OAT RAW MATERIALS AND BAKERY PRODUCTS – AMINO ACID COMPOSITION AND CELIAC IMMUNOREACTIVITY*

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ABSTRACT

Background. The aim of this study was to compare the biochemical and immunochemical properties of avenins in some special oat raw materials and additionally the possibility of using them as a raw material for the gluten-free bakery products.
Materials and methods. The compared oat raw materials were – oat flakes, commercial oat flours (including gluten-free oat flour) and residual oat flour, which is by-product of β-glucan preparation. Biochemical characteristic included amino acid compositions and SDS-PAGE profiles of extracted avenins. The immunochemical reactivity with polyclonal anti-gluten and monoclonal anti-gliadin antibodies was evaluated qualitatively and quantitatively by immunoblotting and ELISA methods. Additionally, experimental bakery products made of examined raw materials were assessed according to their suitability for the celiac patients' diet.
Results. The highest protein content was measured in the β-glucan preparation "Betaven" and gluten-free oat flour. Proteins of all materials are rich in glutamic and aspartic acid, leucine and arginine. Proportions of amino acids in avenins extracted from most of oat raw materials are similar, excluding gluten-free oat flour, which has a very low avenin content and proportions of individual amino acids are different. The SDS-PAGE protein pattern consisted of proteins with molecular weight of about 25–35 kDa. Polyclonal anti-gluten antibody recognized all protein fractions of molecular weight higher than 20 kDa. Quantitative ELISA analysis shows that the majority of samples has a gliadin-like protein content within the range of 80–260 mg/kg, excluding gluten-free flours and corresponding bakery products. Altogether, β-glucan preparation has extremely high level of gliadin-like proteins.
Conclusion. In the examined oat raw materials and foods the contents of immunoreactive amino acid sequences exceeded the limit of 20 mg/kg (considered as gluten-free) except for gluten-free flours (oat and the prepared mixture) and the bakery products based on gluten-free flours. Unfortunately, the rest of oat raw materials and products cannot be considered gluten-free.

Key words: oat, celiac disease, prolamins, avenins, ELISA, Western blot

INTRODUCTION

The alcohol-soluble proteins of oats (avenins) belong to prolamin proteins, which are rich in glutamine and proline. Prolamins of oat represent only about 10% of grain proteins, whereas in other cereals such as wheat, rye and barley prolamins are the largest group of about 80% (Sontag-Strohm et al., 2008).

Prolamins are the causative agents of celiac disease, but in case of oat there is a dispute if oat has toxic activity during the occurrence of celiac disease and if it can be utilized in gluten-free diets (Fric et al., 2010). Prophylactically, oat has often been excluded from gluten-free diets, or celiac patients were allowed to consume only moderate amounts of oat. Nevertheless, some authors report satisfactory oat tolerance by adult celiac patients (Silano et al., 2007; Størsrud et al., 2003a; 2003c), especially if not contaminated by wheat, rye or barley. Unfortunately, many commercially available oat products are cross-contaminated by gluten-containing cereals and this is especially important when they are used in preparation of products named gluten-free.

Recent research of molecular aspects of oat toxicity, tested by *in vitro* immunoreactivity with antigliadin antibodies showed that a content of toxic, gliadin-like peptides in avenins depends on oat variety. Among examined oat varieties, immunoreactivity varied strongly, but most of them did not present gliadin-like immunoreactivity, so this can be essential in selecting the most suitable oat variety for a gluten-free diet (Ballabio et al., 2011). On the other hand, although none of the wheat gluten epitopes occur in oat avenins, two immunoreactive avenin-specific epitopes are common in oat and these epitopes can be potentially harmful for some celiac disease patients (Londono et al., 2013). Nevertheless, as the differences in immunogenicity among oat varieties were also revealed by immunological methods using gliadin-reactive T-cells, the selection and breeding of "safe" oat varieties may be possible (Mujico et al., 2011).

The aim of this study was to estimate the celiacactive protein content in oat raw materials (including the beta-glucan preparation) and experimental bakery products, based on the same materials, as well as some gluten-free products. The immunoreactivity was measured by the Western blot and the ELISA methods, using polyclonal and monoclonal antibodies, respectively.

