Protein Fractions of Oats and Possibilities of Oat Utilisation for Patients with Coeliac Disease

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Abstract

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The applicability was evaluated of 16 different oats species and varieties of different provenance in the coeliac diet in view of the composition of the protein complex and immunological testing during two-year experiments (2001 and 2002). Determination was carried out of total nitrogen content (average of evaluated oats collection in 2001 was 2.21%, in 2002 2.78%), protein nitrogen content (average 2001 1.94%, 2002 2.28%), and crude protein (N × 6.25) content (average 2001 13.80%, 2002 17.37%). The proportions of different protein fractions play a decisive role for the aims of this study because, based on the existing knowledge, coeliacally active protein components are present particularly in the prolamin fraction. The percentage of prolamins (determined by discontinual fractionation after Osborne) in the author's evaluated collection of oats species and varieties under the conditions of Central Bohemia reached on average 17.68% of the total protein in 2001, and 15.36% in 2002. The average percentage of albumins and globulins of the total protein reached 36.97% in 2001 and 41.04% in 2002, the average percentage of glutelins of the total proteins was 37.61% in 2001 and 34.10% in 2002, and residual was on average 7.55% in 2001 and 8.70% in 2002, respectively, of the total protein. Electrophoretic analysis of reserve (gluten) proteins (SDS-PAGE ISTA) showed in the oats collection evaluated the percentage of LMW + prolamins in the range 56–77% of the total reserve proteins in 2001, and 52–73% in 2002. The results of A-PAGE electrophoretic analysis of prolamin proteins confirmed the presence of α-prolamins, that ranged in the total content of prolamins from 50 to 88% in 2001, and from 77 to 100% in 2002, while β- + γ-prolamins ranged in 2001 from 11 to 49%, and in 2002 from 0 to 22%. These values do not give serious guarantees for the possible utilisation of oats in the gluten-free diet. The results of the immunological evaluation of the amount of prolamins in oats grains using ELISA showed great differences between different varieties and the experimental years. In 2001, 7 oats samples out of 13 evaluated, and in 2002 10 samples out of 12 evaluated were below the limit for the gluten-free diet (10 mg prolamins (gliadins)/100 g of sample dry matter), but the other varieties exceeded the limit, particularly in 2001, very significantly. The results obtained in the evaluated collection of species and varieties of oats revealed a great variability in the structure of the protein complex and in the immunological testing. In addition a significant effect of the year on the results of all analyses was evident. Based on our results, the use of oats in the diet for coeliac disease can be very risky for these reasons.

Keywords: oats; species; varieties; protein fractions; immunological testing; coeliac disease; gluten-free diet

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The genus oats (*Avena*) includes many species – *Avena abyssinica*, *Avena byzantina*, *Avena fatua*, *Avena sativa*, *Avena strigosa*, and others. More than 75% of oat areas in the world falls to oat varieties *Avena sativa*, the majority of the remaining part is covered by *Avena byzantina*, and only a low share is taken by *A. strigosa* and other species (Schrickel 1986).

Oats are a crop of cooler regions; the major percentage of the world oat production is concentrated in the Northern Hemisphere, between 35 and 50° of the northern latitude. At present, the greatest oat producers are Russia and other countries of the former Soviet Union, USA, Germany, Poland, Canada, Sweden, China, and Australia.

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. In addition, oat grains are marked by a high content of dietary fibre and the highest content of fats of all cereal, with a very good ratio between saturated and unsaturated fatty acids. The contents of Mg, Fe, P, Ca, and vitamins E and B_1 are also higher in oats comparing with other cereals (Schrickel 1986).

The discussions on the possibility of the use of oats for the coeliac diet among different countries, medical specialists and researchers have been led more than 40 years and the conclusions are still not unambiguous (Rottmann 1996; Thompson 1997; Kaukinen & Collin 2001).

The composition of the protein complex of oats in view of the possibility of oat utilisation in the diet of patients with coeliac disease was evaluated in this work.

The protein content in oat grains, like in the other cereal species, is much influenced by the environmental conditions (weather conditions during the vegetative season, the level of nitrogen nutrition) as well as by the variety (Burrows 1986).

