Figure 4.** Effects of production year, location and variety on the bread specific volume**.**
585 Measured values are the mean ± 0.95 LSD intervals;
586 **Supplementary Figure 1.** Lab-on-a-Chip gel images of albumins obtained for Pob variety in
587 two production years
588 **Supplementary Figure 2 (a,b).** Cut loaves of bread produced from wheat flour samples with
589 minimum and maximum bread specific volumes in two production year and corresponding
590 results of selected technological quality parameters and enzymes activity **(a)**; Scanning
591 electron micrographs of bread crumbs from wheat flour samples selected on the basis of
592 minimum and maximum bread specific volumes in two production year **(b)**.
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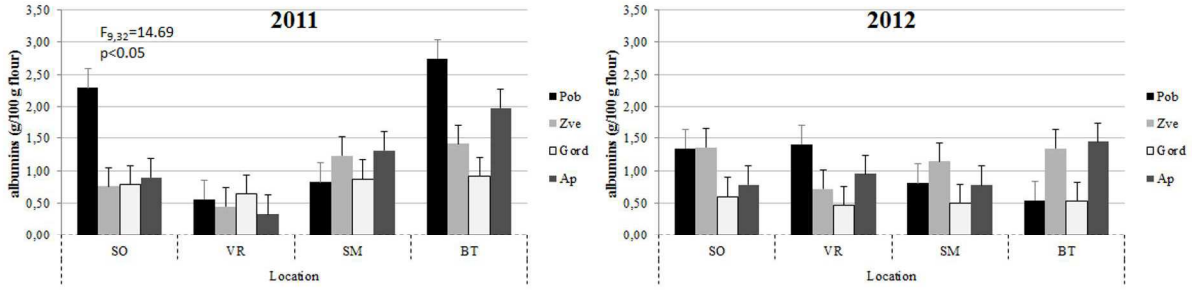

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604 **Figure 1.**

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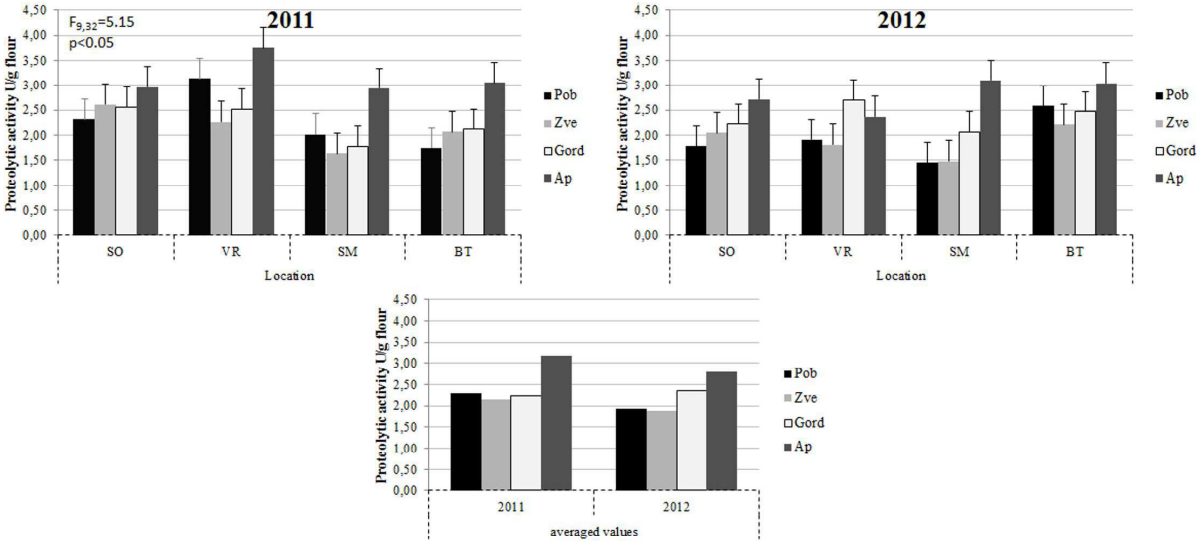