MATERIAL AND METHODS

Material

The tested samples were oat raw materials and products as follows:

1. Raw materials: oat gluten-free flour PROVENA (Raisio Nutrition Ltd., Finland), commercial oat flour (Paweł Bogutyn Młyn, Radzyń Podlaski, Poland), oat flakes, β-glucan preparation "Betaven" and residual oat flour obtained from oat flakes, where residual oat flour is a by-product of "Betaven" production (Microstructure Ltd., Warsaw, Poland), gluten-free mixed flour was prepared in our laboratory as a mixture of rice flour (50%), corn starch (35%) and corn flour (15%).

2. Products: experimental bakery products (four types of muffins) formulation: Flour: 180 g (alternatively: gluten-free oat flour, residual oat flour, commercial oat flour, gluten-free mixed flour), other ingredients: sugar 100 g, buttermilk 50 ml, oil 30 ml, gluten-free baking powder 5 g, 3 eggs (165 g–60 g yolk and 105 g white added separately). Dry and wet ingredients were mixed in separate dishes, blended and finally whipped egg whites were added. Muffins were baked for 25 minutes in 180°C.

Chemicals and laboratory equipment

Molecular weight protein markers (FERMENTAS Int. Inc., Canada), the polyclonal Anti-gluten wheat rabbit antibody (USBiological, USA), the Anti-rabbit HRP antibody (BD Pharmingen, USA), SIGMAFASTTM 3,3'-Diaminobenzidine tablets (SIGMA, USA), RIDASCREEN® Fast Gliadin (R-BIOPHARM, Germany), the PVDF transfer membrane ImmobilonPSQ (Millipore, USA), the amino acid standard solution for calibration of amino acid analyzers (Sigma, USA). All other analytical grade chemicals were from Sigma (USA), Fluka (Switzerland) or Applichem (Germany).

Lyophylizer Christ®Alpha1-2 LD Plus (Martin Christ, Germany), the electrophoresis and transfer equipment (BIO-RAD Laboratories Inc., USA), the amino acid analyzer AAA400 (Ingos, Czech Republic), the ELISA reader TECAN Infinite 200 PRO (TECAN, Switzerland).

The preparation of avenins

The extraction of avenins: samples were milled (if necessary) and proteins were extracted by magnetic stirring with 15 ml of solvent/g meal for 1 hour at room temperature. The extraction was executed with the following series of solvents: 0.5 M NaCl to extract salt-soluble albumins and globulins, distilled water to remove NaCl, and 70% (v/v) ethanol to extract prolamins and alcohol soluble proteins. The extracted alcohol-soluble proteins were lyophilized.

The analysis of amino acids

The analysis of amino acid composition of raw materials and extracted prolamins was performed according to the method of Moore and Stein (Smith, 2003). The liquid-phase hydrolysis of proteins was performed in 6 M HCl containing 0.5% phenol at 110°C for 24 hours in argon atmosphere. Sulphur-containing amino acids were analysed as oxidized derivatives obtained by performic acid oxidation followed by standard hydrolysis procedure with HCl. The hydrolysates were lyophilized and dissolved in the appropriate volume of sodium citrate buffer pH 2.2. Amino acids were determined by the ion-exchange chromatography with a strong cation ion-exchanger and sodium-citrate elution buffers system followed by post-column derivatization with ninhydrin and the spectrophotometric detection at $\lambda = 570$ and 440 nm, according to the standard protocol of the manufacturer of the amino acid analyzer. Asparagine and glutamine were determined in form aspartic and glutamic acid.

Statistical analysis

All analyses were performed at least in duplicate, and results were subjected to univariate analysis of variance (ANOVA) with the help of Statistica 10. The significance of differences were shown by Duncan's test at $\alpha \leq 0.05$. The results are presented as a mean and standard deviation (SD).