Regarding the applicability of oats for the gluten-free diet, the primary characteristics resides 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 weight about 30 kDa. Prolamins (soluble in 50–70% ethyl alcohol or 40% 2-propyl alcohol) together with glutelins (soluble in 0.2–2% NaOH) form the so-called reserve proteins located in the grain endosperm. The reserve proteins form about 60–70% of the grain proteins of cereals. Prolamins are marked by a high percentage of glutamic acid, glutamin, and proline. On the other side, they have a low content of essential amino acids, above all of lysine. Liability to hydrolysis and hence also digestibility of the prolamin proteins are very low which has also been proved by experiments with laboratory animals (Michalík & Karlubík 1988).

The present studies assent to the fact that the prolamin content in oats is remarkably lower compared with those in wheat, rye or barley (Shewry 1995; Thompson 1997). Oats contain approximately 10–15% prolamins of the total content of proteins, while wheat contains 40–50%, rye 30–50% and barley 35–45% of them (Janatuinen *et al*. 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.

Similarly to the composition of the protein fractions, the proportions of amino acids are different from those in other cereals. Particularly, higher contents of lysine, threonin, and methionin is a characteristic property. The different protein fractions are distinguished by differences in the composition of amino acids. As mentioned above, the prolamin fraction is marked by a high percentage of glutamine and proline and it is poor in lysine as compared with the other fractions. The albumin fraction shows higher contents of lysine and alanine and a lower one of glutamine. The compositions of amino acids of globulins and glutelins are similar and form the middle between albumins and prolamins (Draper 1973).

In spite of the fact that, regarding the coeliac diet, in comparison with wheat, rye or barley, oats are marked by a more favourable composition of protein fractions as well as by the nutritionally more valuable composition of amino acids, oats toxicity for coeliac patients is still the subject of discussions. Dicke *et al*. (1953) consider necessary to fully eliminate wheat, rye, barley, and oats from the food for coeliac patients. Based on the trials of Baker and Read (1976), they recommended a considerable limitation of oats in the food for coeliac patients. On the other hand, Janatuinen *et al*. (1995) in their experiments with the daily uptake of oats 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. Storsrud *et al*. (1998) monitored twenty coeliac patients who consumed higher oats rations, 100 g/day, for two years. The participants of the experiment could choose the form of oats consumed – oatmeal, bread, biscuits, scones etc. The results of the study did not show any negative impacts of the frequent consumption of greater amounts of oats neither in biopsy or nutritional status nor in the level of antibodies with the patients studied.

Rispin *et al*. (1992) reported that the introduction of oats into gluten-free diet has also other positive effects – the insoluble fibre helps to control the activity of bowels and increases the sensation of fullness, and the soluble fibre decreases the level of cholesterol. Gluten-free diet given to patients with coeliac disease can positively affect also some other diseases – recently, it has been found that in gluten-free diet the secretion of insulin in persons endangered by diabetes of the first type improved (Pastore *et al*. 2003). After Ryan (1996), however, the contamination of oats by wheat during harvest or processing may cause problems; therefore, it is necessary to pay maximal attention to these processes.

MATERIAL AND METHODS

A collection of sixteen different oats species of different provenance was used in the experiments. The samples of these oats were obtained from the gene bank of the Research Institute of Crop Production, Prague-Ruzyně. After the propagation, we sowed them in springs of 2001 and 2002 at the Experimental Station of the Czech University of Agriculture in Prague, at Uhříněves. The Experimental Station Uhříněves is situated on the periphery of Prague in the sugar-beet growing region of Central Bohemia. Oats were sown after a cereal forecrop, the area of the experimental plots was 10 m^2, 4 replications; neither mineral nor organic fertilisers and pesticides were applied.

A survey of oats species and varieties used is presented in Table 1, the weather pattern in the years 2001 and 2002 at the Experimental Station Prague-Uhříněves in Table 2.

