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Content of free amino groups during postharvest wheat and flour maturation in relation to gluten quality

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Abstract

The objective of this study was to monitor the changes in the content of free amino groups during postharvest wheat and flour maturation. The content of free amino groups of wheat flour was analysed immediately after wheat harvest, after 50 days of wheat storage and after 14 days of flour storage varying by wet gluten samples incubation temperatures and incubation times (0, 90 or 135 min at 30 °C and after that 180 min at 37 °C). The results were observed in relation to wheat-bug damaged kernels content, gluten index values, proteolytic activity and electrophoretic properties of gliadins and glutenins. The content of free amino groups increased during postharvest wheat and flour maturation periods. Proteolytic activity values were the highest 50 days after the wheat storage. The electrophoretic determination indicated a macromolecular redistribution of the gluten proteins from the moment of the wheat harvest until the moment of flour stabilisation.

Key words: free amino groups, wheat and flour maturation, gluten quality

1. Introduction

The study of the biochemical changes in wheat gluten proteins during wheat postharvest maturation as well as the study of the after-stabilisation of flour milling dates back to early 1940s (Bayfield, Anderson, Geddes & Hildebrand, 1943; Jones & Gersdorff, 1941; Shellenberger, 1939), continues in the early 1970s (Evers & Redman, 1973; Pyler, 1973; Skupin & Warchalewski, 1971) and still remains a topic of interest. The wheat postharvest maturing begins immediately after harvest, continues during storage, and depends on time, ambient conditions of storage and the grain moisture content. During the wheat maturation a number of biochemical and colloidal changes occur until the final technological maturity is reached. Newly harvested wheat has poor milling and baking quality, therefore, the postharvest maturation of wheat and wheat flour is a necessary part of improvement of their technological quality (Chen & Schofield, 1996; Wang & Flores, 1999).

After the milling of mature wheat, the maturation process continues in the wheat flour. Pyler (1973) described the complex biochemical changes during the flour maturation which started 4 to 5 days after milling and lasted for approximately 3 weeks.

Mature wheat flour has higher water absorption, better mixing tolerance, improved rheological properties, greater gas retention capability and produces bread with greater loaf volume (Mi□, 2003; Wang & Flores, 1999).

The above mentioned complex biochemical changes include several groups of enzymes present in wheat grain and flour. These are: amylases, proteases, oxygenases, polyphenol oxidases and peroxidases. Proteases are concentrated in the endosperm, germ and aleurone layer (Evers & Redman, 1973). In contrast to protease, peptidase was found largely in the endosperm (Kruger, 1973). Bleukx, Roels and Delcour (1997), in their research on the presence and activities of

71 proteolytic enzymes in vital wheat gluten, concluded that one or more aspartic and serine
72 endoproteases were causing gluten hydrolysis. Each time when a peptide bond is hydrolysed a
73 free amino group and a free carboxyl group are released. The progress of hydrolysis is
74 determined on the basis of the increase in the concentration of these groups. The measurement of
75 the free amino groups content in wheat proteins is directly proportional to the degree of
76 hydrolysis of proteins (Nielsen, Petersen & Dambmann, 2001), i.e. to the degradation of the
77 polymeric structure of protein up to the water-soluble free amino acids and small peptides (Aja,
78 Pérez & Rosell, 2004).
79 Although these enzymes are inactive during grain and flour storage after finished maturation
80 process, when water is added they become active and play a significant role in determining the
81 functional attributes of the flour. Lin, Lookhart and Hoseney (1993) reported the positive effect
82 of proteolytic enzymes on wheat dough rheological properties in production of fermented
83 products. To our knowledge, in the available literature of recent date there are no similar data on
84 monitoring biochemical changes during wheat postharvest and wheat flour maturation through
85 the concentration of free amino groups in wet gluten.The exception to this assertion is the study
86 of Zhao, Li, Liu, Liu and Li (2012) who investigated the changes in the amount of free amino
87 groups in the frozen gluten samples stored from 0 to 120 days. The negative effects of
88 proteolytic enzymes activity in durum wheat products were described by Petrova (2002) and
89 Olanca, Ozay and Koksel (2009). Pérez, Bonet and Rosell (2005) reported the increase in the
90 amount of free amino groups during incubation of gluten of damaged wheat as a consequence of
91 an activity of insect protease (saliva *Eurygaster* spp.).
92 The aim of this study was to determine the status of free amino groups in two different maturing
93 periods of the harvested wheat and flour obtained from it. For the determination of free amino

94 groups content, two temperatures were chosen (30 °C and 37 °C) and different incubation
95 periods of the wet gluten on those temperatures (0, 90 or 135 min at 30 °C and after that 180 min
96 at 37 °C). The temperature of 30 °C was chosen in order to simulate the measurement conditions
97 of the rheological properties of the dough on the extensograph and the duration of processing of
98 the dough in practise. The second temperature, 37 °C, was chosen as the optimum temperature
99 for proteolytic enzyme activity, in order to determine the potential of the content of free amino
100 groups as an indicator of the proteolytic activity.
101 The additional aim was to relate the content of free amino groups with the selected quality
102 parameters of the wheat/flour protein complex which included the gluten index at 30 °C and 37
103 °C, and the proteolytic activity.
104

105 **2. Materials and methods**

106 **2.1 Samples**

107 Three wheat varieties (*Triticum aestivum*) Pobeda (Pob), Zvezdana (Zve) and Apache (Ap)
108 grown in 2011 in three areas in Serbia: Ba□ka Topola (BT), Sremska Mitrovica (SM) and Vr□ac
109 (VR) were selected for the study. Pobeda and Zvezdana were bred by the Institute of Field and
110 Vegetable Crops, Novi Sad, Serbia, whereas Apache was bred by Limagrain, Chappes, France.
111 The measurements of temperature and precipitation at meteorological stations in the three
112 examined areas were observed from the beginning of May until the time of harvest (8-9 July).
113 During May and June, the VR area had sufficient precipitation and the smallest number of days
114 with a maximum temperature higher than 30 °C; two times lower compared to the BT area and
115 SM. The greatest number of days with a temperature higher than 30 °C was recorded at the SM