SDS-PAGE and the immunoblotting of extracted prolamins

The SDS-PAGE electrophoresis under reducing conditions was performed according to the Schägger-von Jagow method (Schägger and von Jagow, 1987). The electrotransfer to the PVDF membrane was performed for 1.5 hour at 170 mA using 10 mM CAPS transfer buffer pH 11 (containing 10% methanol), followed by the Coomasie Brillant Blue membrane staining. In the Western blot the membrane was blocked in 1% Bovine Serum Albumin (in Tris-Buffered Saline buffer, pH 7.6) for 1 hour, washed with TBS buffer four times for 15 min, and placed in the primary antibody solution (anti-gluten wheat antibody diluted 1:1,000 in a blocking solution) for 1.5 hour and finally washed with TBS buffer five times for 15 min. After that blot was incubated with the secondary antibody (the anti-rabbit antibody labelled with horseradish peroxidase, diluted 1:2,000 in a blocking solution) for 1 hour and washed with TBS buffer six times for 15 min. The chromogenic detection was conducted with diaminobenzidine according to the manufacturer's instructions.

ELISA assay

Samples preparation: samples (after drying and milling if necessary) were extracted with the cocktail solution (delivered by R-BIOPHARM, Germany), followed by 80% ethanol extraction and appropriate sample dilution for immunodetection by ELISA method. RIDASCREEN® Fast Gliadin kit based on monoclonal antibody R5 was used and the analysis was performed according to the manufacturer´s instructions.

RESULTS AND DISCUSSION

In most of the cereals, prolamins are the dominating seed protein fraction, whereas the amount of avenin in oat is significantly lower (about four times). Moreover, there are also differences between the amino acid composition and the sequence of avenins and their counterparts in wheat, barley and rye. Especially, avenins contain lower amount of proline residues, which is important for the T-cell stimulation, and lack of proline residues changes recognition and binding properties of peptides for HLA-DQ2. Additionally, proline preserves protein degradation by proteinases, so avenins are more susceptible to proteolytic degradation. All these differences are the molecular basis of the lower celiac disease activity of avenins (Vader et al., 2003).

In the present study, we examined some oat raw materials and experimental bakery products in the context of the biochemical and immunochemical characterization of avenins.

The amino acid compositions of proteins of the examined oat raw materials are shown in Table 1. The amino acid compositions of the bakery products proteins were not determined due to the significant share of proteins from other raw materials used to muffins preparation (eggs and buttermilk). The highest protein content was measured in the β-glucan preparation "Betaven" and gluten-free oat flour. Both samples are richer in proteins in comparison to other flours, which have similar amounts of protein. Proteins of all materials are rich in glutamic and aspartic acid, leucine and arginine, while the proline level does not distinguish itself from other amino acids.

In the amino acid profiles of oat flours glutamic acid is dominating, followed by aspartic acid, leucine and arginine. Sulphur-containing amino acids and histidine are present at the lowest levels. The described amino acid compositions are similar to literature data (Hahn et al., 1990; Koehler and Wieser, 2013; Wu et al., 1972).

The comparison of the obtained amino acid composition shows that oat flakes contain more aspartic

Table 1. Amino acid composition of proteins of oat raw materials

Amino acid	Amino acid amount*, % of protein				
	gluten-free oat flour	commercial oat flour	oat flakes	oat residual flour	"Betaven"
Asx**	8.20 ±0.12 b***	8.06 ±0.15 ab	8.53 ±0.04d	7.94 ±0.13 a	8.39 ±0.07c
Thr	3.26 ±0.08 ab	3.25 ±0.07 ab	3.32 ±0.01 b	3.18 ±0.07 a	3.32 ±0.12 b
Ser	4.67 ±0.24 a	4.70 ±0.03 a	4.65 ±0.07 a	4.70 ±0.03 a	4.96 ±0.20 b
Glx**	24.27 ±0.28 c	23.96 ±0.10 bc	23.32 ±0.06 b	23.93 ±0.17 bc	22.26 ±0.03 a
Pro	5.41 ±0.05 a	5.14 ±0.20 a	4.96 ±0.21 a	5.14 ±0.05 a	5.66 ±0.07 a
Gly	5.03 ±0.03 a	4.99 ±0.04 a	5.13 ±0.04 b	5.04 ±0.02 a	5.73 ±0.03 c
Ala	4.72 ±0.04 b	4.64 ±0.05 a	4.71 ±0.01 b	4.64 ±0.03 a	5.03 ±0.03 c
Val	5.33 ±0.15 ab	5.34 ±0.02 ab	5.35 ±0.03 b	5.33 ±0.04 ab	5.24 ±0.17 a
Ile	4.07 ±0.12 b	4.15 ±0.01 b	3.99 ±0.05 b	4.14 ±0.04 b	3.74 ±0.13 a
Leu	7.75 ±0.05 b	7.89 ±0.04 c	7.82 ±0.02 bc	7.87 ±0.08 c	7.34 ±0.07 a
Tyr	3.87 ±0.05 a	4.09 ±0.02 bc	4.14 ±0.04 c	4.08 ±0.04 b	4.04 ±0.04 b
Phe	5.46 ±0.05 b	5.46 ±0.02 b	5.42 ±0.02 b	5.48 ±0.04 b	4.95 ±0.01 a
His	2.63 ±0.06 b	2.85 ±0.02 d	2.30 ±0.07 a	2.85 ±0.02 d	2.74 ±0.13 c
Lys	3.98 ±0.04 a	3.91 ±0.03 a	4.10 ±0.02 b	3.93 ±0.04 a	4.26 ±0.03 c
Arg	6.93 ±0.40 a	7.16 ±0.10 a	7.04 ±0.06 a	7.14 ±0.05 a	7.06 ±0.14 a
Cys	2.74 ±0.07 a	2.68 ±0.10 a	2.76 ±0.10 a	2.81 ±0.14 a	3.66 ±0.14 b
Met	1.65 ±0.03 a	1.73 ±0.06 ab	1.62 ±0.09 a	1.83 ±0.08 b	1.61 ±0.05 a
Protein content* % w/w	16.24 ±0.17 c	11.26 ±0.13 a	13.03 ±0.13 b	10.93 ±0.30 a	20.33 ±0.68 d