Oat samples were taken after the harvest for the following analyses:

(a) total nitrogen (determined by the method of Kjeldahl),

(b) protein nitrogen (determined by the method of Berstein),

(c) the composition of the protein fractions (discontinual fractionation after Osborne, modification by Michalík *et al*. 1994, 2002). The whole-grain groats were used for the determination. Albumins + globulins were determined by extraction with 10% NaCl (45 min/20°C/mixing; repeated three times), prolamins by extraction with 70% ethanol (45 min/20°C/mixing; repeated three times), glutelins by extraction with 0.2% NaOH (45 min/20°C/mixing; repeated three times),

(d) the composition of the reserve proteins by electrophoresis (SDS-PAGE ISTA). Standard vertical discontinual electrophoresis in polyacryl amide gel under the presence of sodium dodecyl sulphate (SDS), equipment SE 600 electrophoresis unit, Hoefer Pharmacia Biotech,

(e) the composition of prolamin proteins by electrophoresis (A-PAGE),

(f) immunological determination of the prolamin amount (ELISA, RIDASCREEN-Gliadin kit). The principle of the test resides in the reaction in which monoclonal antibodies detect gliadin fractions from wheat and the corresponding prolamins from rye, barley, and oats. For the determination, whole-grain groats were used (1 g of groats was extracted with 10 ml 60% ethanol, after centrifugation the supernatant was applied on a micro-plate with antibody). After incubation and rinsing, the antibody conjugated with peroxidase was applied, the reaction with the substrate and chromogene followed, and after its termination, the optical density was read. According to the data presented in "Codex Alimentarius", foods that contain less than 10 mg gliadins per 100 g of sample dry matter can be considered as suitable for gluten-free diet.

RESULTS AND DISCUSSION

The results of the determination of total and protein nitrogen and of crude protein (average of two determinations) in the oats varieties studied are presented in Table 3.

After Robbins *et al*. (1971) and Briggle *et al*. (1975), there exist a wide range of the protein concentration in oat grains in the dependence on the species and variety. Together with it, the content of proteins in oat grains is significantly influenced, like in the other cereals, also by the environmental conditions, mainly by the weather pattern during the growing season as well as by the agricultural practices of cultivation, particularly by the level of nitrogen nutrition. Peterson and Brinegar (1986)

Table 1. Survey of evaluated oat samples

Sample No.	Oat species	Genotype	Origin
1	*Avena strigosa* SCHREB.	Rouge de Tithiviers	France
2	*Avena byzantina* C. KOCH	Sierra	USA
3	*Avena sativa* L.	Ankara 76	Turkey
4	*A. sativa* var. *aristata* KRAUSE	Achilles	New Zealand
5	*A. sativa* var. *aurea* KOERN.	Stanton	Argentina
6	*A. sativa* var. *brunnea* KOERN.	Pelso	Finland
7	*A. sativa* var. *flava* KOERN.	Populatic Ratbar	Rumunia
8	*A. sativa* var. *inermis* KOERN.	Vir K 1932 Local	China
9	*A. sativa* var. *montana* ALEF.	Mesdag	France
10	*A. sativa* var. *mutica* ALEF.	Selekty Horsky	Czechoslovakia
11	*A. sativa* var. *obtusata* ALEF.	Dippawski	Poland
12	*A. sativa* var. *pugnax* ALEF.	Schwarzer Tartarischer Fahnen	Germany
13	*Avena sterilis* L.	Brunker	USA
14	*A. sativa* var. *mutica* ALEF.	Mestnyj Mutica	Russia
15	*A. sativa* var. *aurea* KOERN.	Sto Alexio	Spain
16	*A. sativa* var. *brunnea*	Black Diamond	USA

reported that the content of proteins in oat grains ranges usually between 15 and 20%.

Great differences between different varieties followed also between both experimental years are given in Table 3. They are evident in the content of crude protein (N × 6.25), where the Finnish cultivar Pelso reached the lowest content (12.88%), while the highest crude protein content (17.56%) was found in the French cultivar Mesdag. In 2002, the lowest content of crude protein was found in the American cultivar Sierra and the Rumanian cultivar Populatic Ratbar (15.81%), the highest in the Czechoslovak cultivar Selekty Horsky (19.06%).

