JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CHARACTERISTICS OF CEREALS, PSEUDOCEREALS AND LEGUMES FOR THEIR COELIAC ACTIVE POLYPEPTIDES

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doi: 10.15414/jmbfs.2019.9.special.390-395

ARTICLE INFO

Received 8. 7. 2019
Revised 6. 9. 2019
Accepted 17. 9. 2019
Published 8. 11. 2019

Regular article

OPEN ACCESS

ABSTRACT

The aim of this study was to analyze two types of cereals (wheat, barley), two types of pseudocereals (buckwheat, amaranth) and one type of legumes (peas) in terms of their use in gluten-free diet. Protein content, a fractional protein complex of grain, electrophoretic separation of storage proteins in SDS-PAGE and detection of coeliac active polypeptides by ELISA and Western blot were determined in whole grain samples. Significant differences in the proportion of individual protein fractions in cereals, pseudocereals and legumes were detected. Prolamins and glutelins (63.35% - 65.25%) prevailed in cereals, while albumins and globulins (49.2% - 73.24%) showed the highest proportion in pseudocereals and legumes. The ELISA method quantified of coeliac active polypeptides, with a lower limit of gluten proteins in peas, amaranth and buckwheat. On the contrary, in wheat and barley grain, gluten protein content was found above the permitted limit of 0.02 $g.kg^{-1}$. Western blot has confirmed that foods made from wheat and barley are not suitable for gluten-free diet, whereas peas, amaranth and buckwheat do not contain coeliac active polypeptides, and therefore represent a suitable source of protein for the preparation of gluten-free foods and food for coeliac patients.

Keywords: coeliac disease, cereals, pseudocereals, peas, SDS-PAGE, ELISA, Western blot

INTRODUCTION

Cereals are an essential resource in human nutrition and together with legumes have a significant impact on the nutritional balance. They are particularly important in terms of high carbohydrate, protein, fat, mineral and vitamin content **(Marko *et al.*, 2015)**. Legumes are considered to be the richest source of protein, essential amino acids and beneficial substances in the plant kingdom. Our most important "bread" cereal is wheat (*Triticum aestivum* L.), whose gluten proteins significantly affect the properties of the final product, but on the other hand, they constitute a group of allergens whose consumption causes health problems and coeliac disease to the genetically predisposed individuals **(Socha *et al.*, 2011).**
Adverse food responses can be divided into food allergies and food intolerances, with food allergy specifically referring to an immune-mediated adverse reaction, while food intolerance is not an immune-mediated response **(Mills and Shewry, 2004).** Food allergens include proteins or glycoproteins with a molecular weight from 5 kDa to 100 kDa and the ability to bind IgE receptors **(Breiteneder and Radauer, 2004).**
Coeliac disease, gluten-sensitive enteropathy, is a permanent intolerance to gluten prolamins in wheat (gliadins), barley (hordeins) and rye (secalins). The oat itself is considered not causing coeliac disease, but it is often contaminated with gluten-containing cereals during storage or processing **(Duta a Culetu, 2015).** Coeliac disease is caused by a faulty immune response to dietary wheat gluten. The disease is caused by immunological intolerance to gluten. The main triggering factor is the 20-30 kDa low molecular weight wheat protein fraction called α-gliadins. All allergenic fragments share two celiac active tetrapeptide fragments in the N-terminal region of the protein, Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro **(Lionetti and Gatti, 2015; Comino *et al.*, 2016; Catassi and Lionetti, 2019).**
The exact course of the disease is unknown, but gluten are thought to trigger a cascade of inflammatory reactions that lead to malabsorption and subsequent damage to the small intestinal mucosa. The disease affects about one in every 300 individuals. The „Draft Revised Standard for Gluten Free Foods„ of the Codex Alimentarius Commission (Berlin, June 2000) included a maximum level of 200 $mg.kg^{-1}$ (referred gluten-free) or 20 $mg.kg^{-1}$ (naturally gluten-free). Consumers rely on correct labeling of "gluten-free products", therefore it is necessary to control raw material as well as final products.
In the case of gluten intolerance, the only appropriate therapy for patients today is the immediate exclusion of gluten from food and strict adherence to a gluten-free diet. In an effort to expand the dietary spectrum of people reliant upon this type of treatment, pseudocereals, such as buckwheat, amaranth, millet, sorghum, quinoa in rational nutrition are now given great attention **(Hager *et al.*, 2012).** These do not cause problems to patients with coeliac disease and help to alleviate fiber deficiencies **(Alvarez-Jubete *et al.*, 2010).**
Currently, great emphasis is being placed on testing the presence or absence of coeliac active polypeptides in foods labeled as gluten-free. There are several analytical methods that allow quantitative and qualitative detection of allergenic food residues. The most commonly used method is an ELISA, which is able to specifically detect proteins from allergenic sources, is sufficiently sensitive and allows for the rapid establishment of residue limits in industrial food processing **(Leonard *et al.*, 2017; Schopf and Scherf, 2018).** Methods such as mass spectrometry are used to detect and quantify allergenic residues **(Baumert, 2014).**
The Western blot (protein immunoblot) is a widely used analytical technique in molecular biology, immunogenetics and other molecular biology disciplines to detect specific proteins in a sample of tissue homogenate or extract. Proteomic techniques, combined with Western blotting, make it possible to identify allergens and contribute significantly to the acquisition of new knowledge to develop diagnostic methods by detecting the binding of IgE antibodies to specific proteins (allergens). Two-dimensional electrophoresis in combination with immunoblotting and mass spectrometry is also used, allowing the identification and sequencing of unknown sample extracts **(Sancho and Mills, 2010).**
The aim of this study was to detect coeliac active polypeptides in two types of cereals (wheat, barley), two types of pseudocereals (buckwheat, amaranth) and one kind of legumes (peas) in terms of their use in a gluten-free diet.

