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PROTEIN COMPOSITION OF SORGHUM AND OAT GRAIN AND THEIR SUITABILITY FOR GLUTEN-FREE DIET

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Abstract

A collection of 12 oats and 6 sorghum species and varieties was evaluated for the suitability for a gluten-free diet. Quantitative evaluation of storage proteins in the oats collection with the use of SDS-PAGE electrophoresis showed the percentage of the most coeliacally active protein components - prolamins+LMW glutelins - between 53.43 and 74.96 % of the total storage proteins, in the sorghum collection between 32.25 and 41.36 % of the total storage proteins. Immunological determination of the quantity of prolamins in oat grains by ELISA varied from 5.57 to 35.60 mg 100 g^{-1} of sample, in sorghum grains from 1.97 to 3.30 mg 100 g^{-1} of sample. Based on these results, the use of oats in a gluten-free diet can be risky and the use of sorghum should be safe.

Key words: gluten-free diet, oats, sorghum, protein composition, immunological evaluation

Introduction

Coeliac disease (gluten-sensitive enteropathy, coeliac sprue) is defined as a permanent intestinal intolerance of gluten contained in some cereal species. The coeliac disease is manifested both in children and adults, with the highest incidences in people around 40-years of age. The consumption of food containing gluten results in the damage to intestinal mucous membrane /Tlaskalová et al., 1999/.

Gluten is a specific protein complex of the cereal grain. Coeliac disease is an intolerance to certain chains of amino acids found mostly in prolamin (gliadin) fraction of wheat gluten and the corresponding protein fractions of other species from the first group of cereals (barley, rye and probably oats) /Tlaskalová et al., 1999/.

The cereals of the second group include thermophilous, mostly short-day, plants such as maize, rice, sorghum, millet, foxtail millet and others, predominantly grasses of tropical and subtropical zones; intolerance to gluten is mostly not manifested in these plants and, consequently, they can be used for a gluten-free diet /Petr et al., 2004/.

Selection of raw materials for a gluten free diet is limited and for this reason it is desirable to find other sources. In this direction, the use of maize and rice is the most widespread. In the remaining species, the possibility of utilization is presumed, but it has not been adequately tested. Great attention should be paid to sorghum in respect of its usage in the gluten-free diet. Even though it is a thermophilous cereal, it is fast

expanding in Europe, where it is cultivated on 220 000 ha. It gives high yields and the technology is identical with that used for maize. Similarly to maize, a change in its cultivation can also be seen with an expansion into zones situated more to the north, owing to its higher tolerance to cold and the earliness of new hybrids. The possibility of using of sorghum for a gluten-free diet was derived from the common use of maize and rice from the same group of cereals. However, its suitability should be tested in respect of its composition of the protein complex and immunology for the elimination of the coeliac disease /Petr et al., 2003; Petr et al., 2004/.

Serious consideration should also be given to oats. Oat grains have a high content of proteins whose qualitative composition, in relation to the consumer's body, is more favourable than in the remaining cereals - wheat, rye and barley. In addition, oat grains are marked by a high content of dietary fibre and the highest content of fats of all cereals. The contents of Mg, Fe, P, Ca and vitamins E and B_1 are also higher in oats compared with other cereals /Moudrý, 2003/.

The discussions of the possibility of the use of oats for the coeliac diet by medical specialists and researchers in different countries have been going on for more than 40 years and the conclusions are still not unambiguous /Thompson, 1997; Kaukinen, Collin, 2001/.

Regarding the suitability of oats for the gluten-free diet, the primary characteristics reside in the composition of the protein fractions – albumins, globulins, prolamins (avenins) and glutelins. With respect to coeliac disease, they belong to the most active fractions of prolamin proteins of relatively low molecular weights about 30 kDa. Prolamins are marked by a high percentage of glutamic acid, glutamin and proline. On the other hand, they have a low content of essential amino acids, above all of lysine. Susceptibility to hydrolysis and hence also digestibility of the prolamin proteins are very low as proved by experiments with laboratory animals /Michalík, Karlubík, 1988/.

The present studies assent to the fact that the prolamin content in oats is lower compared with that in wheat, rye or barley /Shewry, 1995; Thompson, 1997/. Kumar and Farthing (1995) pointed out that if avenins (oat prolamins) are responsible for oats toxicity in coeliac patients, much higher amounts of oats should be consumed than of rye or barley to manifest identically "deleterious" effects.