Relatively significant differences in the contents of the total nitrogen, protein nitrogen and crude protein in both years of 2001 and 2002 can be ascribed to the particularly different weather patterns in the time of grain formation and ripening in both years. In 2002, in which the contents of the total nitrogen, protein nitrogen and crude protein were higher than in 2001, the average daily air temperature in June was higher by 3.1°C and in July by 0.65°C compared with the previous year. Year 2002 was the year with an above normal precipitation that decelerated the ageing of the assimilation apparatus of the upper part of the plant which is usual at high temperatures. Generally, the effect of higher temperatures is manifested by increased respiration of plants which reduces the amount of assimilates of the saccharide nature and thus, the percentage of proteins is increasing. The combination of significantly above-average temperatures and above-average precipitation is relatively rare. A high sum of precipitation should decrease the amount of proteins in grains (Table 2) under the normal pattern of temperatures.

The percentages of different protein fractions play a decisive role for the aims of this study. Based on the existing knowledge, coeliacally active protein components are present particularly in the prolamin fraction. Janatuinen *et al*. (1995) reported the percentage of prolamins (avenins) in oats to be between 10 and 15% of the total protein, Peterson and Brinegar (1986) gave 4–14%. The percentage of prolamins determined by discontinual fractionation after Osborn in 2001 in the authors' evaluated collection of oat species and varieties under the conditions of Central Bohemia reached, on average 17.68%, of the total protein, in 2002 15.36% (Table 4).

The percentage of albumins in water-soluble fractions, that are particularly a part of enzymes

Table 2. Weather pattern at the Experimental Station Prague-Uhříněves in the years 2001 a 2002 and long-time average

Month	Average monthly air temperature (°C)		Difference	Sum of monthly precipitation (mm)		Difference	long-time average of teperature (°C)	Long-time sum of precipitation (mm)
	2001	2002		2001	2002			
January	–0.44	0.78	1.22	25.4	19.6	–5.8	–2.1	28
February	2.41	5.00	2.36	30.9	56.1	25.2	–0.8	27
March	5.17	5.52	0.35	13.0	31.7	18.7	3.4	31
April	8.49	9.15	0.66	71.6	26.5	–45.1	8.2	46
May	15.64	16.54	0.90	67.3	50.1	–17.2	13.4	65
June	15.47	18.57	3.10	71.9	132.7	60.8	16.3	74
July	18.90	19.55	0.65	97.5	113.9	16.4	18.2	74
August	19.49	19.85	0.36	71.5	226.5	155.0	17.5	72
September	12.43	13.22	0.79	75.0	73.7	–13.0	14.0	49
October	12.52	8.36	–4.16	22.4	59.2	36.8	8.6	41
November	2.88	5.25	2.37	41.2	82.7	41.5	3.2	34
December	–1.36	–1.37	0.01	49.2	50.4	1.2	–0.5	34
Average of temperatures	9.30	10.04	0.74				8.28	
Sum of precipitation				636.9	923.1	286.2		575

in oats, ranges between 9 and 20% of the total protein (Peterson & Brinegar 1986). After a majority of authors, globulins are a prevailing fraction of proteins in oat grains. Brohult and Sandegren (1954) reported the percentage of this protein fraction, soluble in salts, to be as high as about 80% of the total protein. After Peterson and Smith (1976), globulins in oats are the predominant protein fraction of oats, their share ranges between 46 and 50%. Peterson (1976) reported in a further study that the percentage of globulins in several oats studied ranged around 52% and fluctuated in the dependence on the environmental conditions, nitrogen fertilisation, and variety. The author also reported that the percentage of another protein fraction, glutelins, amounted to 21–27% of the total protein. Peterson and Brinegar (1986) then reported, on the basis of their further results, the percentage of globulins in oat grains to range between 70 and 80%.

On the other hand, German authors stated on the basis of their results that glutelins are a major protein fraction of oat grains, and that the percentage of globulins ranges only between 12 and 19% (Völker 1975; Wieser *et al*. 1980). The percentage of albumins + globulins in our evaluated collection of oat species and varieties amounted on average to 36.97% of total protein in 2001, and to 41.04% in 2002. The average percentage of glutelins was 37.61% of the total protein in 2001, and 34.10% in 2002. The rest was, on average, 7.55% in 2001 and 8.7% in 2002 (Table 4).

Electrophoretic analysis brings accurate and detailed results on the structure of the protein complex, that is A-PAGE of prolamin proteins and SDS-PAGE as developed for the reserve, gluten proteins.