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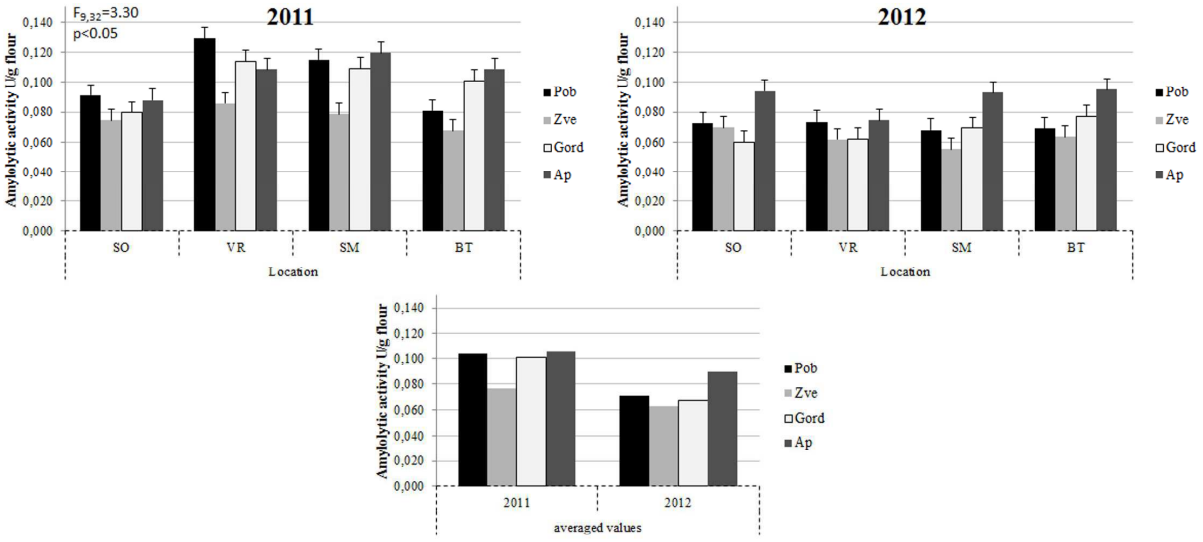

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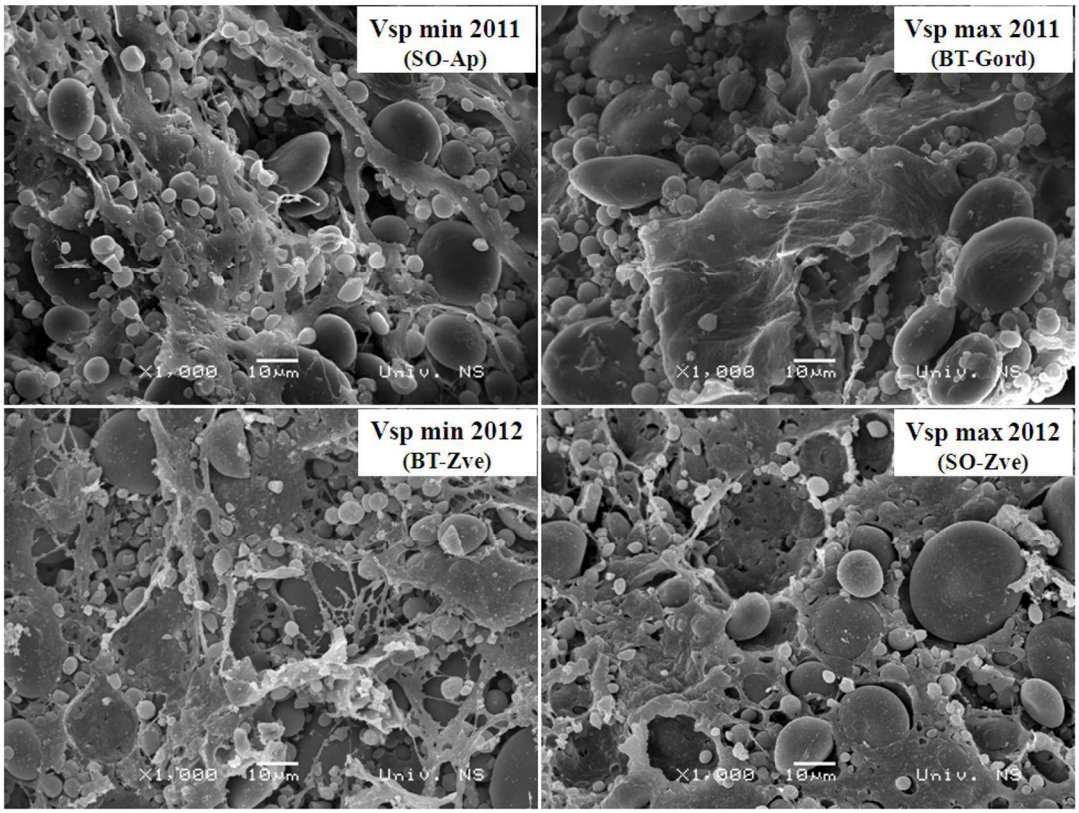

