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WHEAT PROTEIN FRACTION TO GRAIN QUALITY CHARACTERISTICS OF SOFT ALBANIAN WHEAT

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Abstract

The classical Osborne wheat protein fraction (albumins, globulins, gliadins, glutenins), modified according Maes and chemical - technological characteristics, were determined in ten lines of soft wheat, collected during the summer season of 2014. The solvent, used during the process of extracting proteins from wheat flour, consisted of distilled water, 40% isopropyl alcohol, 3.85% lactic acid and 0.5% potassium hydroxide. Wheat lines investigated in the experiment was carried out as a randomized block design in 3 m^2 plots with three replicates. The flour obtained from those lines of wheat has the following quality: protein: 15.05%, Zeleny Sedimentation: 47 ml and gluten index (GI): 53.95%. The protein extraction with this procedure was efficient and provided 90-99% protein recovery.

Key words*: soft lines, quality parameters, protein fraction, gluten index.*

INTRODUCTION

Among the large assortment of food products from wheat, the bread is still the most essential food product from wheat. For this reason, the good quality is important and thus, the studies of bread making quality are of a fundamental nature. The quality of wheat could be affected by several variables, including: physical grain properties, protein content and composition, starch and lipid content. Among the quality characteristics, the baking quality of wheat flour is primarily dependent on the quantity and quality of flour protein (Finney, 1987; Dowell et al., 2008). As most of the proteins in flours are gluten and gluten forming proteins play key roles in baking quality of wheat by improving the water absorption capacity, extensibility and elasticity of the dough. Total protein content, as well as protein quality, as measured by Zeleny sedimentation (K-SDS), wet gluten (WG) and Gluten Index (GI), are influenced by environmental and genetical factors (Curic et al., 2001; Dowell et al., 2008; Šekularac et al., 2018). Wet gluten content is determined by washing the dough obtained from flour, with water or other solutions (e.g. NaCl solution), in certain conditions, to remove the starch and other soluble compounds of the sample (Mis, 2000). The rubbery mass that remains after washing is the wet gluten. The gluten proteins, the gliadins and glutenins, constitute up to 80-85% of the total flour protein (Shewry et al., 1995). The gliadins and glutenins constitute each around 50% of the gluten proteins. In 1907,Osborne classified wheat protein according to the basics of solubility: albumins (soluble in water), globulins (aqueous solution of salt), gliadins (aqueous ethanol) and glutenins (dilute acid or alkali). Water-soluble albumins and salt-soluble globulins constitute anywhere from 10% to 22% of the total flour protein (Singh and MacRitchie, 2001). The gliadins constitute anywhere from 30% to 40% of total flour proteins and are divided into four groups, alpha- (α-), beta- (ß-), gamma- (γ-), and omega- (ω-) gliadins, based on their electrophoretic mobility at a low pH (Woychik et al., 1961; Kasarda et al., 1983). Maes (1962) presented a novel approach of fractionating of flour protein based on solubility. The new solvent sequence consisted of: distilled water,

40% isopropyl alcohol, 3.85% lactic acid and 0.5% KOH.

The goals of the study are as follows: (i) to evaluate the wheat protein fraction according Maes (albumin, globulin, gliadins and glutenins) in ten lines of soft wheat and (ii) to determine the content of protein, wet gluten, gluten index and K-SDS as quality parameters of wheat flour.

MATERIALS AND METHODS

Ten lines of soft wheat (L 1, L 2, L 3, L 4, L 5, L 6, L 7, L 8, L 9, L 10) were grown during the year 2013-2014 in the Experimental Didactics Economy (E.D.E) of the Agricultural University of Tirana (latitude 41°19'39"N, longitude 19°49'08"E; average altitude 89 m). Each plot was planted in five rows; the plot size being 5 m x 1.2 m. The protein content (% N x 5.7) was determined by the Kjedahl method (AOAC, Method 979, 09), and lipid content was analysed based on the Soxhlet extraction method utilizing n-hexane (AOAC, Method 4.5.01). The Wet Gluten and Gluten Index values for all flours samples were determined using the Glutamatic system (Petern Instrument AB, Stockholm, Sweden) with the use of the AACC method 38-12.02. Sedimentation value (K-SDS) was determined according to Zeleny (Zeleny, 1947). The fraction of wheat protein was based on solubility using the continuous extraction procedure proposed by Maes (1966) and applied with some modification by Mattern et al. (1968) and Williams and Butler (1970). Solvent sequence consisted of: distilled water, 40% isopropyl alcohol, 3.85% lactic acid and 0.5% KOH. All chemical analyses were performed in three replications and the results were statistically analysed. Pearson's correlation and the analysis of variance (ANOVA) between the obtained results were performed using StatSoftStatistica 10.0 software.