The evaluation of SDS-PAGE electrophoretic analysis of the reserve proteins is given in Table 5. Glutelins are divided into two groups – high-molecular (HMW) and low-molecular (LMW) ones. They differ from prolamins by that they contain intramolecular and intermolecular disulfidic bonds, while prolamins contain only intramolecular disulfidic bonds (http://www.e-celiaks.org/2%20Celiac%20Disease in Adults.htm).

The representation of the reserve proteins, particularly LMW + prolamins, in our oat samples

Table 3. The content (in %) of total nitrogen, protein nitrogen and crude protein in oat grains

Sample No.	Total N		Crude protein (N × 6.25)		Protein N	
	2001	2002	2001	2002	2001	2002
1	2.24	–	14.00	–	2.03	–
2	2.24	2.53	14.00	15.81	1.89	2.13
3	2.16	2.64	13.50	16.50	1.91	2.10
4	2.19	2.78	13.69	17.38	2.01	2.36
5	2.26	2.72	14.13	17.00	1.96	2.37
6	1.71	2.67	10.69	16.69	1.47	2.16
7	1.82	2.53	11.38	15.81	1.73	2.27
8	2.33	–	14.56	–	1.99	–
9	2.81	–	17.56	–	2.53	–
10	2.06	3.05	12.88	19.06	1.75	2.21
11	2.22	2.81	13.88	17.56	1.95	2.44
12	2.39	–	14.94	–	2.10	–
13	2.27	2.86	14.19	17.88	1.92	2.46
14	–	2.95	–	18.44	–	2.24
15	–	2.92	–	18.25	–	2.22
16	–	2.88	–	18.00	–	2.35
Average	2.21	2.78	13.80	17.37	1.94	2.28

ranged from 56.14 to 77.77% in 2001 and between 49.51 and 73.76% in 2002. These values can be considered risky in view of coeliac disease.

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); ω-prolamins do not contain toxic sequences of amino acids, therefore they are considered non-toxic for the patients with coeliac disease (Skerritt *et al*. 1990; Rottmann 1996).

The results of A-PAGE electrophoretic analysis of prolamin proteins are presented in Table 6. The presence of ω-prolamins was not recorded, β- and γ-prolamins participated in the total content of prolamins in 2001 11–49%, in 2002 0–22%; α-prolamins 50–88% in 2001, in 2002 78–100%. These values do not give serious guarantees on the possible utilisation of oats in gluten-free diet.

Immunological determination of the amount of prolamins in oats grains is an important indicator for the assessment of the suitability for the coeliac diet using ELISA (Table 7).

It can be seen that distinct differences occurred between different oat samples assessed. The values of some samples (samples No. 2, 4, 5, 9, 10, 11 and 13 in 2001, in 2002 all evaluated samples except No. 6 and 7) were below the limit for gluten-free diet – 10 mg of prolamins (gliadins)/100 g of sample dry matter – and thus they should be suitable for the gluten-free diet. On the other hand, samples No. 1, 3, 6, 7, 8 and 12 in 2001 and No. 6 and 7 in 2002 exceeded the limit, particularly in 2001, very significantly, and their usability for the diet in coeliac disease did not come into account.

A significant influence of the year on the amount of prolamins is also evident from the results (Table 7) obtained in immunological testing when the values found in 2002 were much lower and, consequently, also more favourable compared with those in 2001.

The year evidently affected the content of prolamins – lower percentage of prolamins in 2002 as compared to that in 2001 was determined in the evaluation of the protein fractions by the method