*Without Trp.

**Asx = Asp + Asn, Glx = Glu + Gln.

***a, ..., d – mean values in rows and denoted by different letters differ statistically significantly at α ≤ 0.05.

acid, glycine, tyrosine and lysine, and less histidine than analyzed flour samples. The difference in amino acid composition is not surprising, as oat flakes are produced from oat grains and are not deprived of the aleurone layer and only hull.

Oat flakes were the raw material used for the production of both β-glucan preparation and residual oat flour (all samples were delivered by manufacturer – Microstructure Ltd., Warsaw, Poland). Physical treatment of oat flakes (size reduction) was used for isolation of β-glucan preparation "Betaven". The remaining residue was called "residual oat flour", whose particles are much more fine-grained in comparison to commercial flours due to the micronization process. The fraction rich in β-glucans ("Betaven") also contained other low density components, including proteins. The amino acid compositon of residual oat flour does not differ from standard, commercial oat flour, although the technology of production is quite different. Interestingly, β-glucan preparation "Betaven" contains much more proteins than oat flakes and all the other oat flours and its amino acid composition resembles flour.

Commercial oat flour and residual oat flour did not differ significantly in their amino acid profile and total protein content. These flours compared to gluten-free oat flour contained significantly less protein, however gluten-free oat flour contained significantly more alanine and less tyrosine and histidine than others. Both commercial and residual oat flours as a result of the processing lacked anatomical parts of the kernel, whereas the gluten-free flour was wholemeal, which affected total protein content and amino acid profile.

The data obtained for the prolamins extracted from the samples are presented in Table 2. Total protein contents of alcohol extracts demonstrates high variation and extremely low protein content in oat gluten-free flour extract (three or four times less in comparison to other raw materials). Proportions of amino acids in prolamins extracted from most of oat raw materials are similar and comparable with the results obtained for four oat varieties in our previous research (Mickowska et al., 2013). Especially gluten-free oat flour is significantly different as it has a very low prolamin content in relation to other extracts, and proportions of individual amino acids are different, too (mainly aspartic and glutamic acids, serine, glycine, histidine and lysine). Although gluten-free oat flour has the highest

protein content, the content of prolamin-like protein is the lowest among all oat raw materials, which was also confirmed by the ELISA method.

The SDS-PAGE protein pattern was similar in all the tested samples and consisted of proteins with molecular weight of about 25–35 kDa. The immunodetection of prolamins by Western blot analysis was performed using extracted prolamins and a polyclonal anti-wheat-gluten antibody. That antibody recognized all protein fractions of molecular weight higher than 20 kDa and did not react with low molecular weight proteins present in all oat extracts. Some additional bands were visualized by the immunoreactions and their molecular weight was more than 35 kDa. Especially β-glucan preparation "Betaven" is enriched with these large proteins in comparison to other samples (Fig. 1).