Based on the trials of Baker and Read (1976), they recommended a considerable reduction of oats in the food for coeliac patients. On the other hand, Janatuinen et al. (1995) in their experiments with daily uptake of oats from 50 to 70 g did not find any deleterious effect on intestinal mucous membrane, though they claimed that a higher oats consumption should be toxic for coeliac patients due to the similarity of the sequence of peptides in oats and wheat. According to Ryan (1996), however, problems may be caused by contamination of oats by wheat during harvest or processing. It is necessary to exercise maximum caution in these processes.

As mentioned above, the basic treatment of the coeliac disease is a life-long gluten-free diet. The treatment by gluten-free diet is successful if both the diagnosis is made and life-long gluten-free diet is introduced at an early stage. The gluten-free diet allows recovery to normal life without any handicaps and prevents serious complications. The "naturally gluten-free" food may contain no more than 2 mg of gliadin per

100 g of the sample. The food with "decreased gliadin content" may contain up to 10 mg of gliadin per 100 g of the sample (Codex alimentarius WHO/FAO2000).

Materials and methods

The suitability to the coelic diet was evaluated in 12 oats species and varieties of different provenance (Table 1) and 6 sorghum varieties (Table 2) in respect of the protein complex composition and immunological evaluation.

Table 1. Survey of evaluated oat samples
1 lentelė. *Įvertintų avižų bandinių apžvalga*

Sample No. *Bandinio nr.*	Oat species *Avižų rūšis*	Genotype *Genotipas*	Origin *Kilmė*
1	*Avena sativa* L.	Auron	Czech Rep. *Čekijos Respublika*
2	*Avena sativa* L. var. *aristata* Krause	Achilles	New Zealand *Naujoji Zelandija*
3	*Avena sativa* L. var. *brunnea* Koern.	Pelso	Finland / *Suomija*
4	*Avena sativa* L. var. *flava* Koern.	Populatic Ratbar	Romania / *Rumunija*
5	*Avena sativa* L. var. *inermis* Koern..	Vir K 1932 Local	China / *Kinija*
6	*Avena sativa* L. var. *aurea* Koern.	Sto Alexio	Spain / *Ispanija*
7	*Avena sativa* L. var. *obtusata* Alef.	Dippawski	Poland / *Lenkija*
8	*Avena sativa* L. var. *mutica* Alef.	Selekty Horsky	Czechoslovakia *Čekoslovakija*
9	*Avena nuda* L.	Abel	Czech Rep. / *Čekijos Resp*.
10	*Avena nuda* L.	Izak	Czech Rep. / *Čekijos Resp.*
11	*Avena sativa* L. var. *montana* Alef.	Mesdag	France / *Prancūzija*
12	*Avena sativa* L.	Ankara	Turkey / *Turkija*

Table 2. Survey of evaluated sorghum samples
2 lentelė. *Įvertintų sorgų bandinių apžvalga*

Sample No. *Bandinio nr.*	Sorghum species *Sorgų rūšis*	Genotype *Genotipas*	Origin *Kilmė*
1	*Sorghum bicolor* (L.) Moench	Bianca	Hungary / *Vengrija*
2	*Sorghum bicolor* (L.) Moench	GK Csaba	Hungary / *Vengrija*
3	*Sorghum bicolor* (L.) Moench	GK Zsófia	Hungary / *Vengrija*
4	*Sorghum bicolor* (L.) Moench	GK Zsófia F_1Hybrid	Hungary / *Vengrija*
5	*Sorghum bicolor* (L.) Moench	GK Altföldi	Hungary / *Vengrija*
6	*Sorghum bicolor* (L.) Moench x *Sorghum vulgare* Pers. var. *sudanense*	Bovital	Hungary / *Vengrija*

The seed of oats was obtained from the gene bank of the Research Institute of Crop Production in Prague and from the varietal experiments of the Czech Institute for Supervising and Testing. The seed of grain sorghum was obtained from the Cereal Research Institute in Szeged (Hungary) and from the gene bank of the Research Institute of Crop Production in Prague.

The seed samples of the above mentioned sorghum and oats species and varieties were used in the field experiments at the experimental station of the Czech University of Agriculture in Prague – Uhříněves. The Prague – Uhříněves experimental station is located on the outskirts of Prague, in the sugar beet region (elevation above sea-level 295 m, average annual temperature 8.4°C, average annual precipitation totals 575 mm). The weather pattern in the experimental years 2002-2004 at the Prague – Uhříněves experimental station is presented in Table 3. Oats and sorghum were sown after a cereal forecrop. The area of the experimental plots was 15 m^2 with 4 replications in oats and 30 m^2 with 4 replications in sorghum. Experimental plots 15 m^2 are usually used in the field experiments with the first group of cereals, which are cultivated in narrow rows (125 mm). In the case of the second group of cereals (sorghum), it is better to use bigger area of experimental plot, with respect to very different character of plants and growth. In our experiments sorghum was cultivated at an inter-row distance of 750 mm, height of plants of some varieties was about 2 m, number of plants per m^2 only about 8-11. In this case, an experimental plot area of 30 m^2 is able to give more representative character of growth, compared with a smaller experimental plot.