Table 4. Protein fractions of oat grains

Sample No.		Albumins + globulins		Prolamins		Glutelins		Rest	
		2001	2002	2001	2002	2001	2002	2001	2002
1	Content (% N)	0.898	–	0.387	–	0.786	–	0.168	–
	percentage	40.02		17.25		35.03		7.89	
2	Content (%N)	0.814	0.999	0.449	0.404	0.791	0.875	0.177	0.224
	percentage	36.27	39.56	20.01	16.00	35.25	34.65	7.89	8.87
3	Content (%N)	0.746	1.083	0.393	0.387	0.878	0.926	0.140	0.224
	percentage	34.54	41.07	18.19	14.68	40.65	34.12	6.48	8.49
4	Content (%N)	0.758	1.246	0.421	0.438	0.870	0.842	0.140	0.238
	percentage	34.64	44.85	19.23	15.77	39.76	30.31	6.40	8.59
5	Content (%N)	0.814	1.111	0.359	0.407	0.892	0.954	0.191	0.233
	percentage	36.05	40.83	15.90	14.96	39.50	35.06	8.46	8.56
6	Content (%N)	0.687	1.058	0.295	0.421	0.567	0.856	0.152	0.210
	percentage	40.15	39.70	17.24	15.80	33.14	33.90	8.88	8.32
7	Content (%N)	0.729	1.080	0.323	0.365	0.631	0.856	0.140	0.210
	percentage	39.97	42.77	17.71	14.45	34.59	33.90	7.67	8.32
8	Content (%N)	0.887	–	0.407	–	0.856	–	0.168	–
	percentage	38.08		17.48		36.75		7.21	
9	Content (%N)	1.060	–	0.519	–	0.943	–	0.275	–
	percentage	37.78		18.50		33.61		9.80	
10	Content (%N)	0.752	1.173	0.365	0.471	0.786	1.080	0.154	0.269
	percentage	36.47	38.89	17.70	15.62	38.12	35.81	7.47	8.92
11	Content (%N)	0.791	1.136	0.379	0.421	0.898	0.982	0.146	0.253
	percentage	35.69	40.48	17.10	15.00	40.52	35.00	6.59	9.02
12	Content (%N)	0.858	–	0.382	–	0.996	–	0.149	–
	percentage	35.97		16.02		41.76		6.25	
13	Content (%N)	0.794	1.220	0.398	0.426	0.915	0.931	0.163	0.266
	percentage	34.95	42.63	17.52	14.88	40.27	32.53	7.17	9.29
14	Content (%N)	–	1.103	–	0.477	–	1.094	–	0.258
	percentage		37.44		16.19		37.14		8.76
15	Content (%N)	–	1.148	–	0.463	–	1.016	–	0.253
	percentage		40.37		15.87		34.82		8.67
16	Content (%N)	–	1.263	–	0.435	–	0.920	–	0.247
	percentage		43.92		15.13		31.99		8.59
Average	Content (%N)	0.814	1.135	0.391	0.426	0.831	0.944	0.166	0.240
	percentage	36.97	41.04	17.68	15.36	37.61	34.10	7.55	8.70

Table 5. Quantitative evaluation of SDS-PAGE electrophoretic analysis of reserve (gluten) proteins (harvest of 2001 and 2002)