116 area, while the BT area was characterized by a combination of two unfavorable impacts on the
117 wheat development - drought and high temperature.
118 The appearance of wheat bug damage and black point kernels, kernels infected by Fusarium and
119 broken kernels, was registered at all areas but the impact of climatic conditions on the amount
120 and composition of impurities in the wheat could not be determined since the crops were treated
121 with herbicides, fungicides and insecticides.
122 The samples were stored in craft paper bags under laboratory conditions (22 °C, 70% RH) for 50
123 days. The samples were cleaned and tempered and milled using a Bühler MLU 202 (Bühler,
124 Uzwil, Switzerland) according to AACC methods (1999) immediately after harvest (point 1), and
125 after 50 days of wheat storage (point 2). Flour obtained after 50 days of wheat storage was also
126 analysed after 14 days of storage at the above mentioned conditions (point 3). Therefore, the
127 samples originated from point 1 were regarded as freshly harvested wheat, samples originated
128 from point 2 as matured wheat, while samples originated from point 3 were regarded as matured
129 flour. The storage periods 1-2 and 2-3 present wheat postharvest maturation and flour
130 maturation, respectively.
131
132 **2.2 Content of wheat-bug damaged kernels**
133 The content of wheat-bug damaged kernels (WBDK) was determined according to the ICC
134 standard method 102/1 (ICC, 1972) in two replicates (SD=0.08).
135
136 **2.3 Free amino groups content**
137 The content of free amino groups was determined according to the procedure described by Pérez
138 et al. (2005) from wet gluten washed out from flour samples (obtained as described in 2.1

139 section) according to standard ICC method 155 (ICC, 1994). The determination of free amino
140 groups was performed for different incubation times and temperatures in flour from freshly
141 harvested wheat (point 1), flour from matured wheat (point 2) and matured flour (point 3), as
142 presented in Table 1.
143 Every treatment was applied on flour samples of each examined wheat variety (Pob, Zve and Ap)
144 from all three areas (BT, SM and VR) in all three test points (1, 2 and 3).
145 The determination of free amino groups was carried out in four replicates, where the results were
146 calculated against a serine standard curve. The spectrophotometric readings were performed at
147 340 nm (GBC CINTRA 303UV/VIS) (for $SD_{(I)}$=0.03, $SD_{(II)}$=0.02, $SD_{(III)}$=0.03, $SD_{(IV)}$=0.03,
148 $SD_{(V)}$=0.03, $SD_{(VI)}$=0.03).
149

150 **2.4 Gluten index**

151 Gluten index was measured at 1, 2 and 3 points in two different ways: according to the ICC
152 standard method No 155 (ICC, 1994) (GIS) and by modified method (GIM) which includes
153 incubation of dough ball at 37 □C for 90 min (Torbica, Antov, Mastilovi □& Kne□evi □, 2007). All
154 measurements were performed in dublicate ($SD_{(GIS)}$=1.09, $SD_{(GIM)}$=3.62).
155

156 **2.5 Proteolytic activity**

157 The protease assay was performed for flour samples from wheat (point 1 and 2) and maturated
158 flour samples (point 3) stored in defined periods, as described by Rani, Prasada Rao, Leelavathi
159 and Haridas Rao (2001). The proteolytic activity (PA) was measured by using azocasein as
160 substrate. All spectrophotometric readings were performed at 440 nm (GBC CINTRA

303UV/VIS) in dublicate (SD=0.06). One unit of activity is defined as the change in absorbance by 1.0 unit.

2.6 Lab-on-a chip electrophoresis

The extraction of gliadins and glutenins from flour samples of freshly harvested wheat (point 1) and matured flour (point 3), was carried out according to the Osborn fractionation of the wheat protein and reduced by 2xTREATMENT BUFFER (0,125 M tris-Cl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). The extracts were analysed using the Protein 230 Plus LabChip kit (Torbica, □ivan□ev, Nikoli□, □or□evi□ & Nikolovski, 2010) by Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA) in dublicate (SD= 0.75-2.96 for molecular weights range from 14 to 220 kDa) (□ivan□ev, Nikolovski, Torbica, Mastilovi□& □uki□, 2012).

2.7 Statistical analysis

The effects of factors (storage period, gluten incubation temperature and gluten incubation time, variety and locality) on the free amino groups content and protein quality indicators were determined by ANOVA. Where the *F*-test for the ANOVA reached statistical significance ($p<0.05$), the differences among specific means were assessed by Least Significant Difference (LSD) tests. The Principal Component Analysis (PCA) was used for the estimation of the relation between the content of free amino groups and selected quality parameters of wheat/flour samples. Statistical methods were performed using the StatSoft. Inc. (2013) STATISTICA (data analysis software system) version 12.

3. Results and discussion

184 The biochemical changes in the proteins during wheat postharvest and flour maturation were
185 estimated on the basis of the content of free amino groups, gluten index, relative amounts of
186 gliadins and glutenins and flour proteolytic activity. These parameters depend on wheat variety,
187 the production year and the conditions of the harvest and also the milling technology, are
188 important for processing of the flour in bakery and the quality of the final products (Johansson et
189 al., 2013).
190

191 **3.1 Content of wheat-bug damaged kernels**

192 The insect protease might have promoted the gluten breakdown, favouring the accessibility to
193 some amino groups previously hidden in the polymeric structure and, as a consequence, the
194 release of diverse polypeptides (Pérez et al., 2005). In the samples from our study the wheat-bug
195 damaged kernels (WBDK) rangede from 0.30 to 1.32%. According to Serbian regulation
196 (Regulation of methods of physical and chemical analysis for quality control of grain, milling
197 and bakery products, pasta and quickly frozen dough, 1988) the allowed content of WBDK is
198 2%, while according to the U.S. Sample Grade Criteria (Grain inspection handbook, 2013) the
199 allowed number of insect damaged kernels in 1000 g of wheat kernels is 32. In our study, the
200 highest average value of WBDK was registered in the VR area and the lowest content in the SM
201 area.
202

203 **3.1 Content of free amino groups of gluten during incubation at 30 ^{o}C and 37 ^{o}C**

204 The influence of individual factors as well as the simultaneous influence of selected factors
205 (storage periods (1-2, 2-3), gluten incubation temperature, gluten incubation time, varieties and

206 localities) on the content of free amino groups, is shown in Fig. 1(a,b,c) and Supplementary
207 Table 1.

208 The content of free amino groups significantly increased ($p<0.05$) during postharvest wheat and
209 flour maturation (0.130-0.154 μg/mg). The content of free amino groups increased with the
210 increase in incubation temperature of gluten from 30 °C (0.081-0.092 μg/mg) to 37 °C (0.179-
211 0.215 μg/mg) ($p<0.05$) (Fig. 1a,b). The influence of incubation at 30 °C was obvious only after
212 135 min, while the values of free amino groups content at 37 °C for all tested points were
213 significantly higher with the prolongation of incubation time (0.164-0.230 μg/mg) ($p<0.05$) (Fig.
214 1b). The increase in the content of the free amino groups ($p<0.05$) was observed for IV, V and
215 VI sample treatment (Fig. 1b) These results were in accordance with Aja et al. (2004), who
216 showed that under the same conditions of wet gluten incubation, a significantly higher amount of
217 free amino acids and small peptides occurs after 180 min of gluten incubation at 37 °C, as a
218 consequence of the gluten hydrolysis under the optimal conditions for the proteolytic enzymes
219 activity.