Experiments were carried out using organic farming system, and the principles of IFOAM (International Federation of Organic Agriculture Movements), neither mineral nor organic fertilizers and pesticides were applied.

After the harvest, 500 g of sorghum and oats grain samples from each of the four replications were taken and used for the following laboratory analyses:

Table 3. Weather pattern at the Prague – Experimental Station during the years 2002-2004 and the long-term average (1950-2000)
3 lentelė. *Prahos Uhříněves bandymų stoties metereologiniai duomenys 2002-2004 periodu ir daugiametis vidurkis (1950-2000)*

Month *Mėnuo*	Monthly average temperature (°C) *Vidutinė mėnesio oro temperatūra (°C)*			Sum of precipitation (mm) *Kritulių suma (mm)*			Long-term average of temperature (°C) *Daugiametis temperatūrų vidurkis (°C)*	Long-term sum of precipitation (mm) *Daugiametė kritulių suma (mm)*
	2002	2003	2004	2002	2003	2004		
January / *Sausis*	0.78	-0.66	-2.93	25.4	29.4	54.8	-2.1	28
February / *Vasaris*	5.00	-2.70	2.70	30.9	5.3	25.1	-0.8	27
March / *Kovas*	5.52	5.40	4.25	13.0	7.9	42.4	3.4	31
April / *Balandis*	9.15	9.05	10.27	71.6	22.2	15.9	8.2	46
May / *Gegužė*	16.54	16.55	12.73	67.3	72.8	54.8	13.4	65
June / *Birželis*	18.57	20.97	17.04	71.9	30.9	90.2	16.3	74
July / *Liepa*	19.55	21.00	18.91	97.5	76.0	35.4	18.2	74
August / *Rugpjūtis*	19.85	21.82	19.82	71.5	26.5	56.6	17.5	72
September / *Rugsėjis*	13.22	14.48	14.39	75.0	37.3	43.2	14.0	49
October / *Spalis*	8.36	6.46	10.01	22.4	30.1	20.5	8.6	41
November / *Lapkritis*	5.25	5.18	4.68	41.2	7.2	68.7	3.2	34
December / *Gruodis*	-1.37	0.91	0.78	49.2	33.2	12.6	-0.5	34

1. Composition of grain storage proteins using electrophoretic analysis – SDS-PAGE method

Storage proteins are analysed from individual samples by the standard vertical electrophoresis in polyacrylamide gel in the presence of SDS (dodecyl sulfate sodium salt) as a surfactant eliminating a charge, according to method of Wrigley (1992). This method is recommended by the international organization ISTA (International Seed Testing Association). For quantitative evaluation of individual protein subunits special software Bio1D (firm Vilber-Lourmat, France) was used.

Whole, individual grains were used for electrophoretic analysis. From the grain samples from each of 4 replications of the field experimental plots were taken 4 fully developed grains without biological or mechanical damage. Each grain means one sample for electrophoretic analysis - evaluation of each sorghum or oats variety in total consisted of 16 replications (4 analyses from the each of 4 experimental plots).

Individual grain was crushed by hammer and crushed material (about 30 mg) was extracted in a microcentrifuge tube (100-120 μl) for 30 minutes with SDS extraction buffer, which consisted of 1M Tris(hydroxymethyl)aminomethane-HCl (pH 6.8), 2 % SDS (dodecyl sulfate sodium salt), glycerol, mercaptoethanol, pyronin and distilled water. Then the sample was boiled for 10 minutes and centrifuged (10 minutes, 15000 rps). Clear supernatant (15 μl) was pipetted on the polyacrylamide gel. This gel consisted of starting part, which was prepared from 11.43 ml of 1 M Tris (hydroxymethyl)aminomethane-HCl (pH 8.8), 17.48 ml of NN-methylendiacrylamide, 0.30 ml of 10 % dodecyl sulfate sodium salt, 0.76 ml of 1 % amonium persulfate and 0.06 ml of NNNN-tetramethylene diamine and separating part, which was prepared from 12.36 ml of 1M Tris(hydroxymethyl)aminomethane-HCl (pH 6.8), 8.30 ml of NN-methylendiacrylamide, 0.10 ml of 10 % dodecyl sulfate sodium salt, 0.80 ml of 1 % amonium persulfate and 0.03 ml of NNNN-tetramethylene diamine. The electrophoresis was run for 10 hours with maximum voltage 500 V and 5 mA per gel.