MATERIAL AND METHODS

Plant Material

There were analyzed five different important food crops, winter wheat (*Triticum aestivum* L., variety Markola), barley (*Hordeum vulgare* L., variety Antigone), buckwheat (*Fagopyrum esculentum Moench.,* variety Emka), amaranth (*Amaranthus cruentus* L., variety Ficha) and peas (*Pisum sativum* L., variety Jantar). Samples were obtained from the Gene bank of the Research Institute of Plant Production in Piešťany, Slovak Republic and were milled by CU Mill (Lionhill Company, London, United Kingdom) to a homogenous flour with particle size about 0,2 mm.
Total nitrogen content in a homogenous flour of grain was determined by Kjeldahl´s method and fractional composition of proteins by Golenkov **(Michalík, 2002).** Proteins content was calculated based on multiplying total nitrogen content with specific coefficient for each analyzed plant. Coefficient of nutritional quality was calculated based on the formula: (albumins + globulins + residue)/prolamins) x 100.

Electrophoretic separation of storage proteins by SDS PAGE

Storage proteins were isolated from the endosperm of whole, dry single grains. Extraction of gluten proteins was realized according to standard method by ISTA **(Wringley, 1992).** Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS-PAGE) was used for separation of storage proteins. Electrophoresis was running for 6 – 8 hours at 15° C, 10 mA, 50 wats and 500 volts in the vertical discontinual electrophoretic unit Protean II (BioRad). Polyacrylamide gels were stained in the mixture containing 95 ml 10 % trichloroacetic acid and 5 ml 0,5 % Coomassie Brilliant Blue R250 in ethanol overnight and decolorized in distilled water. Electrophoretic profiles were scanned by GS-800 Calibrated Densitometer (BioRad), which cooperates with program Quantity One and individual bands were analyzed by Doc-It LS Image analysis UVP software.

ELISA (Enzyme-linked immunosorbent assay)

RIDASCREEN® Gliadin kit for quantitative determination of gliadins (R-Biopharm, SRN) with monoclonal antibody R5 was used in our experiments. The RIDASCREEN® Gliadin test is a sandwich enzyme immunoassay for the quantitative analysis of gliadins from wheat and corresponding prolamines from rye and barley in food with a detection limit of 3 ppm. The calibration curve was constructed based on the absorbance of standards from which was measured the prolamin concentration in the diluted sample and calculated its corresponding gluten content.

