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PROTEIN COMPLEX OF WHEAT, BUCKWHEAT AND MAIZE IN RELATION TO CELIAC DISEASE

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ABSTRACT

Cereals are the most wide spread and very important plants utilized as a food source for mankind and for animals where they play role in energetical metabolism and proteosynthesis. Cereals contain proteins with unique properties. These properties allow us to produce leavened bread. Technological characteristic of cereal grain is determined by quantity and quality of storage proteins which represent alcohol soluble prolamins and glutenins soluble in acids and basis solutions. Celiac disease is one of the most frequent food intolerance caused by cereal storage proteins. Therapy consists of strict diet without consumptions of cereals or gluten. Pseudocereals are very perspective groups of plants in gluten free diet, due to absence of celiac active proteins, but on the other hand, flour from pseudocereals is not very suitable for baking. There are a lot of analytical methods applicable for detection of celiac active proteins in cereal and pseudocereal grain. Electrophoretical and immunochemical methods are the most utilized. Genotypes of wheat and maize were homogeneous and singlelined in contrast with genotypes of buckwheat. Average content of HMW-GS was highest in genotypes of bread wheat and lowest in buckwheat varieties. A celiac active fraction of storage proteins (LMW-GS and gliadins) was detected at the highest content level in wheat genotypes. Genotypes of buckwheat and maize showed similar low content of this protein fraction. Presence of residual albumins and globulins in buckwheat varieties showed the highest value.

Keywords: Buckwheat, celiac disease, maize, proteins, wheat

INTRODUCTION

Cereals are basic commodity of production and trade. They are main part of human nutrition and strategic resource. Cereals grains are one of the most important sources of energy and nutrients in human and animal being. Mainly, wheat and bread are essential to human life and are the basic food in many countries **(Shewry, 2004/Rev. 2006)**.
The most important impacts on the nutritional quality and functional properties of grains have storage proteins. Amount of storage proteins is about 50% or more of the total proteins in wheat and barley mature grains. **(Shewry and Halford, 2002)**.
Prolamins constitute about 50% of gluten which is defined as the water-insoluble protein fraction of flour from wheat, barley, rye, oats and their crossbred varieties. The prolamins of wheat, barley, rye and oats are called gliadins, hordeins, secalins and avenins respectively. The gluten fraction isolated from wheat flours exhibits an enormous complexity due to the broad genetic polymorphism of several classes of these proteins. As gliadins are the proteins mainly involved in the pathophysiology of coeliac disease and they are also recognized to be major allergens in wheat allergy, they represent good markers to measure gluten traces both with respect to wheat allergy and coeliac disease. Quantification of gliadin levels could therefore provide an appropriate tool for defining an acceptable limit for gluten-free food (**Hischenhuber *et al.*,2006)** .
Parnell et al. (1999), **Fasano (2001)**, **Wieser (2008)**, **Pekárková *et al.* (2009)** defined celiac disease as a disaster caused by high sensitivity of individuals on presence of prolamins. Celiac disease is gluten intolerance. Gluten consists of prolamins and glutenins naturally occurring in wheat, barley and rye (**Petr, *et al.*, 2003**).
World prevalence of celiac disease is more than 1 % of population. Disease occurrence in Europe ranged from 1 : 100 to 1 : 500 with the highest incidence in England and the lowest in Germany and Sweden (**Rimárová, *et al.*,2008**).
Lifelong no gluten diet have been established as the most effective treatment. Patients have to exclude cereal products which contain wheat, barley, rye, triticale or oat flour (**Pruska-Kędzior *et al.*, 2008**).
There is opportunity to substitute these prohibited cereals by products made from maize, rice, soybean, potato, buckwheat, amaranth, fruit, vegetables, eggs or meat (**Pekárková *et al.*, 2009**). Recently, have been attention focused on pseudocereals, due to low content of prolamin fraction and high content of proteins with better aminoacids compositions, lipids, starch and minerals (**Zigone *et al.*, 2010**).
Janesová (2011) presents buckwheat like dietary food utilized in nutrition. Bakery products (buckwheat bread), buckwheat pastas, cakes, cheerios, soups or buckwheat beverage can be made from buckwheat (**Janovská *et al.*, 2008; Pospišil *et al.*, 2012**).
Maize grain contains the highest content of starch and therefore is characterized as the most energetical grain of all cereals, but protein and minerals content is not as high as in other cereals. Yellow colored grain genotypes have higher content of β-caroten, xantophyls and zeaxantins as well as significant volume of tiamin, riboflavin and pyridoxine (**Maize in Human Nutrition, 2009**).
The objective of our study was to evaluate utilization of buckwheat and maize as alternative plants for celiac diet.