RESULTS AND DISCUSSIONS

Data in Table 1 indicates that the content of total protein was significantly higher in ten lines of wheat, which varied from 13.31% (line 8) to 17.34% (line 4). In wheat, the bread - making characteristics of dough is strongly influenced by protein content and protein quality (Johansson and Svensson, 1999).

Table 1.Chemical parameters in ten lines of wheat

Lines	Protein (%)	IG (%)	K-SDS (ml)
1	14.93±0.16^{ab}	90.80±0.14^{h}	49.50±0.71^{b}
2	15.07±0.06^{ab}	53.65±0.21^{c}	47.50±0.71^{b}
3	14.90±0.11^{ab}	59.85±0.21^{f}	48.00±0.00^{b}
4	17.34±0.03^{b}	19.60±0.14^{a}	42.50±0.71^{a}
5	15.07±0.04^{ab}	92.20±0.28^{i}	51.00±1.41^{b}
6	14.93±0.06^{ab}	25.70±0.00^{b}	51.00±1.41^{b}
7	15.68±2.07^{ab}	42.25±0.21^{d}	47.50±0.71^{b}
8	13.31±0.07^{a}	34.35±0.21^{c}	42.50±0.71^{a}
9	14.41±0.02^{a}	42.80±0.14^{d}	41.00±1.41^{a}
10	13.45±0.06^{a}	78.75±0.21^{g}	49.00±1.41^{b}

*The results are expressed as mean ± SD values, followed by different letters in the same column that are significantly different ($p < 0.05$), according to Tukey's HSD test.

However, high value of grain protein content does not reliable indicator for good bread making quality (Zhu and Khan, 2002), while gluten proteins (content and composition of gliadin and glutenins) are associated with viscoelastic properties of dough and with bread making quality (Shewry and Halford, 2002; Bekes et al., 2004; Delcour et al., 2012). Gluten is the rubbery mass that is left when wheat flour is washed with water to remove starch. Gluten structure has a major importance on dough rheological properties (Pedersen and Jorgensen, 2007). Guten proteins (gliadin and glutenins) are responsible for viscoelastic characteristics of dough and bakery products (Singh and Singh, 2013; Wang et al., 2015). Gluten Index (GI) is a method of analysing wheat protein that provides necessary information for gluten quality and quantity (AACC, 2000). The Gluten Index (GI) value was the highest in Line-1 (92.20%) and the lowest in Line 4 (19.60%). In this study the GI range between 19.60% (L-4) and 92.20% in L-5. According to scale of Oikonomou et al. (2015) we can classify analyzed wheat line in three groups. First group had strong gluten with GI over the 80% (L-1 with GI = 90.80% and L-5 with GI = 92.20%), second group of line L-2, L-3, L-7, L-8, L-9 and L-10 which have normal gluten which gluten index was between 30% and 80% and third group of wheat with weak gluten which GI is lower than 30% L-4 with GI = 19.60% and L-6 with GI = 25.70%) (Table 1). The line 10 with GI = 78.75% can classify in the group with strong gluten if we consider criteria of to Ćurić et al. (2001) who are reported that the optimum

value of gluten index is between 75% and 90%. The wheat line (L 1, L 5 and L 10) had high protein content (14.93%, 15.07%, 13.45%) and an optimum level of gluten index (90.80%, 92.20%, 78.75%), belongs the group of wheat which can provide optimum bread making quality (Table 1). According to Šekuralec et al., (2018), in two years of experimental study values of gluten Index (GI) were higher than 80% for all six wheat varieties in both years and GI varied from the lowest 80.50% in Zvezdana to the highest 96.50% in NS 40S. Zeleny sedimentation (K-SDS) is another test used to determine protein quality. In this study K-SDS value varies from 41 ml (line 9) to 51 ml (lines 5, 6). The significant differences among the investigated parameters were established for protein content and GI.Similar data of significat differences for gluten index (GI) among wheat cultivars were found in investigation of Šekularac et al. (2018). The protein composition is strongly influenced by the genetic background (Ćurić et al., 2001).