Western blotting

Western blot was performed according to the BioRad MiniProtean II methodology. Protein extraction was performed from whole grain samples with the methodology described by **Schägger (2006).** Before loading the protein samples to the gel, these were denatured at 100° C for 5 minutes in a water bath. Protein electrophoretic analysis was performed by Tris-tricine SDS-PAGE **(Schägger, 2006)** and run in a Bio-Rad Mini-Protean Tetra System. Protein separation taken 40 minutes at 30 volts, 60 minutes at 60 volts and 60 minutes at 90 volts. The gel was stained in Coomassie Brilliant Blue R-250 overnight and then the background was destained/decolored in 10 % acetic acid.
Electrotransfer of proteins from the gel to Immobilon-P polyvinylidene fluoride membrane (Millipore) was performed in an OmniBLOT Mini Blotting system (Cleaver Scientific) in a buffer solution for 90 minutes at 170 mA. Primary antibody (Anti-gliadin antibody produced in rabbit, Sigma-Aldrich) was diluted to 1.5 $\mu g.ml^{-1}$ and secondary antibody (Anti-rabbit antibody, Abcam) diluted to 0.2 $\mu g.L^{-1}$. The resulting immunocomplexes were detected by the chromogenic substrate SIGMAFAST 3,3'-diaminobenzidine (Sigma Aldrich). The membranes were read by the GS-800 Calibrated Densitometer (Bio-Rad), imaged by Quantity One (Bio-Rad) and evaluated by Image Lab (Bio-Rad).

RESULTS AND DISCUSSION

Conventional cereals, including wheat, barley, rye, and oat, contain gluten proteins which, at low concentrations in the diet, can cause genetically predisposed individuals an immunological inflammatory response of the small intestine. Coeliac disease is a metabolic genetic disease caused by increased sensitivity of some individuals to the presence of gluten in the diet. The only precaution for predisposed individuals is to maintain a lifetime gluten-free diet, which may cause some complications in terms of a limited number of products manufactured for coeliac patients **(Wieser, Koehler, 2008).**
According to **Kopálová (2008)** a gluten-free diet is necessary in coeliac disease, the patient does not need any medication if diet is followed. **Vici *et al.* (2016)** report the need to design new strategies and approaches to a gluten-free diet for coeliac patients. Currently, the focus is on the use of pseudocereals and legumes to extend the range of foods for gluten-free diet. Following this we focused in our work on the evaluation of two kinds of cereals (wheat, barley), two kinds of pseudocereals (buckwheat, amaranth) and one kind of legumes (peas) in order to detect the presence of coeliac active polypeptides.

Characteristics of cereal, pseudocereal and leguminous protein

Proteins are important in terms of nutritional and technological quality, while only protein content but fractional protein composition and their digestibility in individual crops are crucial factors for their utilization. Albumins and globulins are characterized by a high proportion of essential amino acids and thus exhibit high nutritional quality. On the other hand, prolamins and glutelins are characterized by a high proportion of non-essential amino acids, indicating their low nutritional value. The technological quality of the crop is influenced by the content of gluten-forming proteins (prolamins and glutelins), which is important for bakery use **(Mattila *et al*., 2018).**
Legumes, whose consumption has been declining in recent decades due to increased meat consumption, are also important sources of food for human nutrition. While cereals and pseudocereals are a good source of energy due to their high starch content, legumes are a source of high protein content. Chemical composition of agriculturally important crops is influenced by various factors such as crop type, genotype and agroecological conditions of cultivation **(Schoenlechner, 2016).**

Table 1 Proteins content, fractional composition of proteins and coefficient of nutritional quality in analyzed varieties of cereals, pseudocereals and legumes