MATERIAL AND METHODS

Material

We have analyzed:
10 genotypes of bread wheat (*Triticum aestivum* L.): Rupert, Federer, Leguan, Pepino, SU-177, Natanael, Granny, JB Asano, IS Jarissa, Sophytra
12 genotypes of buckwheat (*Fagopyrum esculentum* L.): Východ, Darja semenarna, Orebovico, Kostanjevico na Krki 2009, Mihovo, Šentjenersko polje, Bilje SE, Bilje brez, Ajoke čemežav kleče, Bela krajina Slovenia, Vrhopolje, Gorenje vrhopolje.
10 genotypes of maize (*Zea mays* L.): Adamcova budynska, Aranyozon šarga lofogu, Belaja mestnaja, C-44 Juhoslavska, Moldavskaja, Slovenska florentinka, Partizanka, Slovenska biela perlova, Stodnova a Samorinsky konsky zub.
All analyzed materials were obtained from the Gene Bank Piešťany, Slovak Republic.

Methods

Sample preparation

Proteins were extracted from individual grains according to standard ISTA method (**Wrigley *et al.*, 1992**). Seed storage proteins were isolated from whole, dry and mature grains. There were analyzed 20 individual grains from each genotype.

Electrophoretical separation of proteins in SDS-PAGE

Seed storage proteins separation was realized in vertical discontinual electrophoretic system Hoefer SE 600 DeLuxe. SDS polyacrylamide gel was used as a separation medium according to standard ISTA method (**Wrigley *et al.*, 1992**). 5µl of each sample was loaded into gel. Seed storage proteins separation was running for 20 hours with constant current 10 mA.

Gel staining and image analysis

Protein fraction of separated seed storage proteins were stained in solution of Commasie Brilliant Blue R 250 in ethanol and 10% TCA. Image analysis of SDS-PAGE gels was carried out using DocIt-LS software (Ultraviolet Products) using an automated process supplemented with occasional manual adjustments. Each lane was first defined where the average intensity across the width of these lanes was depicted as a function of the distance in pixels from the top of the image. The background was subtracted from each profile (parameters adjusted on a case-by-case basis), after which the bands were identified. The DocIt-LS software was also used for statistical interpretation of the eletrophoreographs.

RESULTS AND DISCUSSION

Grain protein complex consist of two major groups of proteins. Protoplasmatic proteins are formed by albumins and globulins subgroups and represent mainly metabolic active proteins. Storage proteins are formed by prolamins and glutelins and are important source of nitrogen for germinating grain. Knowledge of protein complex composition provides us essential information about technological and nutritional quality of grain. Electrophoretic spectrum of grain proteins obtained by electrophoretical separation indicates qualitative attributes of proteins subfractions **(Michalík *et al.*, 2006).**
Sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) proved that genotypes of wheat and maize were homogeneous and singlelined in contrast with genotypes of buckwheat which were heterogeneous and mulitlined (Table1, 2, 3 and Figure 1, 4). We detected high molecular weight glutenin subunits (HMW-GS), low molecular glutenin subunits (LMW-GS), gliadins, albumins and globulins by SDS-PAGE.

