

Albumins Characterization in Relation to Rheological Properties and Enzymatic Activity of Wheat Flour Dough

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ABSTRACT

The aim of this study was to characterize albumin fractions of four different wheat varieties grown in a local climatic context, as well as to find the relationship between the particular albumin fractions and enzyme activity of the tested wheat flours and dough rheological properties. The determined content of albumin fractions ranged from 2.64 to 17.50% of total proteins. More than 50% of the albumins total amount was in the range of 5-15 kDa of molecular weight. The obtained results indicated that albumin fraction 15-30 kDa was related to certain rheological properties of dough, especially those related to water absorption and uniaxial and biaxial resistance to extension. Regarding the enzymatic status of flour, no strong relationship was found among the 5-15, 30-50 and 30-60 kDa albumin fractions and proteolytic and α -amylolytic activity. However, the albumin fraction 15-30 kDa showed significant correlation with proteolytic activity.

Keywords: α -amylolytic activity, Lab-on-chip electrophoresis, Proteins, Proteolytic activity.

INTRODUCTION

Wheat flour quality is strongly influenced by protein content and composition, but their complexity prevents a clear differentiation in terms of the specific role they have in the overall quality. Wheat flour quality has been generally estimated on the basis of the characteristics and content of gluten proteins.

The content of albumins and globulins of wheat endosperm represents about 20% of the total wheat proteins (Merlino *et al.*, 2009; Žilić *et al.*, 2011). Singh *et al.* (2011) reported the slightly smaller proportion of albumins and globulins which ranged between 11.48 and 16.03%. Non-gluten proteins are mainly monomeric but both albumins and globulins tend to form polymers by forming inter-chain disulphide

bonds. The molecular weights of non-gluten proteins are mainly lower than 25 kDa, but subunits of molecular weight between 60 and 70 kDa could also be observed (Veraverbeke and Delcour, 2002).

The albumins are mostly monomeric physiologically active or structural proteins (Goesaert *et al.*, 2005). 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 *et al.*, 2001). The inhibition activity of albumin fractions was confirmed by the study of Silano *et al.* (1975), who investigated the inhibition activity of the three albumin fractions (60, 24, and 12.5 kDa) toward amylases. Shewry *et al.* (1984) characterized certain albumins as trypsin/ α -amylase inhibitor. Gao *et al.* (2009) analyzed the

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non-prolamin expression profiles during grain development of bread wheat and found that most of the proteins had masses of 14–97 kDa, which were mostly distributed in the pH 4–7 range. Out of 400 protein spots, 230 proteins were identified and more than 85% of the identified proteins were enzymes possessing different physiological functions. Similar findings were reported by Dong *et al.* (2012) who 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%). Moreover, some high molecular weight albumins and certain globulins are considered to have a storage function (Gao *et al.*, 2009; Dong *et al.*, 2012).

There are indications in the literature for the contribution of certain albumins to wheat flour quality (Osipova *et al.*, 2012). Weegels *et al.* (1995) reported that despite the fact that low *Mr* non-gluten proteins have inconsistent effects on loaf volume, they have profound effects on the biochemical and rheological properties of dough. On the other hand, Unbehend *et al.* (2003) and Prabhasankar (2002) revealed that content of albumin and globulin proteins did not show an influence on loaf volume of wheat bread or chapati-making quality.

While the influence of gluten proteins on technological quality of flour has been extensively studied, the limited number of studies have dealt with the influence of non-gluten proteins (albumins and globulins) on flour quality (Chiang *et al.*, 2006; Gao *et al.*, 2009; Osipova *et al.*, 2012). Considering the general opinion that albumins and globulins of wheat endosperm have a secondary role in influencing the flour quality, they have been insufficiently studied (Gianibelli *et al.*, 2001). The intensive changes of climate during the last few decades have been followed by breeding the genotypes more

resistant to the unfavorable conditions during cultivation. In this regard, it could be assumed that the role of albumins might have changed in terms of their influence on the technological quality of wheat.

In order to gain more knowledge on the wheat flour albumins and their influence on its properties, the aim of this work was: (1) to characterize albumin fractions of four different wheat varieties grown in four different locations in Serbia, and (2) to determine the existing relations between the particular albumins fractions and rheological properties of the tested wheat flours as well as to connect them with the enzyme activity.