Crop	Proteins (%)	Alb+Glo (%)	Prolamins (%)	Glutelins (%)	Pro+Glu (%)	CNQ (%)
wheat	9.03	28.71	33.63	29.72	63.35	109.01
barley	9.43	25.21	35.47	29.78	65.25	90.89
buckwheat	10.1	49.2	3.15	14.32	17.47	2567.3
amaranth	9.83	59.83	2.26	23.07	25.33	3296.46
peas	22.5	73.24	1.96	11.24	13.2	4384.69
Average value (%)	**12.18**	**47.24**	**15.29**	**21.63**	**36.92**	**2 089.67**
Standard deviation (%)	**5.78**	**20.41**	**17.60**	**8.59**	**25.38**	**1 928.06**
Coefficient of variation (%)	**0.47**	**0.43**	**1.15**	**0.40**	**0.69**	**0.92**

Legend: Alb+Glo **–** albumins and globulins, Pro+Glu – prolamins and glutelins, CNQ – coefficient of nutritional quality

The results show (Tab. 1) that the protein content of the individual samples ranged from 9.03 % (wheat) to 22.5 % (peas). Comparison of the protein content in different crops shows that the lowest protein content was in cereals (average value was 9.23 %), followed by pseudocereals (average value was 9.97 %) and peas (22.5 %). **Schoenlechner (2016)** reports a 3 % higher protein content in cereals (12.20 % - 11.84 %), while in peas it is 4.5 % more than in our sample.

The largest range of values was found in pseudocereals (11.04 % - 17.49 %). **Mattila *et al.* (2018)** indicates the peas protein content of 10 % (31,2 ± 0,4 %) and buckwheat 5 % (14.8 ± 1.6 %) higher than in our samples. These disproportions can be explained by the different number of genotypes analyzed from each crop type as well as by the agroecological conditions of cultivation.

Our analyzes also confirmed the results of **Muchová (2001),** which states that the protein content of wheat grain ranges from 8 % to 20 % depending on the variety, while the average content in the analyzed wheat samples was 12.6 %. **Gálová *et al.* (2006)** gives the average value of buckwheat protein content of 6.7 %. According to **Michalík *et al.* (2006)** climatic conditions, especially heat, light and air humidity determine the use of primary photosynthetic products for targeted protein and starch biosynthesis.

Protein content is an important indicator in terms of grain production, but the fractional composition of the protein complex refers the grain quality and its subsequent use. The albumin and globulin fractions are characterized by a high content of essential amino acids and therefore their nutritional value is high. The lowest value of albumins and globulins (Tab. 1) were reported for cereals 26.69 %, followed by pseudocereals with an average of 54.52 % and the highest content was determined in peas 73.24 %. This confirms the well-known fact that legumes have a high nutritional value **(Alonso-Miravalles and O´Mahony, 2018).** The prolamin and glutelin fractions (storage proteins) together with the starch form gluten which is important for the baking process of the wheat flour. Storage proteins have a low content of essential amino acids and a high proportion of non-essential amino acids, so their nutritional value is low. The highest proportion of gluten proteins (Tab. 1) reached cereals (63.35 % - 65.25 %), followed by pseudocereals (17.47 % - 25.33 %) and the lowest proportion was recorded by peas (13.2 %), confirming the use of cereals for bread production. Our results correspond to other works confirming large-scale prolamin and glutelin fractions (from 10 % to 80 %) depending on various crops **(Pellegrini and Agostoni, 2015; Taylor *et al.*, 2016; Schoenlechner, 2016; Kannaujia *et al.*, 2018; Kumar *et al.*, 2019).**

From the point of view of the presence of coeliac active proteins, it is important to monitor the prolamin fraction. The lowest proportion of prolamins was found in peas (1.96 %), followed by amaranth (2.26 %) and buckwheat (3.15 %), which is consistent with **Michalik *et al.* (2006), Gálová *et al.* (2012)** who recommend pseudocereals and legumes as suitable food sources for the preparation of gluten-free foods.