The Western blot analysis was performed with a polyclonal anti-wheat gluten antibody, which recognized proteins extracted from all the samples. Even prolamins of the oat gluten-free flour reacted with that antibody. Moreover, β-glucan preparation "Betaven", which contains approximately 60% of dietary fiber and 20% of proteins, reacts very strongly with the antibody and some additional protein bands were visualized in comparison to other samples (Fig. 1).

The cross-reactivity of all the samples with the monoclonal R5 anti-gliadin antibody was investigated by ELISA assay. Additionally, prepared experimental bakery products (muffins) were tested to check their safety from the perspective of gluten-like proteins. The majority of examined samples has a gliadin-like protein content within the range of 80–260 mg/kg, excluding gluten-free flours and muffins prepared with these gluten-free flours. Altogether, β-glucan preparation "Betaven" has extremely high level of gliadin-like proteins, which corresponds to the immunoblot results. Quantitative data obtained from the ELISA analysis are presented in Table 3.

The ELISA assay is based on a monoclonal antibody R5, which recognizes epitopes of amino acid sequences QQPFP, QQQFP, LQPFP, and QLPFP in wheat gliadins and corresponding proteins from barley (hordeins) and rye (secalins) (Kahlenberg et al., 2006; Konic-Ristic et al., 2009; Van Eckert et al., 2010). Although R5 antibody is declared to have no cross-reactivity with oat proteins, avenins may contain QQQPF sequences and they can also react properly with R5

Table 2. Amino acid composition of prolamins extracted from oat raw materials

| Amino acid	Amino acid amount*, % of protein				
	gluten-free oat flour	commercial oat flour	oat flakes	oat residual flour	"Betaven"
Asx**	2.70 ±0.09 b***	1.76 ±0.01 a	1.81 ±0.03 a	1.76 ±0.05 a	1.79 ±0.05 a
Thr	1.66 ±0.08 b	1.26 ±0.02 a	1.28 ±0.01 a	1.29 ±0.02 a	1.27 ±0.01 a
Ser	2.50 ±0.05 c	1.77 ±0.04 a	1.82 ±0.03 a	1.79 ±0.03 a	1.94 ±0.03 b
Glx**	41.58 ±0.53 a	45.33 ±0.07 c	45.37 ±0.09 c	45.29 ±0.11 c	44.58 ±0.11 b
Pro	8.79 ±0.19 a	8.85 ±0.13 a	8.69 ±0.10 a	8.71 ±0.02 a	9.81 ±0.07 b
Gly	1.62 ±0.02 d	0.90 ±0.01 a	0.95 ±0.01 b	0.90 ±0.01 a	1.09 ±0.01 c
Ala	3.98 ±0.01 b	3.50 ±0.02 a	3.50 ±0.05 a	3.53 ±0.01 a	3.54 ±0.01 a
Val	6.67 ±0.07 b	6.93 ±0.02 a	6.90 ±0.01 a	6.97 ±0.03 a	6.54 ±0.05 a
Ile	3.03 ±0.02 a	3.09 ±0.01 b	3.09 ±0.03 b	3.07 ±0.01 ab	3.21 ±0.01 c
Leu	10.18 ±0.05 a	11.41 ±0.02 c	11.40 ±0.06 c	11.37 ±0.03 c	11.17 ±0.04 b
Tyr	2.65 ±0.02 c	2.21 ±0.01 a	2.24 ±0.01 a	2.27 ±0.01 a	2.36 ±0.01 b
Phe	7.28 ±0.10 b	7.22 ±0.04 ab	7.19 ±0.06 ab	7.25 ±0.01 ab	7.11 ±0.03 a
His	2.49 ±0.05 d	1.92 ±0.01 c	1.69 ±0.02 b	1.84 ±0.01 c	1.50 ±0.08 a
Lys	1.15 ±0.01 e	0.65 ±0.01 a	0.75 ±0.01 c	0.70 ±0.02 b	0.79 ±0.02 d
Arg	3.73 ±0.03 c	3.19 ±0.04 a	3.30 ±0.03 b	3.25 ±0.03 ab	3.30 ±0.07 b
Protein content in dry extract* % w/w	9.81 ±0.14 a	42.76 ±0.14 e	30.35 ±0.17 b	41.60 ±0.36 d	36.93 ±0.28 c

*Without Trp, Cys, Met.