Sample No.	HMW (PI)[a]	LMW + prolamins (PI)[a]	Residual albumins + globulins (PI)[a]	HMW (%)[b]	LMW + prolamins (%)[b]	Residual albumins + globulins (%)[b]
2001						
1	0.00 (0)[c]	381.63 (20)[c]	146.50 (8)[c]	0.00	63.81	36.19
2	0.00 (0)[c]	325.27 (18)[c]	133.95 (9)[c]	0.00	63.11	36.89
3	0.00 (0)[c]	284.71 (16)[c]	97.15 (9)[c]	0.00	74.73	25.27
4	14.04 (1)[c]	272.16 (15–17)[c]	98.12 (10–12)[c]	3.06	63.91	33.03
5	17.41 (1–2)[c]	324.64 (18)[c]	101.93 (8–12)[c]	2.26	74.29	23.45
6	17.13 (1)[c]	324.45 (19–22)[c]	169.83 (15–16)[c]	2.64	61.94	35.42
7	15.75 (1)[c]	323.70 (17–20)[c]	143.35 (10–14)[c]	2.23	64.31	33.46
8	24.02 (2)[c]	313.70 (17–18)[c]	98.79 (11–12)[c]	3.00	71.11	25.89
9	18.30 (1)[c]	285.17 (18–20)[c]	103.96 (11)[c]	2.73	62.68	34.59
10	15.61 (2)[c]	335.53 (18)[c]	192.51 (11)[c]	2.78	56.64	40.58
11	27.73 (2)[c]	333.74 (18)[c]	121.94 (11)[c]	3.76	66.00	30.24
12	18.67 (2)[c]	384.71 (16)[c]	104.85 (10)[c]	1.66	77.77	20.57
13	28.47 (1)[c]	267.00 (16)[c]	146.11 (11)[c]	5.37	56.14	38.49
2002						
1	–	–	–	–	–	–
2	21.54 (4)[c]	240.34 (15)[c]	179.02 (16)[c]	2.89	52.60	44.51
3	28.31 (3–4)[c]	206.12 (10)[c]	131.75 (11–16)[c]	4.99	68.38	26.63
4	6.35 (1)[c]	272.14 (14)[c]	135.31 (13)[c]	1.05	73.76	25.19
5	18.32 (3)[c]	289.95 (17)[c]	187.85 (12)[c]	1.51	58.70	39.79
6	27.98 (3)[c]	266.87 (17)[c]	197.36 (14)[c]	4.05	57.52	38.43
7	22.82 (4)[c]	215.78 (12–15)[c]	189.90 (9–15)[c]	3.40	46.40	50.20
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	19.57 (1–5)[c]	236.50 (15–17)[c]	166.73 (9–10)[c]	2.93	54.34	42.73
11	15.83 (3)[c]	242.84 (15)[c]	167.03 (14)[c]	2.15	63.86	33.99
12	-	-	-	-	-	-
13	10.02 (2)[c]	236.71 (16–17)[c]	187.09 (12–13)[c]	1.57	49.51	48.92
14	28.08 (3)[c]	250.73 (15)[c]	134.00 (13)[c]	4.15	61.64	34.21
15	34.96 (6)[c]	242.65 (16)[c]	184.56 (13)[c]	4.30	61.21	34.49
16	12.08 (2)[c]	251.50 (11)[c]	191.91 (13)[c]	1.08	52.85	46.07

[a]pixel intensity; [b]relative percentage; [c]number of bands

after Osborne (Table 4) and quantitative SDS-PAGE of reserve proteins (Table 5). Much lower content of β- + γ-prolamins in comparison with the previous year was found in 2002 by quantitative A-PAGE electrophoretic analysis of prolamin proteins (Table 6) in a majority of samples.

The comparison of the results of the fraction composition of proteins 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 gluten-free diet. It can be presumed that the starting mechanism

Table 6. Quantitative evaluation of A-PAGE electrophoretic analysis of prolamin proteins (harvest of 2001 and 2002)

Sample No.	ω-prolamins (PI)[a]	β- + γ-prolamins (PI)[a]	α-prolamins (PI)[a]	ω-prolamins (%)[b]	β- + γ-prolamins (%)[b]	α-prolamins (%)[b]
2001						
1	0.00 (0)[c]	172.24 (6)[c]	197.66 (4)[c]	0.00	48.60	51.40
2	0.00 (0)[c]	114.61 (3)[c]	342.24 (5)[c]	0.00	13.35	86.65
3	0.00 (0)[c]	52.44 (3)[c]	162.67 (7)[c]	0.00	20.65	79.35
4	0.00 (0)[c]	141.80 (2–8)[c]	180.74 (7)[c]	0.00	44.23	55.77
5	0.00 (0)[c]	126.16 (3)[c]	236.68 (5–7)[c]	0.00	31.41	68.59
6	0.00 (0)[c]	112.59 (2)[c]	233.57 (6–7)[c]	0.00	26.86	73.14
7	0.00 (0)[c]	46.17 (2)[c]	129.37 (7)[c]	0.00	15.11	84.89
8	0.00 (0)[c]	83.75 (3–4)[c]	146.56 (4–9)[c]	0.00	30.38	69.62
9	0.00 (0)[c]	104.49 (3–6)[c]	120.69 (8–9)[c]	0.00	49.27	50.73
10	0.00 (0)[c]	51.82 (2)[c]	171.47 (6)[c]	0.00	14.80	85.20
11	0.00 (0)[c]	81.72 (1)[c]	279.56 (7)[c]	0.00	20.45	79.55
12	0.00 (0)[c]	107.23 (2)[c]	295.44 (5)[c]	0.00	17.04	82.96
13	0.00 (0)[c]	65.10 (2)[c]	272.70 (5)[c]	0.00	11.80	88.20
2002						
1	–	–	–	–	–	–
2	0.00 (0)[c]	110.15 (2)[c]	368.97 (11)[c]	0.00	22.28	77.72
3	0.00 (0)[c]	60.55 (2)[c]	271.92 (11)[c]	0.00	20.06	79.94
4	0.00 (0)[c]	93.18 (2)[c]	382.90 (10)[c]	0.00	14.89	85.11
5	0.00 (0)[c]	62.75 (1)[c]	251.26 (8)[c]	0.00	16.02	83.98
6	0.00 (0)[c]	31.12 (1)[c]	184.74 (10)[c]	0.00	14.80	85.20
7	0.00 (0)[c]	0.00 (0)[c]	345.05 (6–9)[c]	0.00	0.00	100.00
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	0.00 (0)[c]	14.03 (1)[c]	330.31 (9–10)[c]	0.00	4.47	95.53
11	0.00 (0)[c]	93.18 (1)[c]	427.83 (9)[c]	0.00	20.04	79.96
12	–	–	–	–	–	–
13	0.00 (0)[c]	39.10 (1)[c]	316.84 (8–11)[c]	0.00	7.85	92.15
14	0.00 (0)[c]	10.40 (1)[c]	369.76 (11)[c]	0.00	1.04	98.96
15	0.00 (0)[c]	30.21 (1)[c]	201.00 (11)[c]	0.00	12.51	87.41
16	0.00 (0)[c]	24.75 (1)[c]	246.36 (10)[c]	0.00	9.18	90.82