After electrophoresis finishing electrophoreograms were coloured, then dried and transferred by means of the special camera into software Bio1D /Lourmat, 1999/ for quantitative evaluation of individual protein subunits. In this software electrophoreogram (Fig. 1) is transfered to the graphic form (Fig. 2). Separation of individual protein subunits during electrophoresis runs according to their molecular weight – first are separated HMW glutelins, then LMW glutelins+prolamins and finally residual albumins and globulins. An expert in SDS-PAGE method is able to specify position of individual subunits on the basis of their specific characteristics; software is able to calculate their percentage according to number and area of individual peaks.

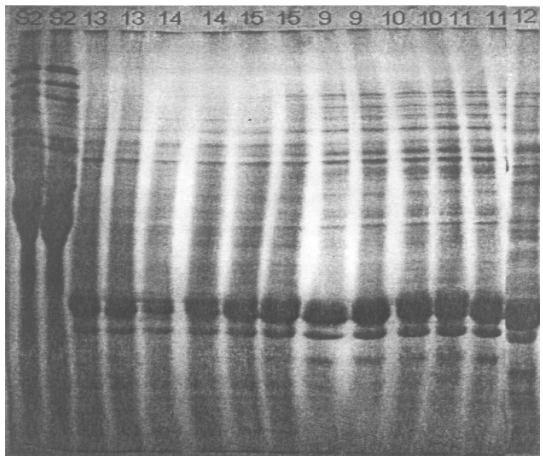

Figure 1. An example of electrophoreogram of SDS-PAGE method (each of bands is one evaluated sample)
1 paveikslas. *SDS-PAGE metodu gautos elektroforegramos pavyzdys (kiekvienas takelis atskiras bandinys)*

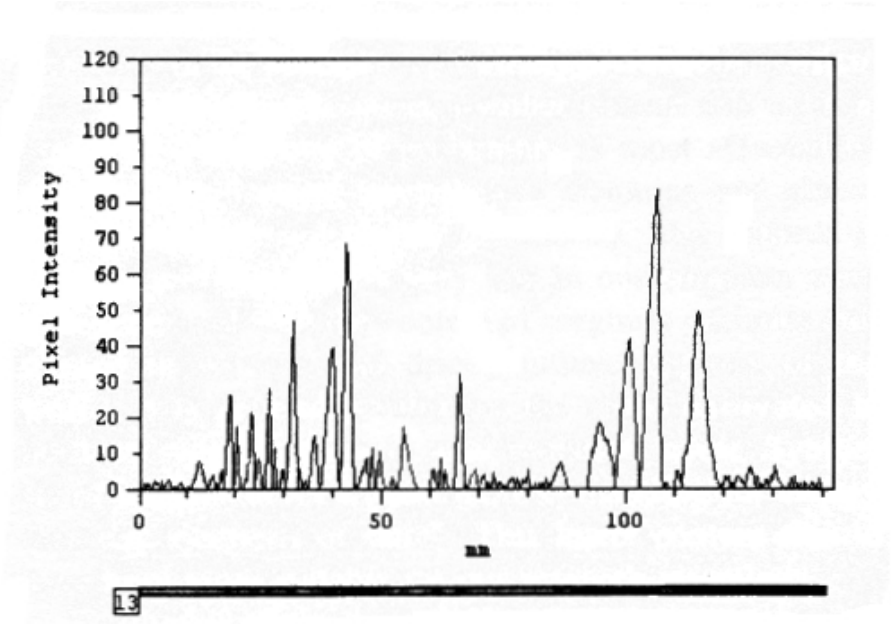

Figure 2. An example of one sample from electrophoreogram, transferred to the graphic form in evaluation software
2 paveikslas. *Vieno bandinio elektroforegramos grafinė išraiška pagal vertinimo programą*

2. Immunological determination of the prolamin quantity (ELISA method)

The principle of the test resides in the reaction in which commercially produced monoclonal anti-gliadin antibodies detect certain chains of amino acids, which are found mostly in prolamin (gliadin) fraction of the cereals and which are toxic for the coeliac patients.