MATERIALS AND METHODS

Samples

Four wheat varieties of *Triticum aestivum*, namely, Pobeda (Pob), Zvezdana (Zve), Gordana (Gord), and Apache (Ap) that were grown in 2011 in four locations i.e. Bačka Topola (BT), Sremska Mitrovica (SM), Sombor (SO), and Vršac (VR) in Northern Serbia were selected for the study. Pobeda, Zvezdana and Gordana were bred by the Institute of Field and Vegetable Crops, Novi Sad, Serbia, whereas Apache was bred by Limagrain, Chappes, France. The wheat samples were cleaned, tempered and milled using a Bühler MLU 202 (Bühler, Uzwil, Switzerland) according to AACC (1999).

The climatic conditions of 2011 growing season were to a certain extent different from the usual climate, with 1.3°C higher mean temperature and 17% lower mean precipitation.

Protein Extraction

The extraction of albumins was conducted following the sequential Osborne extraction procedure (Osborne, 1907) with modifications. The wheat flour (30 mg) was extracted with 300 µL of deionized water and vortexed for 10 seconds. After 24 hours

extraction at room temperature, the obtained extracts were clarified by centrifugation at 14,000 rpm for 20 minutes. The supernatant was collected as the albumin fraction and evaporated in a Reacti-Therm I (Thermo Fisher Scientific Bellefonte, PA, USA) to dryness at room temperature.

Lab-on-a-Chip Electrophoresis

Extracted samples were diluted with 2X treatment buffer (0.125M 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 minutes. The chip-based separations were performed on the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) in combination with the Protein 80 Plus LabChip kit and the dedicated Protein 80 software assay on 2100 expert software. Bovine serum albumin was used as the standard for quantification of the non-gluten proteins. For each investigated wheat sample, analysis was conducted in two independent replications.

Rheological Properties of Wheat Dough

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 and Protein Content

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 minutes (Torbica *et al.*, 2007). Protein content was determined by Infratec 1241

Grain Analyzer (Foss Analytical AB, Hillerød, Denmark).

Measurement of α -amylase Activity

Alpha-amylase activity (CU/g) of flour was determined using the alpha amylase assay procedure (Ceralpha method). At least three replicates were performed for each analysis.

Measurement of Proteolytic Activity

Proteolytic activity of wheat flour was determined following the procedure reported by Strelec *et al.* (2007) and Calucci *et al.* (2004) with some modifications. Flour (2.5 g) was extracted in 5 mL sodium acetate buffer (50 mM, pH 5.0). The proteolytic activity was measured by using 1% (w/v) hemoglobin (Hb) as substrate, dissolved in sodium acetate buffer (0.1M, pH 4.0). The reaction was initiated by adding flour extract (600 μ L) in 2.7 mL of Hb and after incubation at 45°C for 1 hour terminated by adding 25% (w/v) trichloroacetic acid (TCA) and vortexing. The obtained suspension, after keeping at 4°C for 15 minutes, was cleared by centrifugation (10 minutes at 15,000 \times g) and 0.5 mL of the supernatant was utilized to determine the TCA-soluble products by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as standard protein. At least three replicates were performed for each analysis.

Statistical Analysis

For an overview of the effect of two factors (variety and location) in the complex of dataset on albumin content (g 100 g⁻¹ flour), a two-way ANOVA was calculated. The comparison among means was done by the Fisher's least significant differences regarded significant at $p < 0.05$. The Principal Component Analysis (PCA) was performed to determine the relationship between the tested properties of wheat flour samples. For that purpose, relative amount of albumins, categorized into four



intervals according to their molecular weights (5–15; 15–30; 30–50, 50–65 kDa), was used. Relative albumin 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. In order to illustrate the variability of a chosen sample set, descriptive statistic was performed. Statistical methods were performed using the Software XLSTAT, version (2012.2.02).

RESULTS AND DISCUSSION

Albumin Characterization by Lab-on-a-Chip Electrophoresis

Lab-on-a-Chip gel images of albumins obtained for wheat varieties selected for the study originated from different locations are presented in Figure 1 (a and b).