220 During the total period of storage the highest content of free amino groups was observed in the
221 third point, i.e. after the completed biochemical processes in wheat and flour at 30 and 37 °C
222 (Fig. 1a). Referring to the claim of Shelke, Hoseney, Faubion and Curran (1992) that the cake-
223 baking quality of the flour improved with both wheat and flour maturity, it can be assumed that
224 the increase in the content of free amino groups is a factor that affects the formation of the final
225 quality of the flour. In the present study, the increase in the content of free amino groups was
226 more obvious during the wheat postharvest maturation in comparison to the period of flour
227 maturation. That was in accordance with the claims of Shelke et al. (1992) who found that the
228 flour milled from freshly harvested soft wheat changed rapidly immediately after milling.

229 According to Fig. 1c, the big difference between Pob and Ap varieties in their free amino groups
230 content is clear. The greatest impact of the growing area was noticed for variety Ap and the
231 lowest was for variety Pob.
232
233 **3.2 Gluten index determination**
234 The standard gluten index values determined without previous incubation increased during
235 postharvest wheat maturation ($p<0.05$), and then stay constant during flour maturation period (14
236 days) (Fig. 2a). These results were in accordance with the results of Mi□(2003), who reported no
237 major changes in the characteristics of gluten during four weeks of flour storage, unlike the
238 longer time of storage which influenced the rheological properties of gluten as determined by
239 GIS. The GIM values obtained with previous incubation were lower ($p<0.05$) than those
240 obtained without incubation (Fig. 2a) (Aja et al., 2004; Torbica et al., 2007), which was expected
241 as a consequence of applying the optimum temperature for proteolytic enzyme activity; the
242 highest value was in the point 2. The varieties had almost the same GIS values while the
243 differences between varieties were evident for the GIM values. Ap exhibited the highest, while
244 Zve the lowest values of GI (Figure 2b). Regarding the area, SM compared with the other two
245 areas, had the highest GIM values which were in accordance with lowest content of WBDK (Fig.
246 2b). The obtained results complied with the results of Har Gil, Bonfil and Svoray (2011), which
247 indicated that the genotype, climatic factors and agricultural practices applied had an important
248 impact on the values of GI. All samples reached their maximum at the end of the first period
249 (point 2) of maturation (data not shown) and stayed constant until the end of flour maturation
250 (point 3). Only variety Pob showed the changes in GIM values during the tested period.
251

252 **3.3 Proteolytic activity determination**

253 On the basis of previously reported results of Pérez et al. (2005), it was assumed that the time
254 period of 180 minutes would be sufficient to observe the clear distinction between different
255 gluten qualities in regard to free amino groups content.
256 The proteolytic activity of tested samples was in the range of 1.75-3.05 U/g flour (data not
257 shown). The obtained values of the total proteolytic activity were significantly higher ($p<0.05$)
258 after 50 days of wheat maturation (point 2) than those immediately after harvest (point 1) and
259 after 14 days of flour maturation (point 3) (Fig. 3a). In the period of storage from point 1 to point
260 2 the samples were stored in the grain form, while from point 2 the storage continued in the form
261 of flour, which due to the removed aleurone layer exhibited a significantly reduced level of
262 acting enzymes. At the end of the second phase of storage (from point 2 to point 3), a decreasing
263 trend of the overall proteolytic activity was observed compared to their initial values (Fig. 3a).
264 Figure 3b shows the proteolytic activity depending on varieties and the areas from which they
265 originated. No significant differences between the proteolytic activity of varieties in the area of
266 SM were observed. On the other hand, the Ap variety in the areas of BT and VR exhibited the
267 highest total proteolytic activity. Regarding the values of free amino groups content, gluten index
268 and proteolytic activity (Figs. 1c,2b,3b), variety Pob manifested very small differences in
269 relation to the origin of the samples, while varierty Ap showed statistically significant difference
270 between the values of the same indicators depending on the area. Those findings imply that the
271 varieties had a bigger influence on the values of the determined parameters than the climate
272 conditions.
273

274 **3.4 State of the protein complex**

The relative amounts of the analyzed glutenins and gliadins fractions at the beginning and in the end of wheat and flour maturation (from point 1 to point 3) were statistically significantly different ($p<0.05$). During the tested period, a clear trend in the relative amount changes was not observed in both cases (glutenins and gliadins). The exceptions were Glu 40-80 kDa, Glu 80-120 kDa and Gli>120 kDa fractions (Supplementary Figs. 1 and 2). Thus, it can be presumed that from the moment of the wheat harvest until the moment of flour stabilization, changes on the macromolecular level of the protein complex structure took place (Supplementary Figs. 1 (a,b) and 2 (a,b)). Hence, glutenins showed greater stability than gliadins in respect to the total amount of fractions that participated in macromolecular redistribution. This also indicates that proteolytic enzymes were more susceptive to gliadins than high molecular weight glutenins, which is in agreement with Rosell et al. (2002). During flour maturation, the proteolytic activity decreases as a result of flour aeration. Namely, specific sulfhydryl blockings and oxidising agents such as oxygen exhibits an inhibitory effect on the sulfhydryl nature of the proteolytic enzymes of wheat grain (Skupin & Warchalewski, 1971), so it is possible that the final redistribution and polymerization of the protein macromolecules occurred in this phase.

3.5 Principal Component Analysis (PCA) of flour quality data and content of free amino groups during wheat postharvest and flour maturation

Statistical analysis of obtained data included all the tested flour samples and the protein quality indicator values. In order to get an overview of the progress of the biochemical changes in proteins during wheat postharvest and flour maturation a Principal Component Analysis (PCA) was performed. The PCA was applied to visualize the relationships between all the measured variables and to present the results in plots that can be used for simple interpretation. The loading plot shows a projection of the variables in the factors space. When two variables are far from the

299 center (close to the circle line), then, if they are close to each other, they are significantly
300 positively correlated (r close to 1); if they are orthogonal, they are not correlated (r close to 0); If
301 they are on the opposite side of the center, then they are significantly negatively correlated (r
302 close to -1). From the resulting dependences, we have chosen to observe only the most
303 important.

304 PCA was separately performed for period of postharvest wheat maturation and after-milling flour
305 maturation. Fig. 4(a) shows PCA loading plot for freshly harvested wheat (point 1). The first two
306 components explained 69.66% of the total variance in the biochemical indicators of protein
307 properties determined immediately after wheat harvest (point 1). The first PC1 (50.34%) was
308 related to the free amino groups content of wet gluten determined following the patterns listed in
309 section 2.3, with PA and with the content of wheat-bug damaged kernels (WBDK) (Fig. 4a).
310 Karababa and Ozan (1998) and Hariri, Williams and El-Haramein (2000) reported that wheat
311 samples which have more than 5% bug damaged kernels showed significantly low-quality
312 properties. Even though the content of infested kernels in the tested samples of freshly harvested
313 wheat was less than 2%, it partly affected the increase of the content of free amino groups in wet
314 gluten (treatment NH2-I) ($r=0.678$, $p<0.05$). The greatest impact of WBDK (1.32%) on the
315 content of free amino groups (NH2-I, 0.10 μg/mg) was observed for variety Zve from the VR
316 area (data not shown). The content of free amino groups determined after 90 minutes of gluten
317 incubation at 30 □C and further prolongation for 180 minutes at 37 □C (NH2-V) was significantly
318 correlated with proteolytic activity ($r=0.74$, $p<0.05$).