For the determination, the same oats and sorghum grain samples as for electrophoresis were used. About 100 g of grain sample was milled using a laboratory mill. The obtained whole-grain sorghum and oats flour was used for analysis - 1 g of flour was extracted with 10 ml of 60 % ethanol and then centrifuged. After centrifugation the supernatant was pipetted on a special micro-plate with the anti-gliadin antibody and a special colour (the commercial kits for detection of gliadins were used). Kits contain control sample, special colour and monoclonal anti-gliadin antibody in lyophylized form; dilution of antibody is necessary before pipetting on the micro-plate (10 μl of anti-gliadin antibody + 2 ml of distilled water). After 180 minutes of rising (developing of colour reaction between the supernatant and anti-gliadin antibody) reaction was terminated. Then the whole micro-plate with evaluated samples was put to the special computer reader and through the special software the prolamin quantity was calculated according to the colour scale, which is part of software.

Analysis of variance of double classification was used for statistical evaluation (Statgraphics Plus, Version 5.1 statistical programme). Values of all 16 replications (4 individual analyses from each of 4 field experimental plots) were implicated in statistical evaluation (in Tables 4-7 only average values of evaluated varieties were used). Least significant differences in the protein subunits and prolamins quantity among the evaluated varieties and among the experimental years were calculated by the LSD test, at the significance level $\alpha = 0.05$.

Results and discussion

Possibilities of oats use in a gluten-free diet

The question of the cereal protein composition and its quantification is still problematic. Webster (1986) reported that the protein fractionations obtained with Osborne method, based on the solubility of individual protein fractions and the homogenous polypeptides detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) are hardly comparable.

Based on the existing knowledge, coeliacally active protein components are present particularly in the prolamin protein fraction with relatively low molecular weight about 30 kDa. However, many recent studies assent to the fact, that the representation of prolamins in the oat grains is lower compared with wheat, barley and rye /Shewry, 1995; Thompson, 1997; Štěrba, 2003; Moudrý, 2003/.

Prolamins are classified into α-, β- and γ-prolamins containing intramolecular disulfidic bonds and ω-prolamins that do not contain these bonds. It was found that α-, β- and γ-prolamins contain coeliacally toxic sequences of amino acids – in the case of oats it is the sequence – Gin-Gin-Gin-Pro (Gin = glutamine, Pro = proline). Glutelins are divided into two groups – high-molecular weight (HMW) and low-molecular weight

(LMW) ones. They differ from prolamins by that they contain not only intramolecular, but also intermolecular disulfidic bonds /Skerritt et al., 1990; Rottmann, 1996/.

According to some authors, glutelins are a major protein fraction of oat grains /Wieser et al., 1980; Štěrba, 2003/. On the other hand, the results of Peterson and Brinegar (1986) and Sanne (1988) indicated that globulins are a prevailing fraction of proteins of the oat grains.

In the SDS-PAGE method of electrophoretic analysis, it is not possible to separate prolamins and LMW glutelins, due to the similarity of their molecular weights; that is why these protein subunits are presented commonly. The percentage of LMW glutelins+prolamins in our oat samples ranged from 53.43 % to 74.96 % of total storage proteins (Table 4). These values can be considered risky in respect of coeliac disease.

Table 4. Quantitative evaluation of SDS-PAGE electrophoretic analysis of the oats storage proteins. Analysis of variance – significant differences in protein subunits among evaluated varieties and years (LSD, $\alpha = 0.05$)

4 lentelė. *Avižų atsarginių baltymų SDS-PAGE elektoforegramų kiekybinė analizė. Dispersinė analizė – patikimi baltymų sudėtinių dalių skirtumai tarp įvertintų veislių ir metų (LSD, $\alpha = 0,05$)*

	Sample No. *Bandinio nr.*	HMW Glutelins DMM gliutelinai			LMW Glutelins +Prolamins *MMM gliutelinai+ prolamin*ai			Residual Albumins+Globulins *Liekamieji albuminai+globulinai*		
		$\bar{x}$	LSD	signifi-cance *patikimumas*	$\bar{x}$	LSD	signifi-cance *patikimumas*	$\bar{x}$	LSD	signifi-cance *patikimumas*
Variety *Veislė*	1	1.73		a	60.51		bcde	37.76		fgh
	2	1.93		a	57.90		abc	40.18		ghi
	3	3.03		ab	69.07		f	26.23		ab
	4	1.99		a	66.08		ef	31.94		cde
	5	1.80		a	64.46		def	33.74		def
	6	3.37	1.33	b	59.28	5.87	abcd	37.35	5.58	afgh
	7	2.92		ab	53.43		a	43.65		i
	8	2.79		ab	69.98		fg	27.23		abc
	9	2.59		ab	61.82		cde	35.60		defg
	10	2.81		ab	55.32		ab	41.87		hi
	11	3.06		ab	65.24		ef	31.69		bcd
	12	1.78		a	74.96		g	23.26		a
Year *Metai*	2002	2.66		a	60.09		a	36.83		b
	2003	2.32	0.67	a	66.38	2.93	c	31.30	2.79	a
	2004	2.46		a	63.04		b	34.50		b