The gel images of albumin patterns showed diversity in separated fractions, both in qualitative and quantitative sense. Albumins of all tested wheat flour samples were characterized by rich protein pattern – the number of bands varied from 17 (SO-Pob) to 31 (SM-Gord)- corresponding to molecular weights from 5 to 65 kDa. Similar findings were reported by Chiang *et al.* (2006) and Baláz *et al.* (2012). By observing the albumin patterns of individual varieties, it could be noticed that albumin patterns of variety Gordana showed high similarity regardless of growing locations. However, in the case of the other varieties, the variations were more apparent in the region of higher molecular weights. This was particularly related to protein patterns of Pobeda, Zvezdana, and Apache from location BT. Thus, the differences between the protein profiles of the tested wheat flour were not

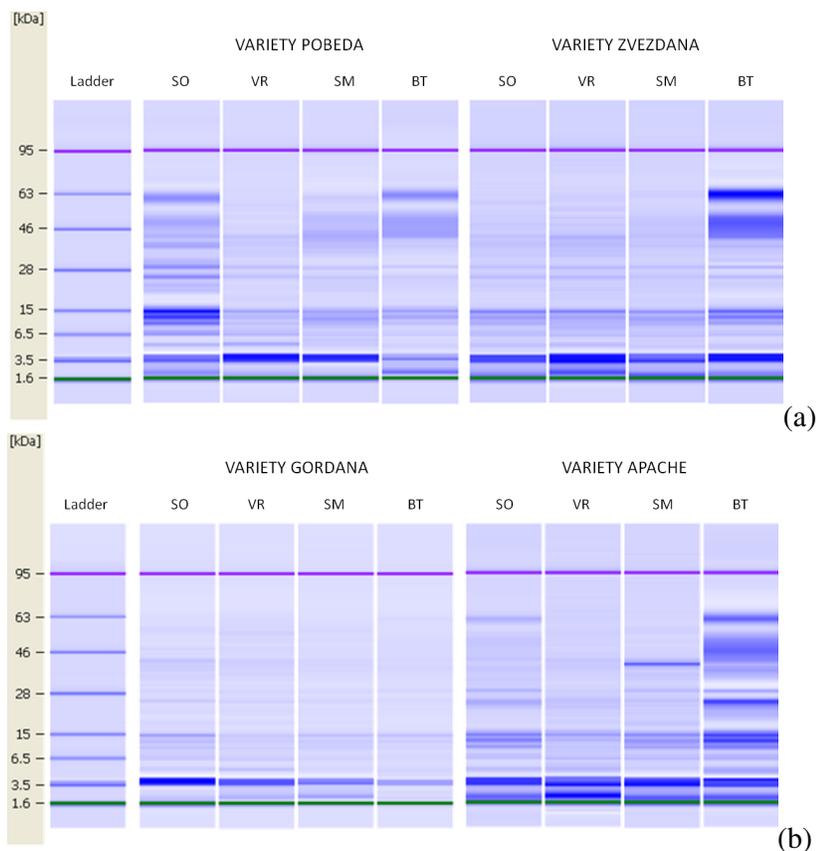


Figure 1. Loac gel images of albumins from four different wheat varieties (a and b).

solely influenced by variety but also by environmental factors.

In Table 1, the data of the albumin content and number of bands obtained by the LoAC method in the examined wheat flours are presented.

Evaluation of the protein fractions content of wheat flour, including albumins, has been conditioned with method, wheat variety, and growing conditions. However, the proportion of albumins has been influenced by flour protein content (DuPont *et al.*, 2005). The protein content of the tested flour samples was in the range from 7.0 to 11.2% (based on 13% moisture content). There are also indications about the presence of albumin in the gluten phase after the removal of water-extractable proteins trapped in the gluten network, which also contributes to the complexity of the separation and quantification of albumins (Kuktaite *et al.*, 2003). HMW-albumins tend to form polymers between themselves and certain HMW-albumins are considered as β -amylases which can form disulfide bonds with LMW-glutenins (Gianibelli *et al.*,

2001).

In the tested samples of wheat flour, the content of albumin fractions ranged from 2.64 to 17.50% of total proteins (i.e. 0.29 to 2.39% of flour) (based on 13% moisture content). Similar results were obtained by Chiang *et al.* (2006) who found that content of albumin and globulin fractions was in the range of 1.1 to 2.1% of flour. On the other hand, Žilić *et al.* (2011) reported that content of albumin and globulin fractions, as percentage of total protein for bread and durum wheat samples, was about 38%. It has to be taken into account that there are a few studies associated with the content of total flour protein composition and they are mainly based on the determination of albumin and globulin fractions together. Data in Table 1 indicate that the content of albumins obtained by Lab-on-a-Chip electrophoresis, was almost significantly different ($P < 0.05$) for all the tested flour samples regardless of varieties and growing locations. An exception was the variety Gordana, which had a balanced proportion of albumin fractions in all tested locations. The statistical analysis