**Asx = Asp + Asn, Glx = Glu + Gln.

***a, ..., d – mean values in rows and denoted by different letters differ statistically significantly at α ≤ 0.05.

antibody (Comino et al., 2011; Ellis et al., 1998; Osman et al., 2001). However, some doubts can occur in case of the extrapolation of the results obtained with gliadin-detecting antibodies in order to measure the toxicity of other protein species. Nevertheless, the ELISA method still remains the best way of the quantitative determination of the prolamin content in cereal raw materials and foods.

According to Codex Alimentarius (Codex Stan 118:1979) and the EU regulation (the European Commission regulation No. 41/2009), only food whose gluten content is less than 20 mg/kg is considered gluten-free. In the examined oat raw materials and foods the contents of immunoreactive amino acid sequences exceeded the limit except for gluten-free flours (oat and the prepared mixture) and the muffins based on gluten-free flours. Unfortunately, the rest of oat raw materials and bakery products cannot be considered gluten-free. Additionally, in our experiment the amount of prolamins determined in bakery products does not directly correspond to the amount of these proteins in the used flours. Probably processing can change the yield of the extraction of proteins, although an extraction solution should provide so strong denaturing and reducing conditions to be efficient for different matrices. Usually the heat processing results in cross-linked and denatured proteins, which are more difficult to dissolve in extraction

A

B
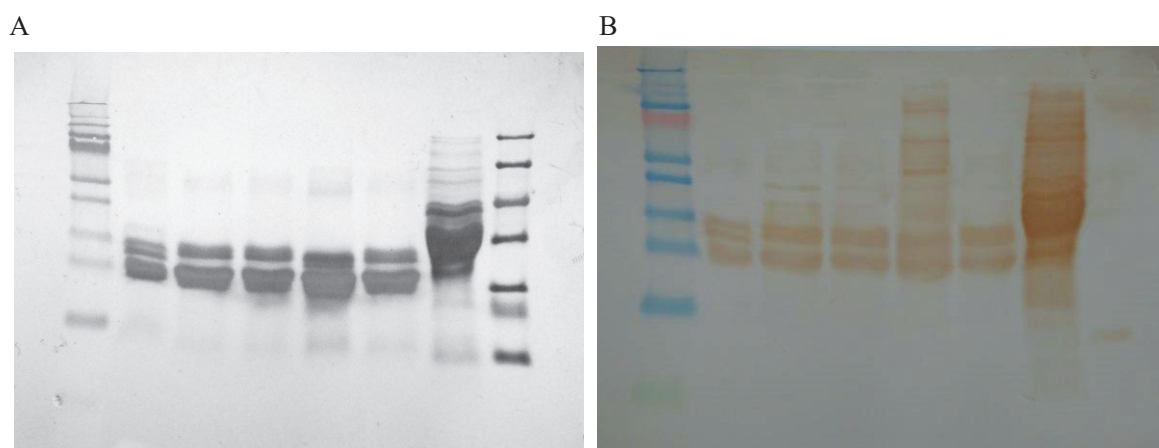
Fig. 1. SDS-PAGE transferred on the PVDF membrane (A) and the Western blot of prolamins extracted form oat raw materials (B) (the amounts of protein/lane are in brackets). Lanes: 1 – pre-stained molecular weight markers (170, 130, 100, 70, 55, 40, 35, 25, 15, 10 kDa); 2 – oat gluten-free flour (3.9 µg); 3 – oat commercial flour (6.4 µg); 4 – oat residual flour (7.9 µg); 5 – Betaven (14.8 µg); 6 – oat flakes (3.7 µg); 7 – a positive control sample – wheat flour (17.4 µg); 8 – molecular weight markers (116, 66.2, 45.0, 35.0, 25.0, 18.4, 14.4 kDa, invisible on blot)

Table 3. Gliadin-like proteins content in examined oat raw materials and experimental bakery products (muffins)