$\bar{x}$ = average values of the percentage of individual protein subunits (%) in evaluated varieties and years; LSD = least significant difference

$\bar{x}$ = atskirų baltymų sudedamųjų dalių, įvertintų procentais, vidutinės vertės veislėms ir pagal metus; LSD = mažiausias patikimas skirtumas

Percentage of HMW glutelins in oat grain is low; from the coeliac point of view HMW glutelins do not play an important role, because the coeliacally active protein components are not present in them.

Albumins and globulins are protein fractions with the most suitable amino acid composition, in relation to the consumer's body /Moudrý, 2003; Štěrba, 2003/. Oat samples evaluated in our study reached between 23.26 and 43.65 % of these fractions.

It is evident from our results, that the variability in protein composition among evaluated species and varieties was higher than the variability among evaluated experimental years. These results indicate relatively high genetic dependence of oats protein composition and coincide with the findings of Briggle et al., (1975), who evaluated a wide range of the protein concentrations and compositions in relation to the species and variety.

On the other hand, protein composition, especially the percentage of LMW glutelins+prolamins and albumins+globulins, were also influenced by the environmental conditions, mainly by the weather pattern during the growing season.

On the basis of the survey of the weather pattern at the Prague - Uhříněves Experimental Station (Table 3), the higher percentage of LMW glutelins+prolamins in the experimental year 2003 seems to be positively influenced by the very high air temperature during the time of grain formation – June, July and beginning of August.

Immunological determination of the quantity of prolamins (respective quantity of coeliacally active parts of prolamins) in oat grains by ELISA is an important indicator for the assessment of the suitability for the coeliac disease (Table 5).

It can be seen that a distinct occurred among the different oat samples assessed. The values of some samples (samples No. 2, 7, 10) were below the limit for gluten-free diet – 10 mg of prolamins (gliadins)/100 g of sample, and thus they should be suitable for the gluten-free diet. On the other hand, other evaluated samples exceeded the limit, some of them – No. 3, 4, 8, 11, 12 very significantly, and their usability for the diet in coeliac disease did not come into account. As in the case of protein composition, higher differences were found among the evaluated varieties compared with the differences among the evaluated experimental years.

The comparison of the results of the protein composition with the results of the immunological evaluation is interesting mainly in view of the interpretation of their toxicity and the assessment of the suitability for a gluten-free diet. It can be presumed that the starting mechanism of coeliac disease resides in the presence of protein fragments with a high frequency of glutamin and proline and a certain conformational state of these proteins. A pathological reaction is triggered by their interaction with receptors of the small intestine. A great variability was unambiguously found in the results obtained in the evaluated collection of species and varieties of oats concerning the structure of the protein complex and the results of immunological testing. In addition, a significant effect of the year on the results of analyses was evident. Based on our results, the use of oats in the diet for coeliac disease can be very risky for these reasons.

Table 5. Immunological evaluation (ELISA) of prolamins quantity in the oats grains. Analysis of variance - significant differences among evaluated varieties and years (LSD, $\alpha = 0.05$)

5 lentelė. *Prolaminų kiekio avižų grūduose imunologinis įvertinimas ELISA metodu. Dispersinė analizė – patikimi skirtumai tarp įvertintų veislių ir metų (LSD, $\alpha = 0,05$)*

	Sample No. *Bandinio nr.*	Quantity of prolamins *Prolaminų kiekis*		
		$\bar{x}$	LSD	significance *patikimumas*
Variety / *Veislė*	1	14.47		ab
	2	8.73		ad
	3	21.17		b
	4	20.97		b
	5	18.00		bc
	6	10.90		acd
	7	5.57	7.19	d
	8	29.73		e
	9	14.56		abc
	10	8.97		ad
	11	19.43		bc
	12	35.60		e
Year / *Metai*	2002	14.31		a
	2003	21.31	3.60	b
	2004	16.58		a

$\bar{x}$ = average values of the prolamins quantity (mg 100 g^{-1} of sample) in evaluated varieties and years; LSD = least significant difference