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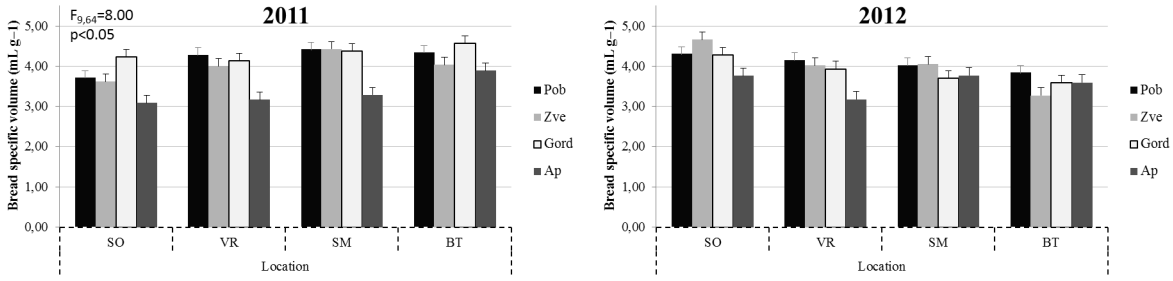
612 **Figure 3.**



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614 **Figure 4.**

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Table 1

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

	ALB1	ALB2	ALB3	ALB4	PA	AMYL
WA		-0.50			-0.65	
R		-0.60				
Ex		0.61			0.60	
R/Ex		-0.65			-0.56	
P		-0.68			-0.68	
L					0.55	0.70
G					0.53	0.71
W		-0.55				
P/L		-0.64			-0.71	-0.60
WAMix		-0.55			-0.59	
DevMix		-0.65			-0.66	
C5					0.54	
C3-C4	-0.51			0.52		
C5-C4					0.57	
β					0.52	
γ		-0.55				
Vsp		-0.56			-0.72	
Hard		0.52			0.66	
Spring					-0.55	
Cohes		-0.59			-0.66	
Chew					0.68	

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); β, Gelatinization rate (Nm/min); γ, 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, α- amylase activity (U/g flour).

Table 2

Correlations between biochemical and wheat quality parameters of wheat flour samples from 2012 production year

	ALB1	ALB2	ALB3	ALB4	PA	AMYL
WA					-0.71	-0.80
P					-0.69	-0.87
L						0.67
G						0.63
W					-0.58	-0.72
P/L						-0.66
WAMix					-0.75	-0.79
DevMix	0.53			-0.52		
ElastMix	-0.59			0.57		
C3						0.67
C4						0.62
C5				0.56		0.60
C5-C4				0.58		
α	0.66			-0.55		
γ					-0.56	-0.65
GI					0.60	
Vsp	0.65			-0.51		
Hard	-0.63			0.53		0.50
Spring					-0.65	-0.83
Cohes	0.52			-0.55	-0.59	-0.74
Resil	0.52			-0.61	-0.56	-0.72

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), Cohesiveness (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).