The representation of individual protein fractions in grain proteins was used to calculate the nutritional quality coefficient (CNQ), which indicates the nutritional quality of individual crops. CNQ values ranged from 90.89 % (barley) to 4384.69 % (peas), which is also confirmed by the results of the fractional composition of proteins in individual crops. Peas showed the highest nutritional value, then the amaranth, buckwheat and the lowest nutritional value reached cereals, which corresponds to the results of **Michalík *et al.* (2006), Gálová *et al.* (2012).**

Electrophoretic separation of storage proteins of analyzed samples

Evaluation of protein fraction composition due to their different solubility in different solvents does not allow to explain presence of allergenic protein determinants **(Michalík *et al.*, 2006).** Therefore, storage proteins from all analyzed wheat, barley, buckwheat, amaranth and peas genotypes were further electrophoretically separated into individual protein subfractions by standard ISTA SDS-PAGE reference method. Based on the results we identified high molecular weight glutelin subunits (HMW-GS), low molecular weight glutelin subunits LMW-GS), monomeric prolamins and residual albumins and globulins (Fig. 1). The obtained results provide information about species differences in the fractional composition of storage proteins, which influence the technological and nutritional quality of the grain.

Electrophoreogram of tested samples (Fig 1) show that HMW-GS are separated in the first third of the polyacrylamide gel, LMW-GS are separated in the second third of the gel and residual albumins and globulins in the bottom of the gel, while the content of HMW-GS in all samples ranged from 5.24 % (amaranth) to 11.75 % (wheat) (Tab. 2). The highest content of HMW-GS was obtained by cereal samples (wheat, barley), then peas and pseudocereals (buckwheat, amaranth) showed the lowest ones. On the other hand, content of LMW-GS was about 70 % higher than content of HMW-GS and ranged from 51.2 % (buckwheat) to 64.56 % (wheat). The proportion of residual albumins and globulins fractions was 23.69 % - 26.69 %. Comparable results were also achieved by **Visioli *et al.* (2016),** who identified content of HMW - GS in cereals from 6 % to 10 %. **Dangi and Khatkar (2018)** detected LMW-GS variability from 43.19 % to 69.74 % in four wheat varieties. **Michalík et al. (2006)** reported average value of LMW-GS in cereals 58.72 % and in pseudocereals 41.71%, which is significantly lower than in our study.

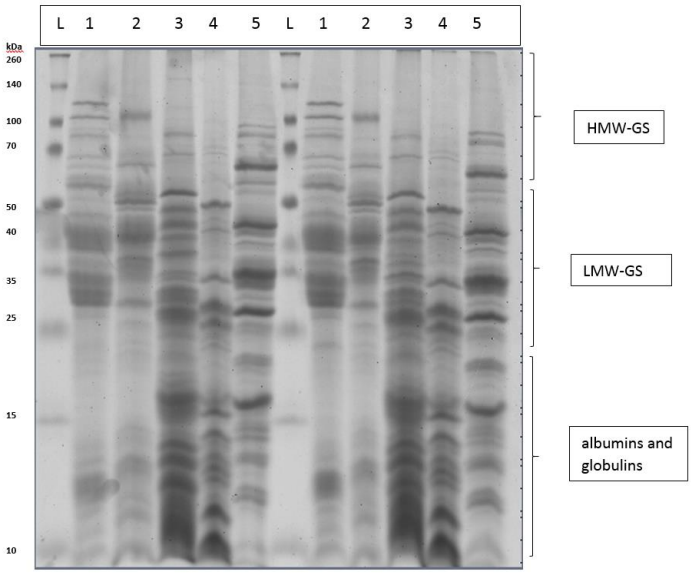

Figure 1 Electroforetic profiles of storage proteins in grains of analyzed samples using SDS-PAGE

Legend: L – ladder marker, 1- wheat, 2 - barley, 3 - buckwheat, 4- amaranth, 5 – peas, HMW-GS - high molecular weight glutelin subunits, LMW-GS - low molecular weight glutelin subunits

Table 2 Content of protein subfractions in analyzed varieties of cereals, pseudocereals and legumes

Crop	HMW- GS (%)	LMW- GS (%)	Residues of albumins and globulins (%)
wheat	11.75	64.56	23.69
barley	10.48	62.83	26.69
buckwheat	5.57	51.2	43.22
amaranth	5.24	52.59	42.17
peas	8.92	61.05	30.03
Average value (%)	**8.39**	**58.45**	**33.16**
Standard deviation (%)	**2.60**	**5.48**	**8.05**
Coefficient of variation (%)	**30.99**	**9.38**	**24.27**