Because grains were collected from plants grown under equal conditions in the field at the same location during the same growing season, the influence of environmental factors can be ignored. The fraction of wheat protein was based on solubility using the continuous extraction procedure proposed by Maes (1962). Solubility fractions of wheat are presented in Table 2. Data are expressed as the percentage of total protein (protein solubility index - PSI). Compared to the Osborne method, this method investigates the whole spectrum of wheat proteins. The following fractions were understood to be present; in water, there are albumin extractions, in isopropyl alcohol 40% of the fractions are globulins extractions, in lactic acid 3.85% are gliadins extractions and in KOH 0.5% are glutenin extractions. The protein fraction content soluble in water (albumin) and protein fraction content soluble in isopropyl alcohol 40% (globulin) ranged from 18.10% (line 7) to 22.68% (line 3); 10.26% (line 4) to 17.89% (line 5), respectively. According to Stehno et al., (2008), albumin - globulin constitute from 22.29% to 30.81%, in soft wheat. Similar mean values of albumin contents (23.12 g kg^{-1}) for 15 analyzed bread wheat found Branković et al. (2015). In most of the lines of wheat samples it was found that the protein fraction content soluble in lactic acid 3.85% (gliadin) was lower and ranged from 23.30% (line 4) to 42.16% (line 8). Although the albumin and globulin fractions are not known to play a direct role in bread making, they may be necessary for normal baking properties (Peruffo et al., 1996).

Table 2.The content of protein fraction (%) in 10 lines of wheat

Lines	H_2O	Isopropyl alcohol 40%	Lactic acid 3.85%	KOH 0.5%
1	21.81±0.08^{f}	13.49±0.04^{f}	30.66±0.04^{f}	18.37±0.08^{e}
2	20.23±0.06^{d}	15.67±0.07^{h}	26.88±0.03^{c}	20.12±0.06^{c}
3	22.68±0.17^{g}	17.82±0.01^{a}	27.55±0.21^{d}	16.18±0.01^{d}
4	19.50±0.07^{c}	10.26±0.08^{b}	23.30±0.02^{a}	14.98±0.07^{a}
5	19.79±0.08^{c}	17.89±0.06^{a}	25.86±0.04^{b}	19.61±0.11^{b}
6	18.99±0.01^{b}	17.45±0.05^{i}	28.41±0.04^{c}	19.96±0.03^{c}
7	18.10±0.06^{a}	12.38±0.03^{d}	38.45±0.01^{h}	22.89±0.11^{f}
8	20.71±0.11^{c}	15.05±0.01^{g}	42.16±0.03^{j}	19.46±0.01^{g}
9	20.60±0.01^{c}	13.09±0.01^{e}	38.13±0.02^{g}	25.25±0.18^{b}
10	20.90±0.08^{c}	11.65±0.13^{c}	40.16±0.03^{i}	14.85±0.11^{a}

*The results are expressed as mean ± SD values, followed by different letters in the same column that are significantly different ($p < 0.05$), according to Tukey's HSD test.

A higher variation of gliadins was found in ten lines of wheat samples. The protein fraction content soluble in KOH 0.5% (glutenin) ranged from 14.85% (line 10) to 25.25% (line 9). Gliadins and glutenins are recognized as a major wheat storage protein. Glutenin affects the elastic properties of dough where as gliadins affect the viscous properties of dough. Pearson correlation coefficients were calculated between flour quality characteristics and protein fraction.