Table 1. The content of albumins and number of albumin bands of different wheat varieties.^a

Sample	Albumins (g 100 g ⁻¹ flour)	Total proteins (g 100 g ⁻¹ flour)	Number of bands
SO-Pob	2.01 ^{ab}	10.40 ^e	17 ^d
VR-Pob	0.49 ^{fgh}	11.22 ^a	29 ^a
SM-Pob	0.72 ^{efg}	10.96 ^b	23 ^b
BT-Pob	2.39 ^a	10.22 ^f	21 ^{bc}
SO-Zve	0.67 ^{fgh}	9.35 ^h	24 ^b
VR-Zve	0.39 ^{gh}	10.83 ^c	30 ^a
SM-Zve	1.07 ^{cde}	10.79 ^c	24 ^b
BT-Zve	1.23 ^c	10.22 ^f	19 ^{cd}
SO-Gord	0.69 ^{efg}	11.18 ^a	30 ^a
VR-Gord	0.56 ^{fgh}	10.61 ^d	28 ^a
SM-Gord	0.76 ^{defg}	11.18 ^a	30 ^a
BT-Gord	0.79 ^{def}	10.88 ^{bc}	30 ^a
SO-Ap	0.79 ^{def}	7.00 ^j	19 ^{cd}
VR-Ap	0.29 ^h	9.14 ⁱ	31 ^a
SM-Ap	1.13 ^{cd}	9.35 ^h	22 ^{bc}
BT-Ap	1.72 ^b	9.79 ^g	31 ^a

^a Means followed by different letters within the same column are significantly different ($P < 0.05$).



confirmed the visual perception of gel images (Figures 1-a and -b).

In order to find the correlation between albumins and rheological properties as well as enzymatic activity, the total albumins were categorized into four intervals according to their molecular weights: 5–15; 15–30; 30–50, 50–65 kDa. The intervals were selected on the basis of the frequency of specific molecular weights and their quantities in 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.

Figure 2 shows the results of average proportion of certain albumin fractions in the tested flour samples. Fraction in the range of 5-15 kDa, represented more than half of the total amount of albumins (57.13%). Some authors have indicated the existence of α -amylase inhibitors within this range (Singh and Skerritt, 2001; DuPont *et al.*, 2005). The lowest content of albumin subunits was in the range of molecular weight from 50 to 65 kDa.

Analysis of Dough Rheological Properties of Different Wheat Flours

In order to illustrate the variability of chosen sample set, descriptive statistic was performed and results are given in Table 2.

The parameters of empirical rheological methods are commonly used to determine

the optimal flour quality for a particular purpose.

According to the obtained results (Table 2), it could be noticed that values of tested parameters showed wide variability. Water absorption (WA) value presents the amount of water needed by flour to provide dough of optimal viscoelastic characteristics suitable for dough consistency and processing (Peymanpour *et al.*, 2012). WA values were in the range of 53.63–62.73%, showing wide variability in terms of protein water absorption ability. The lowest value of WA belonged to the variety Apache, while the other two varieties had almost the same level of this parameter.

Dough development time (DDT) is the time from the first addition of water to the time when dough reaches the point of maximum consistency (500 BU). During this time, the water hydrates the flour components and the dough is developed. DDT was in the range of 1.5–13.0 min which indicated that tested flour samples had different levels of gluten structure compactness.

Gordana and Pobeda showed the highest dough strength properties based on Farinograph results. Both Farinograph DDT (average values: 10.1 and 7.6 minutes, respectively) and stability (Stab) (average values: 16.5 and 12.1 minutes, respectively)

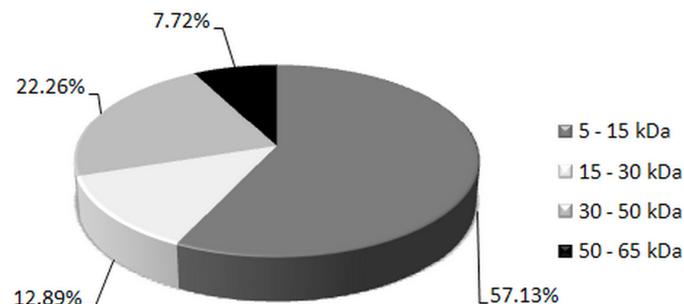


Figure 2. Pie chart of the four MW intervals of albumins. The numbers and percentages of each protein group are indicated.

for these varieties were extremely high, which implied that for the development of gluten complex, these flours required more energy and time. However, these flours could be kneaded for a long time without deterioration of the initial consistency. Moreover, these varieties expressed the lowest values of softening degree indicating stable cohesiveness of dough during kneading.