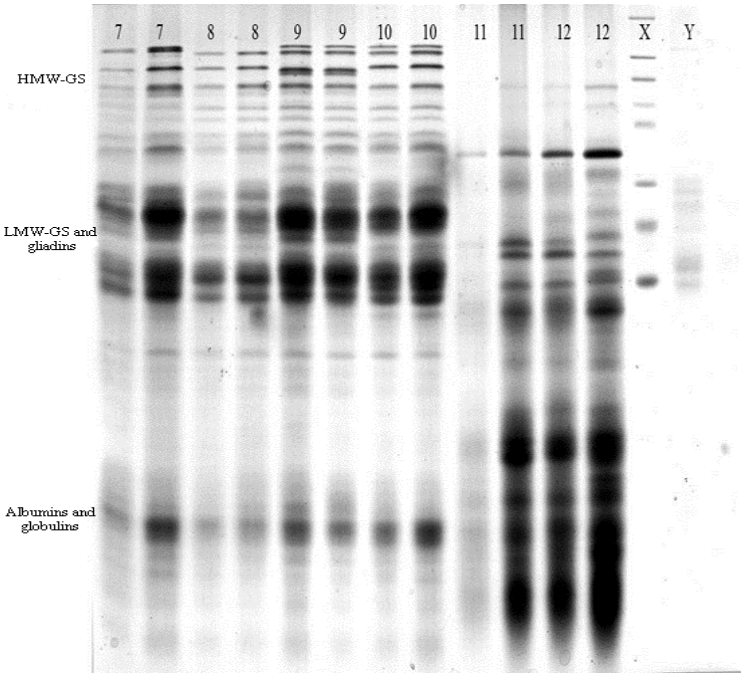

Figure 1 Electrophoretical profile of wheat genotypes storage proteins (*Triticum aestivum* L.) and buckwheat genotypes storage proteins (*Fagopyrum esculentum* L.) (SDS-PAGE): 7 = Granny, 8 = JB Asano, 9 = IS Jarissa, 10 = Sophytra, 11 = Orebovico, 12 = Mihovo, X = standard of molecular weight, Y = Standart – Marquis.

Our results showed (Table 1) that average content of HMW-GS in 10 genotypes of bread wheat was 9.19 % and ranged from 17.26 % (Leguan) to 4.60 % (Federer). Electrophoretical analysis of wheat storage proteins were also performed by **Palenčárová (2010)**, who determined comparable average content of HMW-GS (11.91 %) in bread wheat genotypes.

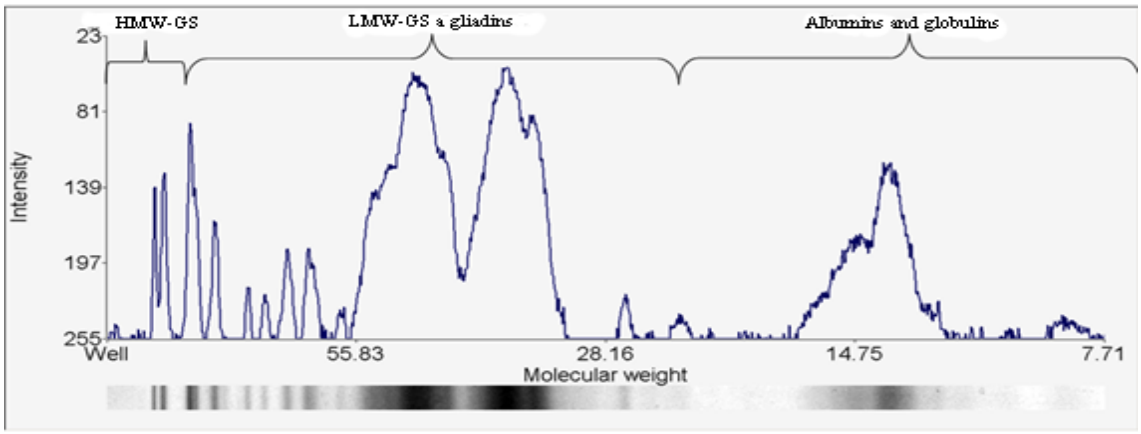

Figure 2 Densitogram of genotype IS Jarissa – *Triticum aestivum* L.