Legend: HMW-GS – high molecular weight glutelins subunits, LMW-GS – low molecular weight glutelins subunits

The content of HMW-GS in buckwheat (Tab. 2) was 5.57 % and in amaranth 5.24 %. **Mlyneková *et al.* (2014)** established content of HMW - GS in amaranth in the range of 0.37 % - 4.4 %, which is lower in comparison to our results. The HMW - GS content in buckwheat they found out from 1.57 % to 8.8 %, which confirms our study. **Gálová *et al.* (2012)** estimated the proportion of LMW-GS in the amaranth genotypes on average 46.76 % and in the buckwheat 45.57 %, the albumins and globulins content ranged from 42.17 % to 43.22 %. We recorded higher values of LMW-GS for amaranth (52.59 %) as well as for buckwheat (51.2 %) in comparison to **Gálová *et al.* (2012).**

The content of HMW-GS in the peas seeds was 8.92 % and LMW-GS 61.05 % (Tab. 2), which is about 13 % lower in comparison to results of **Chen *et al.* (2019)** who reported average values for HMW - GS in the peas seeds 20.68 % and for LMW-GS 45.44 %. **Chen *et al.* (2019)** analyzed the peas protein fractional composition by SDS-PAGE and found that in all samples the molecular size of the electrophoretic bands ranged from 16 to 97 kDa. Peas proteins consist of 70 % globulins, which are considered to have a beneficial effect on human health. Many authors recommend peas for its rational nutrition **(Ma *et al.*, 2017; Mendes *et al.*, 2018).**

Based on protein content, protein fractional composition (Tab. 1) as well as the electrophoretic separation of storage proteins in SDS-PAGE of wheat, barley, amaranth, buckwheat and peas (Fig. 1, Tab. 2) we determined the nutritional and technological differences between the analysed crops. We confirmed that cereals have a higher proportion of storage proteins (prolamins, glutelins), which play very important role in technological quality of grain. On the other hand, pseudocereals and legumes have the higher level of cytoplasmic proteins (albumins, globulins), which are important fractions from a nutritional point of view. Albumins and globulins contain more essential amino acids (lysine, theonine, methionine, isoleucine, arginine) in comparison to prolamins and glutelins.

Detection of coeliac active polypeptides by ELISA

Although the results of the fractional composition of proteins allow to characterize the analyzed samples in terms of their risk in coeliac disease, they do not provide direct evidence of the presence or absence of protein determinants that immediately cause this disease. Currently, an objective conclusion can only be made on the basis of ELISA or Western blot **(Lexhaller *et al.*, 2016).** The most recommended method according to the relevant legislation is the R5 - ELISA Mendez sandwich method, developed by **Osman *et al.* (2001).**
The principle of the RIDASCREEN® sandwich ELISA test, lies in the reaction of monoclonal R5-antibodies with ω-prolamins of wheat, rye, barley, that are directed against epitopes of QQPFP, QQQFP, LQPFP and QLPFP occurring in coeliac toxic gliadin, secalin and hordein amino acid sequences **(Wieser, Koehler, 2008).** These epitopes have a toxic effect on humans and are referred to as celiac active polypeptides **(Koehler *et al.*, 2013; Colgrave *et al.*, 2016).** According to **Jappe and Vieths (2010)**, the sandwich type ELISA method has a detection limit of 1µg.g^{-1}, suggesting a sufficiently sensitive and credible method.
As Tab. 3 shows, the highest content of prolamins and gluten (Tab. 3) was in the cereal varieties (prolamins 87.57-57.53 g.kg^{-1}; gluten 175.14-115.07 g.kg^{-1}), followed by pseudocereals varieties (prolamins 0.09-0.08 g.kg^{-1}; gluten (0.18-0.16 g.kg^{-1}) and ultimately the lowest content of prolamins and gluten was found in the legumes (prolamins 0.07 g.kg^{-1} 0.14 g.kg^{-1}).
Kerpes *et al.* (2016) tested the gluten protein content of cereals by ELISA and confirmed our results. The prolamin content was detected in a wide range from 147 ± 2.1 g.kg^{-1} to 47 ± 0.5 g.kg^{-1}. Likewise, gluten values ranged from 323 ± 2.3 g.kg^{-1} to 93 ± 0.6 g.kg^{-1}. **Socha *et al.* (2010)** confirmed the excess content of prolamins in all analyzed cereals (summer wheat, spelt wheat, durum wheat, oats, spring barley, triticale) by ELISA analysis. The highest content of prolamins was detected in spelt wheat (16.3 g.kg^{-1}) using the R5 antibody ELISA. **Lexhaller *et al.* (2016)** conducted similar research in which they compared 5 different ELISAs. They found a prolamin content in cereals ranging from 49.4 ± 1 g.kg^{-1} to 19.9 ± 1.2 g.kg^{-1}, which is lower than our results. In their work, the RIDASCREEN® Gliadin test, which we have applied in our work, has proved to be the most suitable ELISA method.