Based on the measured values of uni- and biaxial extension properties of wheat dough, the samples were grouped in a different way. Regarding Extensograph data, some variations were observed in both extensibility (Ex) and resistance (R). The higher variations in both parameters were observed between different locations.

Regarding the biaxial elasticity, Apache variety expressed the highest values of resistance to extension (P) and extensibility (L), while varieties Gordana and Pobeda had the maximum values of Alveograph deformation energy (W). Varieties Gordana and Pobeda had the same level of Amylograph peak viscosity (PV) which classified them as wheat suitable for processing in baking industry (Đaković, 1997). Characterization of the rheological behaviour of dough showed wide variability among tested samples for all Mixolab quality parameters. Variability among samples was also indicated by standard deviation values. Water absorption (WAMix) values were in the range of 49.60–60.00%. Dough development time (DevMix) and dough stability (StabMix), which indicate strength of protein (Karaoğlu, 2011) were in the range of 0.92–8.62 and 4.72–11.58 minutes, respectively. Apache differed from the other varieties with low values of WAMix and DevMix, being in agreement with Farinograph results. Moreover, Apache expressed higher value of β slope which refers to gelatinization rate.

Measured gluten index (GI) values were in the range of 81.00–98.65%, showing different gluten strength from strong to very strong gluten (AbuHammad *et al.*, 2012). The gluten index values obtained after

previous incubation of piece of dough at 37°C [GI (37°C)] were lower and within the range of 22.84–91.24%, which could be the consequence of activity of potentially present proteolytic enzymes that caused partial degradation of gluten (Rosell *et al.*, 2002).

The values of proteolytic and amylolytic activity showed wide variability, indicating that these values were highly influenced by the variety, growing location, and their interaction. Over 80% of the tested samples had a peak viscosity values greater than 400 BU, which means that they had optimal or less than optimal values of amylolytic activity, while the proteolytic activity could be indirectly estimated on the basis of gluten index values at 37° C. Obtained values of proteolytic activity showed that variety had strong influence. In particular, the variety *Ap* was clearly separated by higher values of proteolytic activity. Regarding the growing localities, values of proteolytic activity showed statistically significant differences (data not shown).

Relationship between Flour Protein Composition and Wheat Flour Quality Indicators

In order to observe the relationship between the particular albumin fractions, rheological properties and enzymatic status of tested flours, the PCA was performed (Figures 3-a and -b). The first phase of the statistical analysis was to find correlation among albumin fractions and enzymatic status of flour. Obtained results showed that only albumin fractions of 15-30 kDa were correlated with the proteolytic activity (PA) ($P < 0.05$) and α -amylolytic activity (AMYL) ($P > 0.05$), although not statistically significant (data not shown).

Therefore, in further statistical analysis, only the content of 15-30 kDa albumin fraction was considered for the establishment of relations with dough rheological properties in order to define their

This albumin fraction (15-30 kDa) showed lower correlation with the quality parameter of starch component of flour (C4) measured by Mixolab. This result indicated that albumin fraction of 15-30 kDa molecular weight possibly represented mainly the proteolytic enzyme complex, which was confirmed by statistically significant correlation coefficient ($r= 0.75$, $P< 0.05$), in contrast to statistically insignificant correlation coefficient with α -amylolytic activity ($r= 0.42$, $P> 0.05$). Total proteolytic activity values showed moderate correlation with the same rheological parameters as the fraction 15-30 kDa (WA and WAmix, $r= -0.66$ and $r= -0.60$, $P< 0.05$, respectively) and resistance to extension of dough which was measured uniaxial (R/Ex, $r= -0.56$, $P< 0.05$) and biaxial (P and P/L, $r= -0.68$ and $r= -0.55$, $P< 0.05$, respectively). Taking into account enzymatic nature of albumins (Bleukx *et al.*, 1997), flour samples showed considerable decrease of water absorption ability (for which high molecular proteins were responsible) and decrease of extensibility and resistance measured at both axes (due to the cleavage of intra and intermolecular bonds of gluten proteins), which could be attributed to albumins' influence. It could be the consequence of proteolysis which led to degradation of macromolecules.