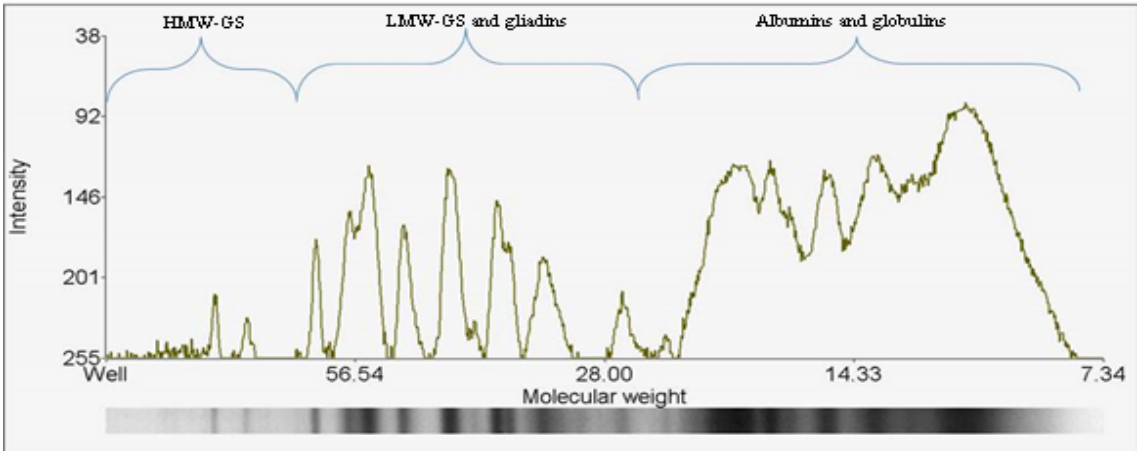

Figure 3 Densitogram of genotype Mihovo - (*Fagopyrum esculentum* L.)

Celiac active fractions of storage proteins (LMW-GS and gliadins) were detected at the highest content level, which represented 69.70 % and varied from 51.38 % (Natanael) to 81.43 % (Federer). Our results were confirmed by other author **(Wieser, 1998 and Palenčárová 2010)**.
As shown Table 1, the average presence of residual albumins and globulins in wheat varieties was 21.10 %. Values of this parameter varied from 38.66 % in variety Nathanael to 13.98 % (variety Federer). **Palenčárová (2010)** determined 17.94 % share of albumin and globulin fraction.

Table 1 Genotype variability of individual protein fraction of bread wheat grain

Genotype	HMW – GS [%]	LMW - GS and gliadins [%]	Albumins and globulins [%]
Rupert	6.13	73.37	20.48
Federer	4.60	81.43	13.98
Leguan	17.26	64.67	18.10
Pepino	15.46	62.46	22.07
SK-177	8.08	76.06	15.87
Natanael	9.94	51.38	38.66
Granny	7.47	63.26	29.26
JB Asano	6.40	79.36	14.25
IS Jarissa	7.95	71.75	20.27
Sophytra	8.64	73.29	18.08
x	**9.19**	**69.70**	**21.10**
σ	**4.08**	**9.17**	**7.60**

Legend x – average, ***σ*** – standard deviation

Our results confirm the findings published by **Michalik *et al.* (2006)**, who determined gluten proteins (represented by gliadin and glutenin) at 80 % of the total protein content of wheat grains. The remaining 20% is accounted for the albumins and globulins. These findings complement **Payne *et al.* (1987), Galova *et al.* (1998)**, who states that 50% of storage proteins is formed by gliadins, 40 % LMW - GS and the remaining 10% is accounted for HMW - GS.
Average representation of HMW-GS in the investigated varieties of buckwheat reached 1.16 % and ranged from 0.14 % to 3.67 % (Table 2). This low level of HMW-GS confirms that buckwheat flour is not satisfying according to the criteria of bakery quality, but it can be used as an additive to wheat or rye flour for bread. Observed low proportion of HMW-GS states is confirmed with work of **Palenčárová (2010)** who reached 3.74 %.