319 Fig. 4(b) shows the PCA loading plot for matured wheat (point 2). The first two components
320 explained 86.51% of the total variance in the biochemical indicators of protein properties
321 determined from flour obtained after 50 days of wheat maturatioThe first PC1 which explained

322 the most variance (67.84%) reflected the content of free amino groups determined following the
323 treatments presented in section 2.3 in addition to PA. The second component was closely related
324 to GIM and GIS (Fig. 4b (loading plot)). The processes that occurred during wheat postharvest
325 maturation affected the enhancement of correlation coefficients between the content of free
326 amino groups and proteolytic activity (Figs. 4a and 4b). This is reflected in Figs. 4a and 4b by
327 closely positioning the variables vectors. The content of free amino groups determined following
328 the treatments that included previous gluten incubation (NH2-II, NH2-III, NH2-IV, NH2-V and
329 NH2-VI) was significantly correlated with the proteolytic activity ($r=0.73$, 0.81, 0.75, 0.91 and
330 0.95, respectively) ($p<0.05$). On the basis of obtained results, it might be assumed that the
331 determination of free amino groups as an indicator of the damage of the protein primary structure
332 due to the proteolytic enzymes present, can be determined by selecting NH2-V and/or NH2-VI
333 treatments ($r=0.908$ and $r=0.950$, respectively) which Fig. 4b clearly shows.
334 Fig. 4 (c) shows the PCA loading plot for matured flour (point 3). The first two PCs explained
335 67.19% of the total variance in the biochemical indicators of protein properties determined from
336 flour that was stored for 14 days as part of the maturation process. The first PC1 (46.97%) was
337 associated with the content of the free amino groups determined following NH2-III, NH2-IV,
338 NH2-V and NH2-VI treatments as well as with GIS. The difference in the correlation
339 coefficients between the content of free amino groups (NH2-III, NH2-IV, NH2-V, NH2-VI) and
340 proteolytic activity before ($r= 0.75$-0.95, $p<0.05$) and after flour maturation process ($r=0.17$-0.53,
341 $p>0.05$) could be explained by the decrease in the total proteolytic activity of the tested samples
342 after flour maturation (Fig. 4c).
343

344 **4. Conclusions**

345 During the wheat and flour maturation the content of free amino groups increased, but GIS, GIM
346 and proteolytic activity increased only during the wheat maturation. According to those findings
347 it could be assumed that the increase in free amino groups content during the 50 days of wheat
348 maturation is a consequence of increased proteolytic activity. After that period, the proteolytic
349 activity decreased. Despite the increase in the content of free amino groups during flour
350 maturation, the GIS values were stabile because gliadins were more susceptible to proteolytic
351 enzymes as revealed using electrophoresis. During the flour maturation process of 14 days the
352 proteolytic activity decreased, and the increase in the free amino groups content in the same
353 period could be explained by changes on a macromolecular level of the wheat flour protein
354 complex structure.
355 The analytical method, incubation time and temperature treatments applied for the determination
356 of the content of free amino groups indicated an increase in free amino groups content with
357 increasing the incubation temperature and time. The highest content was determined at 37 °C by
358 V and VI sample treatments, suggesting that the damage of the proteins' primary structures, due
359 to proteolytic enzymes activity, could be obtained by applying the treatments NH2-V and/or
360 NH2-VI.
361 The present study generated knowledge on the changes of the free amino groups content during
362 the chosen period of wheat and flour maturation. However, in order to obtain more reliable data
363 further examinations should be conducted covering a wider range of wheat varieties from
364 different production years.
365

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Figure captions

Fig. 1 (a,b,c). Changes in free amino groups content during wheat and flour maturation as a function of two different gluten incubation temperatures (30 and 37 ^{o}C) **(a)**, gluten incubation time (I, II, III, IV, V and VI) **(b)** and variety and locality **(c)**. Measured values are the mean ± 0.95 LSD intervals.

Fig. 2 (a,b). Effects of **(a)** the storage period and gluten incubation temperature, **(b)** variety and locality on the gluten index. Measured values are the mean ± 0.95 LSD intervals.

Fig. 3 (a,b). Effect of the storage period **(a)**, variety and locality **(b)** on the proteolytic activity values. Measured values are the mean ± 0.95 LSD intervals.

Fig. 4 (a,b,c). Principal component loading plots for **(a)** flours from freshly harvested wheat, **(b)** flours obtained after 50 days of wheat maturation, and **(c)** flours after 14 days of its maturation for quality indicators: NH2/I-VI - free amino groups content at different time and temperature of gluten incubation (μg/mg), GIS – standard gluten index; GIM – modified gluten index at 37 ^{o}C, PA-proteolytic activity (U/g flour) and WBDK-wheat-bug damaged kernels (%).

Supplementary Fig. 1 (a,b). Lab-on-a-Chip gel images of glutenins in first **(a)** and third **(b)** test points. Glutenin fractions 40-80 kDa and 80-120 kDa are framed with red line.

Supplementary Fig. 2 (a,b). Lab-on-a-Chip gel images of gliadins in first **(a)** and third **(b)** test points. Gliadin fractions >120 kDa are framed with red line.

Fig.1a

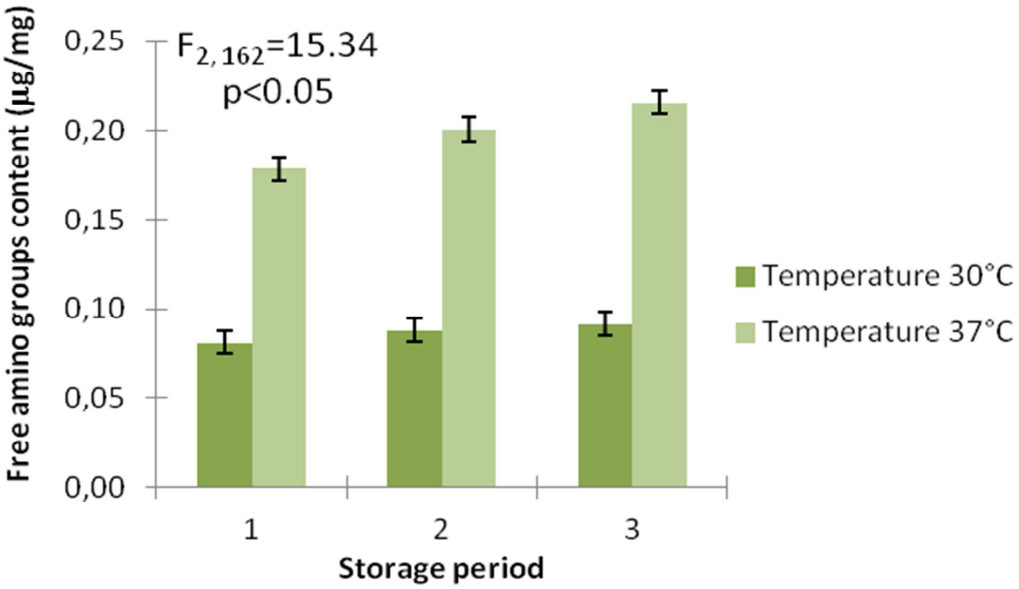

Free amino groups content (µg/mg)
0,25
0,20
0,15
0,10
0,05
0,00
$F_{2, 162}$=15.34
p<0.05
1
2
3
Storage period
Temperature 30°C
Temperature 37°C