Table 3. Correlation between chemical parameters and protein fraction content

	Lipids	IG	K-SDS	H_2O	Isop. alc	Lactic ac.	KOH
Protein	-0.071	-0.297	-0.079	-0.369	-0.237	-0.649^{+}	-0.120
IG			0.562*	0.459*	0.209	-0.031	-0.172
K-SDS				-0.028	0.491*	-0.317	-0.229
H_2O					0.209	-0.010	-0.381**
Isop. alc						-0.322	0.119
Lactic ac.							0.340

$^{+}$Correlation is statistically significant at $p < 0.01$ level; *correlation is statistically significant at $p < 0.05$ level; **correlation is statistically significant at $p < 0.01$ level.

Table 3, indicates a significant positive linear correlation between gluten index (GI) and Zeleny sedimentation (K-SDS) (r = 0.592). It was not possible to find a correlation between the variation of the protein fraction and quality characteristics. The only correlation of practical interest found, was between K-SDS and isopropyl alcohol fraction (r = 0.491). According to the previous study conducted by Maes (1966), it was not possible to confirm either the positive correlation between K-SDS and alcohol soluble fraction or the negative correlation between protein content and the lactic acid fraction. This is because the flour quality characteristics, in most cases, are strongly influenced by recessive or incompletely dominant genes (Fortini et al., 1976; Weegels et al., 1996).

CONCLUSIONS

From the results of this research study, all the lines of soft wheat are characterized with high protein content and good bread making characteristics. There were significant variations in means of soluble protein fraction between ten lines of wheat. The correlation between wheat protein fraction and quality parameters, characterizes the flour and the quality of finished the product, and shows a positive correlation between GI– K-SDS (r = 0.592) and K-SDS - isopropyl alcohol fraction (r = 0.491). It was not possible to find a lot of correlations between the variation of protein fraction and quality characteristics due to the fact that the quality characteristics are influenced by genetic background. The protein extraction with this procedure was efficient and provided 90-99% recovery of proteins.

REFERENCES

AACC International (2000). Approved Methods of the American Association of Cereal Chemists. 10th Ed. [Method 38-12.02] and [46-30.01], Latest edition (Dumas) The Association: St. Paul, MN.

AOAC (1995). Official Methods of Analysis of AOAC International Method., No. 08-01 and No. 44-10.

Branković, G., Dragičević, V., Dodig, D., Knežević, D., Kobiljski, B., Šurlan-Momirović, G. (2015). Albumin content in bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* Desf.) as affected by the environment. *Zemdirbyste-Agriculture*, *102*(3), 281–288.

Bekes, F., Gianibelli, M.C., Wrigley, C. (2004). Grain proteins and flour quality. *Australian Journal of Agriculture Research*, 416–423.

Ćurić, D., Karlović, D., Tušak, D., Petrović, B., Dugum, J. (2001). Gluten as a standard of wheat flour quality. *Food Technol. Biotechnol*., *39*, 353–361.

Delcour, Ja., Joye, I.J., Pareyt. B., Wilderjans, E., Brijs, K., Lagrain, B. (2012). Wheat gluten functionality as a quality determinant in cereal-based food products. *Annual Review of Food Science and Technology*, *3*, 469–92.

Dowell, F.E., Maghirang, E. B., Pierce, R.O., Lookhart, G.L., Bean, S.R., Xie, F., Caley, M.S., Wilson, J.D., Seabourn, B.W., Ram, M.S., Park, S.H., Chung, O.K. (2008). Relationship of bread quality to kernel, flour and dough properties. *Cereal Chemistry, 85*, 213–236.

Finney, K.F., Yamazaki, W.T., Youngs, V.L., Rubenthaler, G.L. (1987). Quality of hard, soft and durum wheats. *In Wheat and wheat improvement, Agronomy Monograph13* (ASA-CSSA-SSSA, Madison), 677–748 pp.

Fortini, S., Galetrio, G., Zitelli, G., Borghi, B. (1975). Protein fraction in crosses involving lines differing in bread making quality and protein content. *Proceeding of the International winter wheat Conferenc*e, June 9-19, Zagreb, Yugoslavia.

Johansson, E, Svensson, G. (1999). Variation in bread making quality: effect of weather parameters on protein concentration and quality in some Swedish wheat cultivars grown during the period 1975-1996. *J.Sci. Food Agric*., *78*. 109–118.