Proportion of LMW-GS and gliadins in analysed buckwheat varieties has average value of 32.09 % (Table 2). Bela karjina Slovenia variety (63.61%) has the largest representation while the lowest representation has variety Gorenje vrhopolje (14.77%), what reflects the high variability of the varietal diversity.

The average share of albumins and globulins reached the value of 66.72% (Table 2), with considerable variation from 33.77% to 85.08%. As reported by several authors **(Li *et al.*, 2002; Palenčárová, 2010; Socha *et al.*, 2010)**, albumin and globulin fraction is represented by 50 % and is a major fraction of buckwheat protein complex. Compared to these authors, proportion of albumins and globulins in buckwheat seeds determined by us was higher.

Table 2 Genotype variability of individual protein fraction of buckwheat grain

Genotype	HMW-GS [%]	LMW - GS and prolamins [%]	Albumins and globulins [%]
Orebovico	0.36	18.28	81.36
Mihovo 1	0.96	18.70	80.34
Mihovo 2	2.54	35.93	61.53
Mihovo 3	0.96	18.30	80.73
Poh. východ 1	0.81	24.32	74.88
Poh. východ 2	3.67	52.24	44.10
Darja semenarna 1	0.67	19.49	79.82
Darja semenarna 2	1.22	50.93	47.87
Ajoke čemežav kleče 1	0.54	47.28	52.19
Ajoke čemežav kleče 2	0.70	37.22	62.07
Šentjenersko polje 1	0.24	22.58	77.17
Šentjenersko polje 2	0.56	31.09	68.36
Bilje SE	2.03	42.18	55.79
Bilje Brez	2.46	48.00	49.55
Bela karjina Slovenia 1	2.61	63.61	33.77
Bela karjina Slovenia 2	0.70	38.51	60.77
Vrhopolje 1	0.23	21.51	78.24
Vrhopolje 2	0.17	17.19	81.94
Kostanjevico na krki 2009 1	0.52	21.54	77.95
Kostanjevico na krki 2009 2	3.12	43.67	53.22
Kostanjevico na krki 2009 3	1.34	23.45	75.22
Gorenje vrhopolje 1	0.16	27.21	72.64
Gorenje vrhopolje 2	0.14	14.77	85.08
x	**1.16**	**32.09**	**66.72**
σ	**1.04**	**13.93**	**14.65**

Legend: x – average, ***σ*** – standard deviation

Table 3 Genotype variability of individual protein fraction of maize grain

Genotype	HMW-GS [%]	LMW-GS and gliadins [%]	Albnumins and globulins [%]
Adamcova budynska	1.73	29.94	68.32
Aranyozon šarga lofogu	1.28	44.36	54.36
Belaja mestnaja	6.19	28.82	65
C-44 juhoslavska	1.84	50.1	48.05
Moldavskaja	3.47	27.63	68.91
Slovenska florentinka	7.92	30.63	61.47
Partizanka	3.78	34.1	62.11
Slovenska biela perlova	3.04	21.15	75.81
Stodnova	12.13	85.25	2.63
Samorinsky konsky zub	5.76	88.59	5.66
x	**3.38**	**24.09**	**25.99**
σ	**4.71**	**44.06**	**51.23**

Legend: x – average, ***σ*** – standard deviation

Our results also show that the overall average of HMW-GS in analysed maize samples (Table 3 and Figure 4) was very low compared with wheat, represented only by 4.71 % (1.28 % - 12.13 %).
LMW-GS and maize prolamins were again low represented as in wheat with an overall mean value of 44.06 %, with high variability ranged from 21.15% to 88.59%.
Compared with wheat, analysed maize varieties showed significantly higher average content of albumins and globulins (51.23 %). The highest content was detected in variety Slovenska biela perlova 75.81 % and the lowest in variety Stodnova (2.63 %).