Fig. 1b

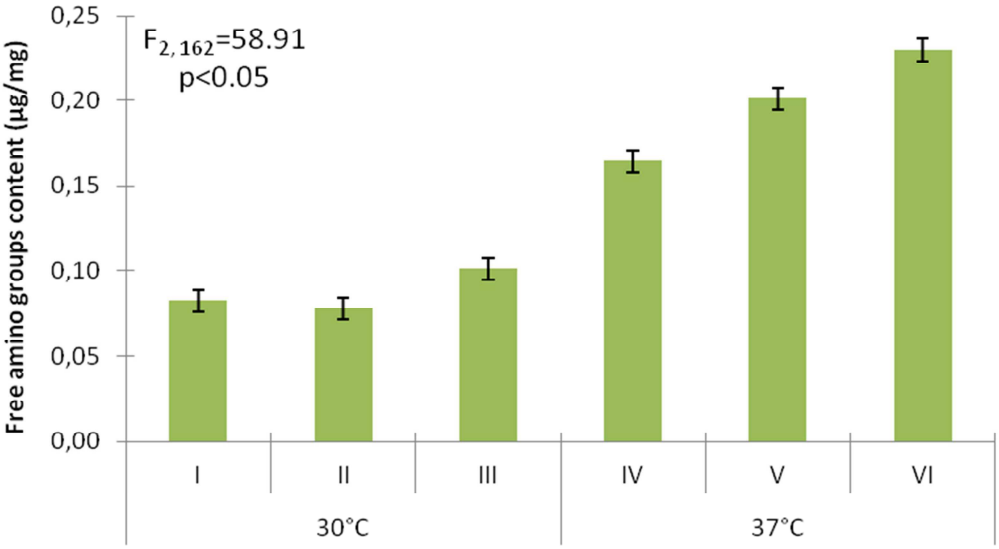

Free amino groups content (µg/mg)
0,25
0,20
0,15
0,10
0,05
0,00
$F_{2, 162}$=58.91
p<0.05
I
II
III
IV
V
VI
30°C
37°C

Fig. 1c

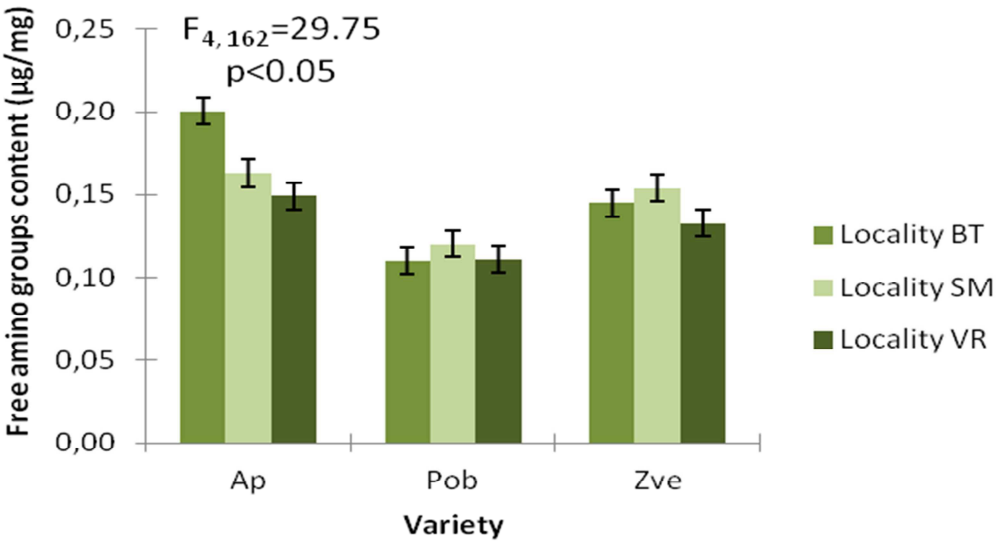

$F_{4, 162}$=29.75
p<0.05
Free amino groups content (µg/mg)
0,25
0,20
0,15
0,10
0,05
0,00
Ap
Pob
Zve
Variety
Locality BT
Locality SM
Locality VR

Fig. 2a

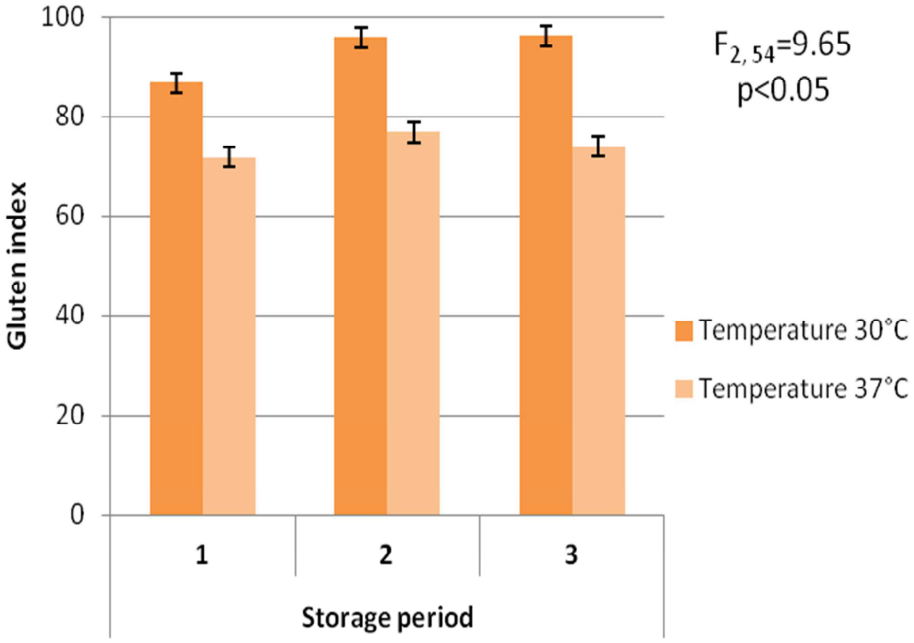

$F_{2, 54}$=9.65
p<0.05
Gluten index
100
80
60
40
20
0
1
2
3
Storage period
Temperature 30°C
Temperature 37°C

Fig 2b.