$\bar{x}$ *= vidutinės prolaminų kiekio vertės (mg 100* g^{-1} *medžiagos) įvertintų veislių augaluose ir pagal metus; LSD = mažiausias patikimas skirtumas*

Possibilities of sorghum use in a gluten-free diet

Compared with wheat gliadins, prolamins of sorghum grain differ by the structure of amino acids. The differences consist in the contents of aspartic and glutamic acids, glycine, alanine, cysteine, leucine and tryptophan. The occurrence of different polypeptide fragments is also presupposed. Despite the presence of prolamin proteins, their sequential domains are not compatible with the gliadin fragments. Prolamins of sorghum grains are represented by the components of α-caphirine, soluble in 95 % ethanol, analogously to α-zeins of maize grain /Sastry, Virupakscha, 1969/.

Results of the quantitative evaluation of SDS-PAGE electrophoretic analysis of sorghum storage proteins are presented in Table 6. It is evident from these results, that albumins and globulins were the prevailing fractions of sorghum proteins – their percentage was between 54.50-62.87 % of total storage proteins. Percentage of LMW glutelins+prolamins varied from 32.25 to 41.36 %. Percentage of HMW glutelins was low – only between 4.01 and 4.88 % of total storage proteins.

These results are in accordance with the findings of Petr et al. (2003), who evaluated protein composition in a collection of varieties of grain sorghum (*Sorghum*

bicolor L. Moench) and silage (sweet) sorghum (*Sorghum saccharatum* L. Moench), and found percentage of albumins+globulins in grain of sorghum varieties between 50.84 and 73.90 %, LMW glutelins+prolamins between 26.10 and 49.32 % and HMW glutelins between 0.00-4.67 % of total storage proteins. Percentage of albumins+ globulins in evaluated varieties of silage sorghum varied from 40.75 to 56.10 %, LMW glutelins+prolamins from 39.39 to 53.68 % and HMW glutelins from 4.51 to 5.57 % of total storage proteins.

Table 6. Quantitative evaluation of SDS-PAGE electrophoretic analysis of sorghum storage proteins. Analysis of variance – significant differences in protein subunits among evaluated varieties and years (LSD, $\alpha = 0.05$)

6 lentelė. *Sorgų atsarginių baltymų SDS-PAGE elektroforegramų kiekybinė analizė. Dispersinė analizė – patikimi skirtumai tarp įvertintų veislių ir metų (LSD, $\alpha = 0,05$)*

	Sample No. *Bandinio nr.*	HMW Glutelins *DMM gliutelinai*			LMW Glutelins +Prolamins *MMM gliutelinai+ prolaminai*			Residual Albumins+ Globulins / *Liekamieji albuminai+globulinai*		
		$\bar{x}$	LSD	signifi-cance *patikimumas*	$\bar{x}$	LSD	signifi-cance *patikimumas*	$\bar{x}$	LSD	signifi-cance *patikimumas*
Variety *Veislė*	1	4.05		a	36.76		b	59.19		b
	2	4.01		a	37.88		bc	58.11		b
	3	4.59	0.51	bc	32.75	2.88	a	62.65	2.75	c
	4	4.88		c	32.25		a	62.87		c
	5	4.14		ab	41.36		d	54.50		a
	6	4.71		c	40.19		cd	55.10		a
Year *Metai*	2002	3.81		a	35.17		a	61.03		b
	2003	4.83	0.36	b	37.80	2.03	b	57.37	1.95	a
	2004	4.56		b	37.63		b	57.81		a

$\bar{x}$ = average values of the percentage of individual protein subunits (%) in evaluated varieties and years; LSD = least significant difference

$\bar{x}$ = atskirų baltymų sudedamųjų dalių procento vidutinės vertės įvertintų veislių augaluose ir pagal metus; LSD = mažiausias patikimas skirtumas

Immunological evaluation of the quantity of prolamins, respectively their fragments, which are toxic for the coeliac patients is a decisive criterion to determine the suitability for gluten-free diet. The results are presented in Table 7. It is evident from this table, that all values are below the limit for the gluten-free diet (10 mg 100 g^{-1} of sample). Similar results of immunological evaluation of grain sorghum and silage sorghum varieties were obtained by Petr et al., (2003) – in their collection of samples the amount of prolamins varied from 2.1 to 3.5 mg 100 g^{-1} of sample.