When characterizing fractional tracks of proteins, interspecies differences are showed. The various fractions of proteins differ not only with different solubility in various solvents, but also with amino acid composition, molecular weight, physic-chemical and biochemical properties, causing significant differences in their biological function, nutritional and technological quality **(Michalik *et al.*, 1988).**

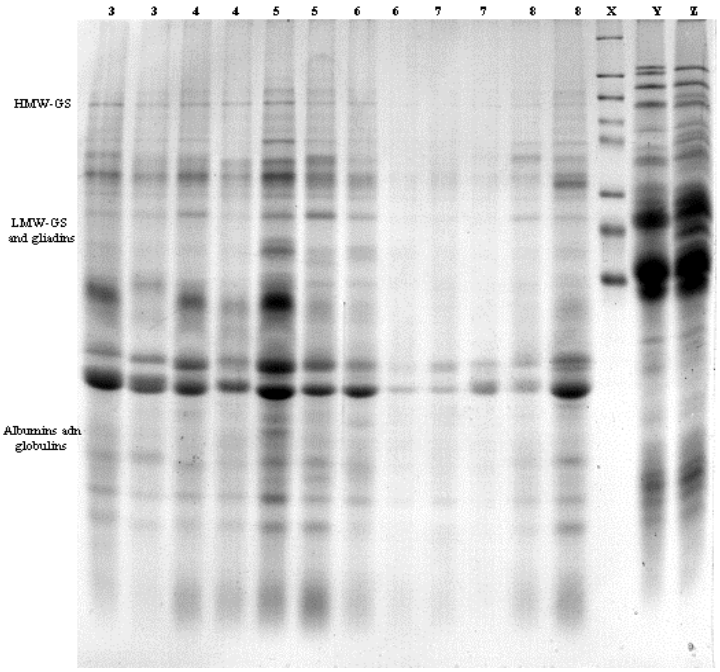

Figure 4 Electrophoretical profile of maize storage proteins (*Zea mays L.)*(SDS-PAGE*)* of genotypes: 3=Belaja mestnaja, 4=C-44 Juhoslavska, 5=Moldavskaja, 6=Slovenska florentinka, 7=Partizanka, 8=Slovenska biela perlova; X=Rebrík, Y=štandard Chinese Spring, Z=štandard Marquis

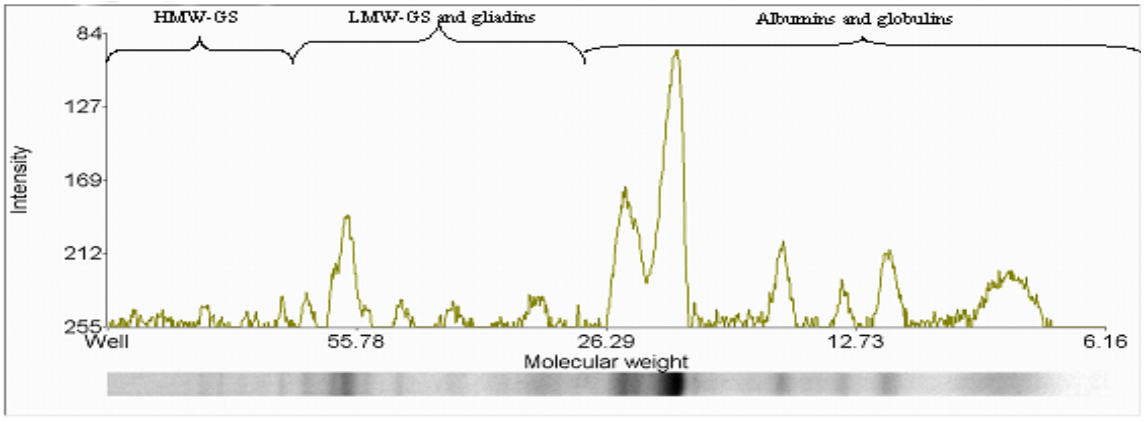

Figure 5 Densitogram of *Zea mays L.* genotype Slovenska biela perlova

CONCLUSION

Protein complex of wheat has a significant negative effect on patients with celiac disease. On the other hand, maize and buckwheat can be included among the major crops in the diet of celiac patients because maize and buckwheat protein doesn't have as much celiac activity as wheat proteins.

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