In contrast to the proteolytic activity, the α -amylolytic activity values showed a statistically significant correlation only with rheological parameters L , G , P/L ($r= 0.70$; $r= 0.71$, and $r= -0.61$, $P< 0.05$, respectively) measured by Alveograph. Albumins (mainly β -amylase) include proteins that occur as polymers stabilized by interchain disulphide bonds (Veraverbeke and Delcour, 2002). It could be assumed that α -amylolytic enzymes act coupled with proteolytic enzymes in the bonds cleavage within the starch and gluten network, consequently, increasing the dough extensibility.

Statistical analysis of the obtained results showed that amount of 5-15 and 50-65 kDa albumin fractions were moderately correlated ($r= -0.52$ and $r=0.55$, $P< 0.05$,

respectively) with starch quality parameter of dough (C3-C4), which indicated the stability of the starch gel when heated (data not shown). The moderate correlation coefficient between albumin fraction of 5-15 kDa and C3-C4 might have indicated the amylase inhibitory activity which, according to many authors, related to this range of molecular weights (Singh *et al.*, 2001; Gao *et al.*, 2009). It was not possible to find stronger relationship between 5-15 kDa albumin fraction and α -amylolytic activity, reflecting the fact that climatic conditions for the studied growing season were unfavorable for high amylolytic activity.

In the corresponding score plot presented as Figure 3-b, the clear distinction between varieties is noticeable. Evidently, Serbian varieties were grouped together on the opposite side of the score plot in relation to the French variety Apache. The corresponding score plot (Figure 3-b) shows clustering of varieties, which might indicate that the variety had the dominant role in enzymatic status of wheat flour in the studied growing season.

CONCLUSIONS

The results obtained in this study showed that the contents of albumin fractions of the tested wheat flour samples were in a wide range depending on their variety characteristic as well as growing locations. Wide variability was also observed in the number of bands corresponding to molecular weights from 5 to 65 kDa. More than 50% of the total amount of albumins was in the range of 5-15 kDa of molecular weight.

The Principal Component Analysis showed that albumin fraction 15-30 kDa might have influence on rheological properties of dough, especially those related to water absorption and uniaxial and biaxial resistance to extension.

Regarding the enzymatic status of flour, no strong relation was underlined among the 5-15, 30-50 and 30-60 kDa albumin fractions and proteolytic and α -amylolytic



activity. On the other hand, albumin fraction 15-30 kDa showed significant correlation with proteolytic activity and the highest correlation, although not statistically significant, with α -amylolytic activity.

Despite the indications of connection among the 5-15 and 30-60 kDa albumin fractions and the amylase inhibitors and α -amylolytic enzymes, the results of this study did not prove a causal relationship. Therefore, further investigations should be conducted covering wider range of wheat varieties in different growing seasons.

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ویژگی های آلبومین در رابطه با خواص خمیره شناسی و فعالیت آنزیم های خمیر آرد گندم

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چکیده

هدف این پژوهش شناخت ویژگی های اجزای آلبومین (albumin fractions) در چهار رقم گندم در یک محیط آب و هوایی محلی و نیز یافتن رابطه بین جزء خاصی از آلبومین با فعالیت آنزیمی آرد خمیر های آزمون شده و خواص خمیره شناسی آنها بود. محتوای اندازه گیری شده اجزای آلبومین در محدوده ۲/۶۴٪ تا ۱۷/۵٪ کل پروتئین ها بود. وزن ملکولی بیش از ۵۰٪ کل آلبومین ها در محدوده ۵-۱۵ kDa بود. نتایج به دست آمده گواه آن بود که جزء ۱۵-۳۰ kDa آلبومین با برخی از خواص معین خمیره شناسی خمیرها، به ویژه آنهایی که مربوط به جذب آب و مقاومت به گسترش تک محوری و دو محوری می شد، ارتباط داشت. در ارتباط با وضعیت آنزیمی آردها، هیچ رابطه قوی بین اجزای ۵-۱۵kDa، ۳۰-۵۰ kDa و ۳۰-۶۰ kDa آلبومین و فعالیت های پروتئولیتیک (proteolytic) و α -amylolytic آنزیم ها به دست نیامد. با این وجود، جزء ۱۵-۳۰ kDa آلبومین همبستگی معنی داری با فعالیت های پروتئولیتیک نشان داد.