It can be said, that immunological determination has limits given by its specificity. Wheat gliadins are used to prepare antibodies that are used in ELISA kits. These antibodies can identify rye, barley and oats prolamins, but we cannot exclude the possibility that the newly proposed plant products (such as sorghum) do contain prolamins whose structure is not accurately recognized by anti-gliadins used in ELISA, exert some immunological activity in patients /Petr et al., 2003/.

Table 7. Immunological evaluation (ELISA) of prolamins quantity in sorghum grain. Analysis of variance - significant differences among evaluated varieties and years (LSD, $\alpha = 0.05$)

7 lentelė. *Prolaminų kiekio sorgų grūduose imunologinis įvertinimas ELISA metodu. Dispersinė analizė – patikimi skirtumai tarp įvertintų veislių ir metų (LSD, $\alpha = 0,05$)*

	Sample No. *Bandinio nr.*	Quantity of prolamins *Prolaminų kiekis*		
		$\bar{x}$	LSD	Significance *patikimumas*
Variety *Veislė*	1	2.13	0.40	a
	2	2.20		a
	3	1.97		a
	4	2.03		a
	5	3.27		b
	6	3.30		b
Year *Metai*	2002	2.18	0.28	a
	2003	2.57		b
	2004	2.70		b

$\bar{x}$ = average values of prolamins amount (mg 100 g^{-1} of sample) in evaluated varieties and years; LSD = least significant difference

$\bar{x}$ *= vidutinės prolaminų kiekio vertės (mg 100* g^{-1} *medžiagos) įvertintoms veislėms ir pagal metus; LSD = mažiausias patikimas skirtumas*

However, on the basis of information by Kabíček (2004), sorghum samples from the experiments of Petr et al., (2003) were used for clinical screening of 20 patients with coeliac disease, which consumed for 3 months in their daily diet 100 g of sorghum bread. This screening showed good results – after sorghum consumption the level of antigliadin antibodies in sera and spittlets of evaluated patients was not increased.

Moreover, our results showed a safe possibility of cultivation of early, Hungarian grain sorghum varieties in Czech warm sugar-beet growing region, sugar-beet and maize-growing regions of Moravia, where grain maize is cultivated with the number of earliness FAO to 300. The cultivation is identical with that of maize, it does not require any special technical equipment and machinery. The progress in breeding of hybrid varieties that are bred for earliness, among other things, is promising /Petr et al., 2003/.

Conclusions

1. On the basis on our results, the use of oats in the diet for coeliac disease can be risky due to the great variability in the results obtained in the evaluated collection of species and varieties of oats concerning the structure of the protein complex and the results of immunological testing.

2. Within the framework of the further examination of the possibilities of oat utilisation in a gluten-free diet, it is necessary to focus on the selection of oat genotypes, that should, in a stable way, under various soil-climatic conditions, reach the results suitable for the diet in coeliac disease. Only then it will be possible to carry out detailed clinical tests on selected groups of patients with coeliac disease to examine their reaction

and to determine the doses of oats acceptable for the patients with various forms of coeliac disease.

3. Our results, concerning the possibilities of the use of sorghum in gluten-free diet gave good results - all values of prolamins amount in sorghum grain were well below the limit for gluten-free diet (10 mg 100 g^{-1} of sample). Furthermore, our results showed that modern Hungarian sorghum varieties are also suitable for the conditions of Central Europe.

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SORGŲ IR AVIŽŲ GRŪDŲ BALTYMŲ SUDĖTIS IR JŲ TINKAMUMAS BEGLIUTENEI DIETAI

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Santrauka

Kolekcija, susidedanti iš 12 avižų ir 6 sorgų rūšių ir veislių, tirta norint nustatyti jų tinkamumą begliutenei dietai. Kiekybinis atsarginių baltymų įvertinimas avižose, naudojant SDS-PAGE elektroforezę rodo, kad patys enteropatiškai aktyviausi baltymų komponentai prolaminai + mažos molekulinės masės glutelinai sudarė nuo 53,43 iki 74,96 %, o sorgų kolekcijoje – nuo 32,25 iki 41,36 % atsarginių baltymų. Prolaminų kiekis, nustatytas imunologiniu ELISA metodu, avižų grūduose svyravo nuo 5,57 iki 35,60 mg 100 g^{-1} mėginio, sorgų grūduose – nuo 1,97 iki 3,30 mg 100 g^{-1} mėginio. Šie rezultatai rodo, kad vartoti avižas begliutenėje dietoje gali būti rizikinga, o sorgus vartoti turėtų būti saugu.

Reikšminiai žodžiai: begliutenė dieta, avižos, sorgai, baltymų sudėtis, imunologinis įvertinimas.

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