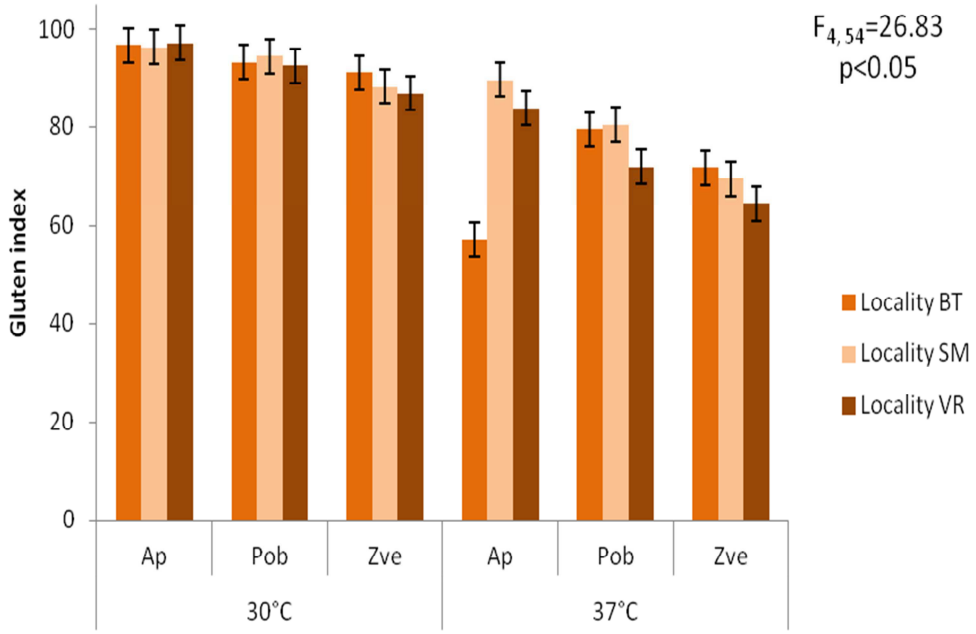

$F_{4, 54}$=26.83
p<0.05
Gluten index
100
80
60
40
20
0
Ap
Pob
Zve
Ap
Pob
Zve
30°C
37°C
Locality BT
Locality SM
Locality VR

Fig. 3

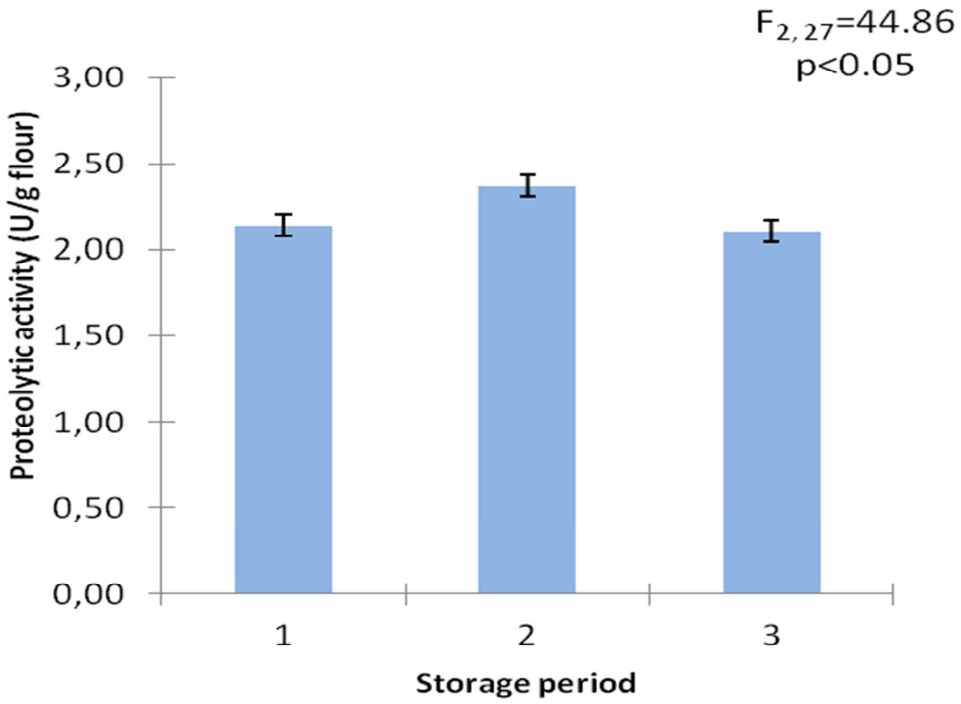

$F_{2, 27}$=44.86
p<0.05
Proteolytic activity (U/g flour)
3,00
2,50
2,00
1,50
1,00
0,50
0,00
1
2
3
Storage period

Fig. 3b

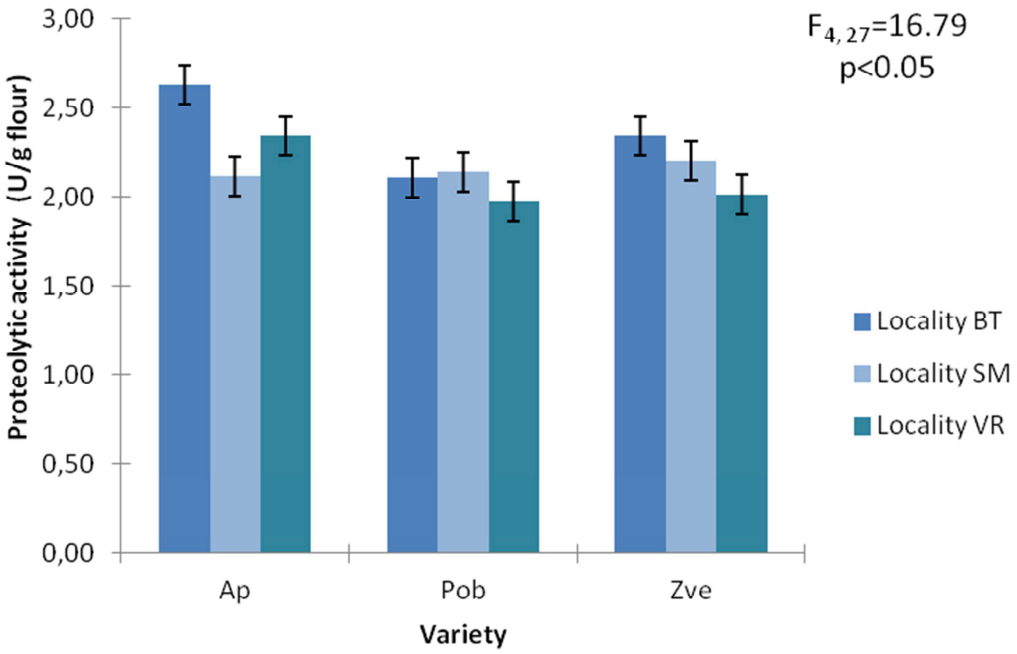

3,00
2,50
2,00
1,50
1,00
0,50
0,00
Proteolytic activity (U/g flour)
F4, 27=16.79
p<0.05
Locality BT
Locality SM
Locality VR
Ap
Pob
Zve
Variety