Table 3 The content of prolamins and gluten in analysed crops by ELISA method

Crop	Prolamins (g.kg^{-1})	Gluten (g.kg^{-1})
wheat	57.53	115.07
barley	87.57	175.14
buckwheat	0.09	0.18
amaranth	0.08	0.16
peas	0.07	0.14
Average value (%)	**29.07**	**58.14**
Standard deviation (%)	**41.09**	**82.18**
Coefficient of variation (%)	**141.4**	**141.4**

Based on our results we can confirm the unsuitability of cereals for food preparation for gluten-free diet. The gluten content in our samples exceeded the permitted limit for gluten content in gluten-free foods several times. **Hischenhuber *et al.* (2006)** note that Codex Alimentarius limits gluten content to 20 mg.kg^{-1} for naturally gluten-free foods. For products that are not naturally gluten-free there is a maximum gluten limit of 200 mg.kg^{-1}, corresponding to 0.02 % gluten **(Palenčárová and Gálová, 2010; Gálová *et al.*, 2012).**
Pseudocereals are characterized by high content of albumins and globulins, while prolamin content in which toxic proteins are found is low **(Comino *et al.*, 2013).** In our analyzed samples of pseudocereals the variability of prolamins ranged from 0.09-0.08 g.kg^{-1} and the gluten content varied from 0.18 g.kg^{-1} to 0.16 g.kg^{-1} (Tab. 3). When we compare our results with the limit given by the relevant legislation we can recommend pseudocereals to gluten-free foods for celiac patients. In addition, pseudocereals are also a very good source of high content of fiber, minerals (calcium, iron) and other bioactive ingredients such as phytosterols, polyphenols, saponins **(Alvarez-Jubete *et al.*, 2010).**
The same results were obtained by **Ballabio *et al.* (2011),** who performed ELISA for detection of gluten content in 40 pseudocereals varieties and recommended the use of analyzed pseudocereals for the preparation of gluten-free foods. The gluten content was below the permissible limit of 0.02 g.kg^{-1}.

Schoenlechner (2016) recommends mixing flour from cereals and legumes for food preparation in order to ensure a better overall balance of essential amino acids. Cereals are characteristic as unfavorable because the content of albumins and globulins is low. On the other hand, leguminous seeds have a high proportion of cytoplasmic proteins with a high content of essential amino acids and therefore have a high nutritional value. The lowest content of toxic prolamins and gluten was found in peas, namely 0.07 g.kg^{-1} of prolamins and 0.14 g.kg^{-1} of gluten, which is below the maximum permissible limit of 0.02 g.kg^{-1} gluten. **Takács *et al.* (2007)** found gluten protein content in leguminous seeds 0.05 g.kg^{-1} and considered legumes suitable for gluten-free diet and safe for celiac patients, as confirmed by other authors **(Colgrave *et al.*, 2016; Schoenlechner 2016; Mattila *et al.*, 2018).**
From the point of view of the overall evaluation of all results from the ELISA it can be stated that the content of gluten proteins in cereals was above the limit and therefore they should be completely excluded from the gluten free diet. At the same time, knowledge of pseudocereals was confirmed. They are suitable for food preparation in a gluten-free diet, because the prolamin and gluten content was below the Codex Alimentarius limit. We also confirmed the results achieved in the peas protein composition, that the gluten content determined by the ELISA satisfies the condition to include peas in the gluten-free diet. In addition, peas are characterized by health-promoting substances and therefore represent good food for celiac patients and for its positive effects on human health.