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

Maes, E. Mehleweissloslichkeit und Backqualitat.(1962). *III. Continuierliche, progressive Extraction*. GeteideMehl, *6*(12), 70–72.

Maes, E.A. (1966). Protein Solubility and baking quality. *Cereal Science Today, 11*, 200–202.

Mattern, P.J., Salem, Ali, Volcmer, G.H. (1968). Modification of Maes continues – extraction process for fraction of hard red winter wheat protein. *Cereal Chemistry*, *45*, 319–328.

Method (a laboratory note), *International Agrophysics* (2000), 14(2), 263–267.

Mis, A. (2000). Some methodological aspects of determining wet gluten quality by the Glutomatic.

Oikonomou, N.A., Bakalis, S., Rahman, M.S., Krokida, M.K. (2015). Gluten Index for Wheat Products: Main Variables in Affecting the Value and Nonlinear Regression Model. *International Journal of Food Properties*, *18*, 1–11.

Osborne, T.B. (1907). The proteins of the wheat kernel. Washington: Carnegie Institution of Washington. p 1–136.

Peruffo, A.D.B, Pogna, N.E, Curioni, C. (1996). Evidence for the Presence of Disulfide Bonds betweenBeta-Amylase and Low Molecular Weight Glutenin Subunits. In *Proceedings of 6th International Gluten Workshop "Gluten 96"*, Sydney, Australia, 2-4 September.

Pedersen, L, Jorgensen, JR. (2007). Variation in rheological properties of gluten from three biscuit wheat cultivars in relations to nitrogen fertilization.*Journal of Cereal Science*, *46*, 132–138.

Šekularac, A., Torbica, A., Živančev, D.,Tomić, J., Knežević, D. (2018). The influence of wheat genotype and environmental factors on gluten index and the possibility of its use as bread quality predictor. *Genetika*, *50*(1), 85–93.

Singh, H., MacRitchie, F. (2001). Application of polymer science to properties of gluten. *Journal of Cereal Science*, *33*, 231–243.

Singh, S, Singh, N. (2013). Relationship of polymeric proteins and empirical dough rheology with dynamic rheology of dough and gluten from different wheat varieties. *Food Hydrocoll*, *33*, 342–348.

Shewry, P.R., Napier, J.A., Tatham, A.S. (1995). Seed storage proteins: structures and biosynthesis. *The Plant Cell*, *7*, 945–956.

Shewry, P.R., Halford, N.G. (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. *Journal of Experimental Botany*, *53*, 947–958.

Stehno, Z., Dvoracek, V., Dotlacil, L. (2008). Wheat protein fractions in relation to grain quality characters of the cultivars. In *Proceedings of 11th International Wheat Genetics Symposium*, *2*, 556.

Wang, P., Jin, Z., Xu, X.(2015). Physicochemical alterations of wheat gluten proteins upon dough formation and frozen storage - a review from gluten, glutenin and gliadin perspectives. Trends *Food Science of Technology*, *46*, 189–198.

Weegels, P.L., Van de Pijpekamp. A.M., Graveland, A., Hamer, R.J., Schofield, J.D., (1996). Depolymerisation and repolymerisation of wheat gluten during dough processing I. Relationships between GMP content and quality parameters. *Journal of Cereal Science*, *23*, 103–111.

Williams, P.C., Butler, C. (1970). Characterization of wheat flour proteins by differential solubility in conjunction with disc – electrophoresis. *Cereal Chemistry*, *47*, 626–639.

Woychnik, J. H., Boundy, J., Dimler, R.J. (1961). Starch gel electrophoresis of wheat gluten proteins wit concentrated urea. *Archives of Biochemistry and Biophysics*, *94*, 477–482.

Zeleny, L.A. (1947). Simple sedimentation test for estimating the bread – baking and gluten qualities of wheat flour. *Cereal Chememistry, 24*. 465–475.

Zhu, J, Khan, K. (2002). Quantitative variation of HMW glutenin subunits from hard red spring wheats grown in different environments. *Cereal Chemistry, 79*, 783–6.