Fig. 4a

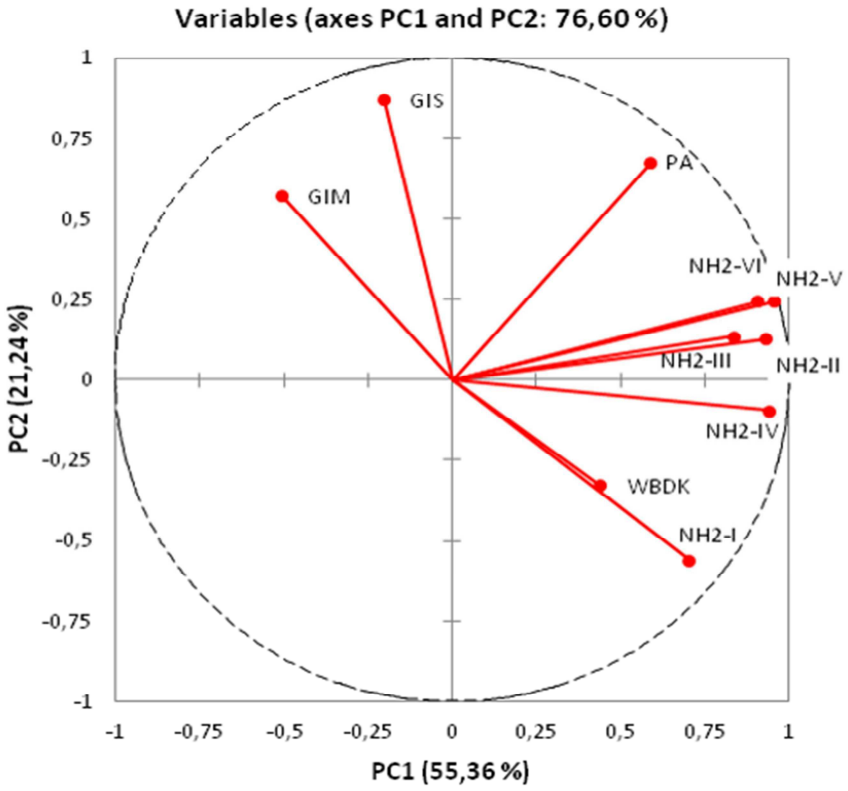

Variables (axes PC1 and PC2: 76,60 %)
GIS
PA
GIM
NH2-VI
NH2-V
NH2-III
NH2-II
NH2-IV
WBDK
NH2-I
PC2 (21,24 %)
PC1 (55,36 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1

Fig. 4b

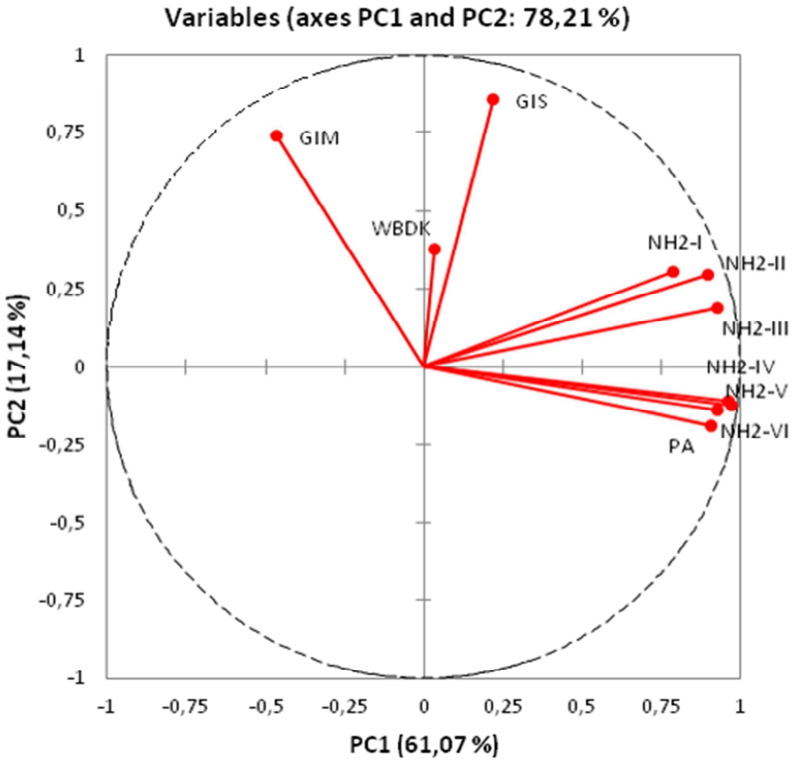

Variables (axes PC1 and PC2: 78,21 %)
GIS
GIM
WBDK
NH2-I
NH2-II
NH2-III
NH2-IV
NH2-V
NH2-VI
PA
PC2 (17,14 %)
PC1 (61,07 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1
-1
-0,75
-0,5
-0,25
0
0,25
0,5
0,75
1