Detection of coeliac active polypeptides by Western blotting

Rosell *et al.* (2014), Su *et al.* (2018) report that the SDS-PAGE method is not sufficient to quantify gluten in raw materials for the preparation of a gluten-free diet due to lack of sensitivity and therefore recommend the use of a Western blot method to confirm or exclude the presence of gluten proteins in food.
Figure 2 demonstrates the separation of wheat, barley, buckwheat, amaranth and peas gluten proteins in SDS-PAGE and subsequently the Western blot for detection of coeliac active polypeptides. Coeliac active polypeptides were detected in wheat with the molecular weight 20-140 kDa and in barley with the molecular weight 35-100 kDa. We detected one 40 kDa band in amarant, assuming that the sample was contaminated. No celiac active polypeptides were detected in buckwheat and in peas.

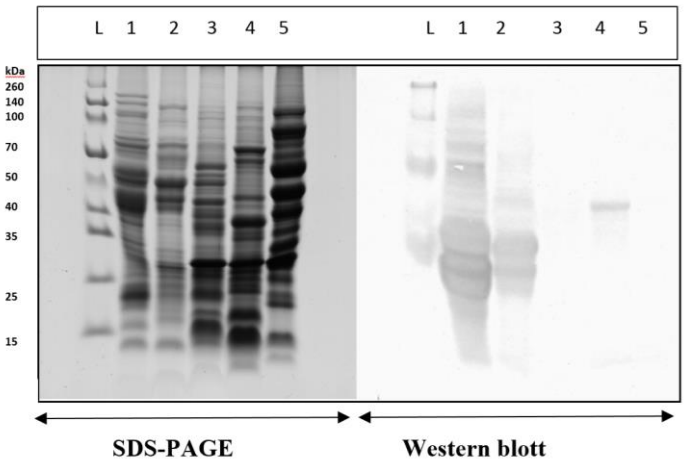

Figure 2 Western blot analyzed samples
Legend: SDS-PAGE: L – ladder marker, 1-wheat, 2-barley, 3-buckwheat, 4-amarant, 5-peas; Western blott: L – ladder marker, 1-wheat, 2-barley, 3-buckwheat, 4-amaranth, 5-peas

Socha *et al.* (2011) note that the Western blot with gluten polyclonal antibodies is a suitable method for the qualitative detection of prolamins in cereals, pseudocereals and legumes. They report that the α-gliadin fraction with a molecular weight of 20-30 kDa exhibits coeliac activity. Our results are approved by **Mickowska *et al.* (2012), Sung *et al.* (2014), Comino *et al.* (2016). Mickowska *et al.* (2012)** compared prolamin proteins of wheat, barley, rye and triticale by electrophoretic and immunochemical methods. They determined, that proteins with a molecular weight of 35 kDa to 45 kDa gave the strongest signal by Western blot.

CONCLUSION

By applying electrophoretic and immunochemical methods it is possible to detect quickly and reproducibly detect coeliac active polypeptides in the raw materials that can be used to produce foods suitable for predisposed individuals. ELISA analysis not only accurately detects, but also quantifies the content of coeliac agents in the analyzed samples. From the point of view of the overall evaluation of the results of our work based on the determination of the total protein content, the fraction of proteins, the separation of the storage proteins in SDS-PAGE,

detection of gluten content by ELISA and Western blot, we do not recommend the consumption of wheat and barley products by coeliac patients. At the same time, we confirmed that buckwheat, amaranth and peas are appropriate in the gluten-free diet.

Acknowledgments: This study was financially supported by VEGA project No. 1/0246/18 (75%), VEGA project No. 2/0109/19 (25%) and KEGA project No. 025SPU-4/2018

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