Oat raw materials*	Gliadin-like protein content mg/kg	Gluten content mg/kg
Oat commercial flour	261.1	522.2
Oat residual flour	145.3	290.6
Oat flakes	158.3	316.6
β-glucan preparation "Betaven"	>1 000	>2 000
Oat gluten-free flour	7.3	14.6
Gluten-free mixed flour	1.9	3.8
Wheat commercial flour 650	41 963.5	83 927
Experimental bakery products**	Gliadin-like protein content mg/kg	Gluten content mg/kg
Muffins with gluten-free mixed flour	7.7	15.4
Muffins with oat commercial flour	83.0	166.0
Muffins with oat residual flour	144.6	289.2
Muffins with oat gluten-free flour	6.4	12.8

 *Results for commercial raw materials.
 **Results for air-dry weight of products.
 Gluten content is calculated by multiplying gliadin content by factor 2.

solutions. Uniquely in case of oat residual flour it seems that the extraction is better in processed bakery products than in the "native" raw material. Possibly, the small size of flour particles can explain this observation as denatured and cross-linked proteins were more susceptible to extraction. Similarly, a high recovery was also observed for gluten-free flours and products, although there are very low levels of prolamin proteins detected. Moreover, β-glucan preparation "Betaven" has a very high content of prolamins, and it certainly cannot be consumed by celiac people. Another reason for the differences in gluten content in the raw material and the final product is the possibility of contamination. In this study, we wanted to reconstruct the home conditions, where in the same places, except gluten-free flour also wheat and rye flours are used. Although all precautions to prevent contamination during baking was taken, earlier processing of wheat or rye can affect the samples. On the other hand, ELISA results obtained for muffins prepared with gluten-free flours suggest, that avoiding of such a contamination is possible. There is only a few publications illustrating the content of gluten in products prepared at home, particularly bakery products. It was shown that there is a slight risk of contamination of gluten-free products at home, but exact conditions of the preparation were not described (Sdepanian et al., 2001).

Other researchers used the ELISA R5 method for the detection of gluten-like proteins in seeds of a wide spectrum of oat varieties and proved that a concentration of these proteins differs greatly among varieties. The content of prolamin-like proteins can vary from less than 3 mg/kg to more than 80 mg/kg (Ballabio et al., 2011). Although it appears to be possible to find many oat varieties potentially non-toxic from the perspective of the prolamin content, the immunoreactivity of oat food products can be quite different and data about gluten content in oat products are hardly available besides some data concerning the number of oat products from the Swedish market. The analyzed products included oat rolls and bran, oat meals (porridge, gruel), oat crunch and drink. Only 50% of tested products were really gluten-free, 37% had gluten content in range of 20–200 mg/kg and 13% of above 200 mg/kg. The gluten content varied strongly among the samples from below 20 mg/kg to

over 4000 mg/kg. It was found by the PCR method that these products are in majority contaminated by wheat, barley and rye. Since the ELISA used in that study was based on an anti-ω-gliadin monoclonal antibody, any results can be under- or overestimated as well (Størsrud et al., 2003b).

Similar results were obtained in a study performed in Canada, by R5-ELISA and AOAC-ELISA (Gélinas et al., 2008). Among twelve examined oat products, only four contained less than 20 mg gluten/kg of product, in other oat products gluten content was in the range 33–2827 mg/kg (R5-ELISA method). According to studies by other authors (Gelinas et al., 2008; Størsrud et al., 2003b), which is also confirmed by our research, oats and oat products must be carefully tested before inclusion in the diet of people with celiac disease. Contaminations are in fact still a problem during the production of flour and other oat products.

According to the Codex Alimentarius (Codex Stan 118: 1979), the oat contain gluten proteins but it may be well tolerated by most people with gluten intolerance. Therefore, the allowance of oats that are not contaminated with wheat, rye or barley in foods covered by this standard may be determined at the national level. However, according to the European Commission regulation (No. 41/2009) the presence of gluten in oat grain is not scientifically confirmed, but not everyone can eat it. Moreover, oat destined for people with celiac disease should be produced, prepared and processed in a special way, so the contents of gluten can not be greater than 20 mg/kg.

To summarize, our study demonstrated that each oat raw material or product has a different immunoreactivity. Before including into diet, it is important to test its source. Suitable variety selection and avoiding of raw material contamination (especially during processing) allows to obtain the products safe for people with celiac disease. The inclusion of oat to the diet of celiac patients may also bring a number of health benefits because of very high nutritional value of oat grains.

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