Fig. 4c

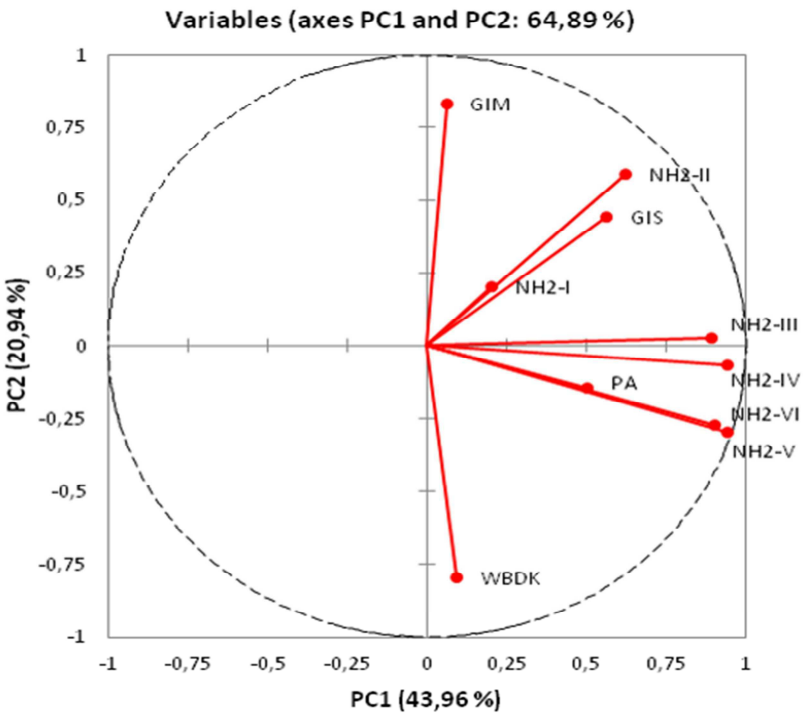
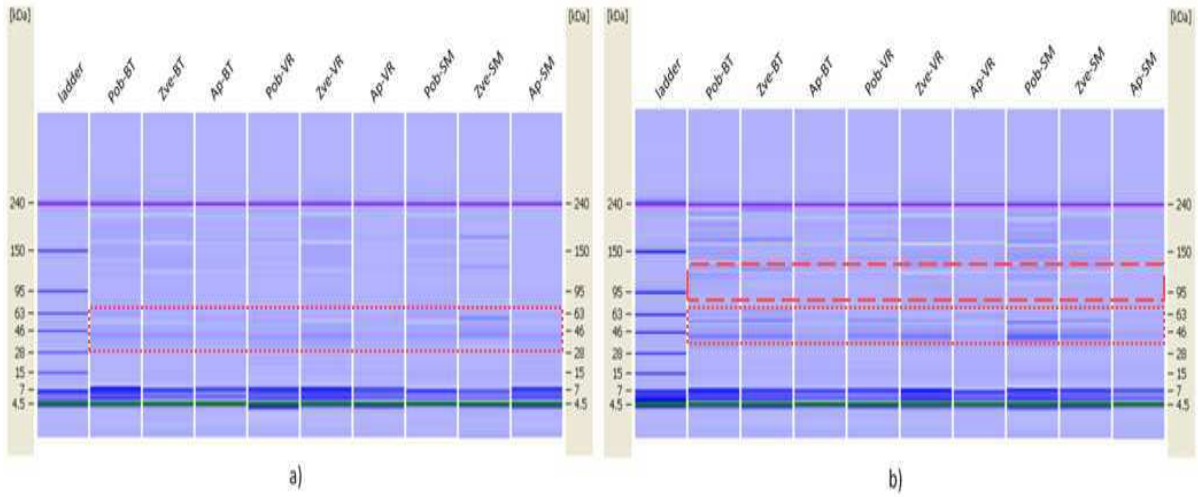

Variables (axes PC1 and PC2: 64,89 %)
GIM
NH2-II
GIS
NH2-I
NH2-III
NH2-IV
PA
NH2-VI
NH2-V
WBDK
PC2 (20,94 %)
PC1 (43,96 %)
1
0,75
0,5
0,25
0
-0,25
-0,5
-0,75
-1
-1
-0,75
-0,5
-0,25
0
0,25
0,5
0,75
1

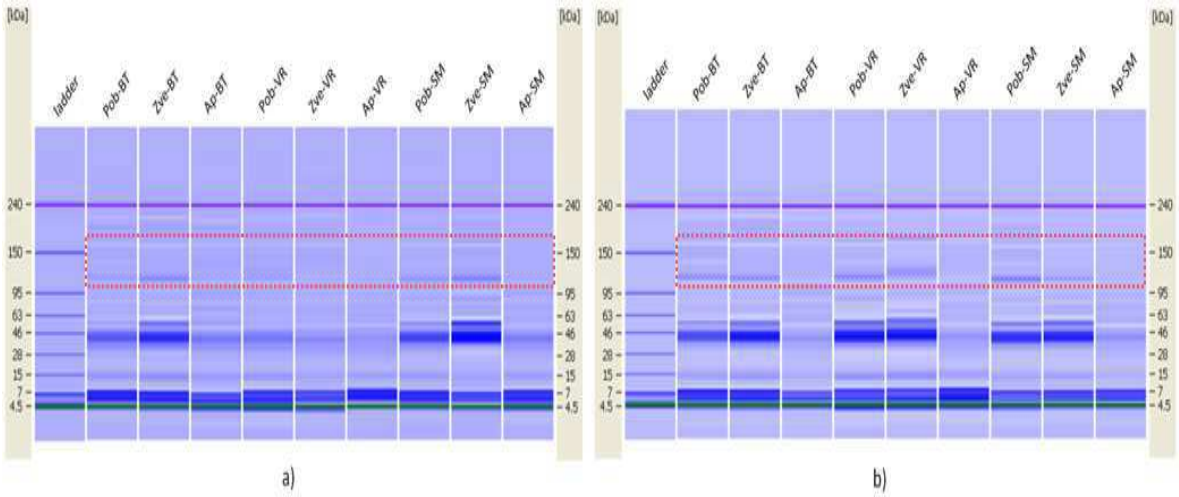
Table 1
Plan of experiment

Treatments labels	Test points	Sample incubation treatment			
		Temperature (°C)	Time (min)	Temperature (°C)	Time (min)
NH2-I	1, 2, 3	-	-	-	-
NH2-II	1, 2, 3	30	90	-	-
NH2-III	1, 2, 3	30	135	-	-
NH2-IV	1, 2, 3	-	-	37	180
NH2-V	1, 2, 3	30	90	37	180
NH2-VI	1, 2, 3	30	135	37	180

Supplementary Figure 1a,b



Supplementary Figure 2a,b



Supplementary Table 1

Effect of storage test point, temperature and time of gluten incubation on the content of free amino groups

Treatments labels	Storage test point	Sample incubation procedure				Free amino groups $_{LS\ means}$ (μg/mg)
		Temperature (°C)	Time (min)	Temperature (°C)	Time (min)	
NH2-I	1	-	-	-	-	0.082^{jk}
NH2-II	1	30	90	-	-	0.070^{l}
NH2-III	1	30	135	-	-	0.092^{ij}
NH2-IV	1	-	-	37	180	0.145^{g}
NH2-V	1	30	90	37	180	0.181^{e}
NH2-VI	1	30	135	37	180	0.211^{cd}
NH2-I	2	-	-	-	-	0.083^{jk}
NH2-II	2	30	90	-	-	0.084^{jk}
NH2-III	2	30	135	-	-	0.097^{i}
NH2-IV	2	-	-	37	180	0.168^{f}
NH2-V	2	30	90	37	180	0.205^{d}
NH2-VI	2	30	135	37	180	0.229^{b}
NH2-I	3	-	-	-	-	0.081^{jk}
NH2-II	3	30	90	-	-	0.080^{kl}
NH2-III	3	30	135	-	-	0.114^{h}
NH2-IV	3	-	-	37	180	0.179^{ef}
NH2-V	3	30	90	37	180	0.217^{c}
NH2-VI	3	30	135	37	180	0.250^{a}

Values followed by the same letter within column are not significantly different ($p<0.05$).