[a]pixel intensity; [b]relative percentages; [c]number of bands

of coeliac disease resides in the presence of protein fragments with high frequency of glutamin and proline and a certain conformational state of these proteins. Pathological reaction is triggered by their interaction with receptors of small intestine.

Table 7. Amount of prolamins in oat grains (immunological evaluation – ELISA)

Sample No.	Amount of prolamins (mg/100 g dry matter)	
	2001	2002
1	21.6	–
2	3.1	< std
3	24.6	4.40
4	8.0	< std
5	4.7	< std
6	43.5	25.0
7	37.3	15.0
8	14.1	–
9	< std	–
10	3.1	< std
11	<std	< std
12	35.8	–
13	< std	< std
14	–	<std
15	–	3.9
16	–	< std

std – standard for the method RIDASCREEN-Gliadin kit is 12.5 mg/kg (1.25 mg/100 g)

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 all analyses was evident. Based on our results, the use of oats in the diet for coeliac disease can be very risky for these reasons.

Within the framework of the further examination of the possibilities of oat utilisation in 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 and in greater number of experimental localities and years, reach the amounts of prolamins suitable for the diet in coeliac disease. Only then will it be possible to carry out detailed clinical tests on selected collections 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.

Of course, it is necessary to take into account "dilution" of the prolamins content in a certain food, for example oats porridge, bakery products, oats flakes etc., in the dependence on the cooking procedure and the eventual portion of other components in concrete foods. However, the basic presumption, especially in alternative crops utilisation for gluten-free diet, should be also "safe" input raw material, that is oats grain.

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Souhrn

Capouchová I., Petr J., Tlaskalová-Hogenová H., Michalík I., Faměra O., Urminská D., Tučková L., Knoblochová H., Borovská D. (2004): **Bílkovinné frakce ovsa a možnosti využití ovsa pro dietu při celiakii**. Czech J. Food Sci., **22**: 151–162.

Ve dvouletých pokusech (pokusné roky 2001 a 2002) jsme ověřovali využitelnost 16 různých druhů a odrůd ovsa rozdílné provenience pro dietu při celiakii po stránce skladby bílkovinného komplexu a imunologického testování. Analyzován byl celkový dusík (průměr odrůd ovsa 2001 2,21 %, 2002 2,78 %), bílkovinný dusík (průměr 2001 1,94 %, 2002 2,28 %) a hrubý protein N × 6,25 (průměr 2001 13,80 %, 2002 17,37 %). Velmi důležitý byl podíl jednotlivých frakcí bílkovin, protože celiakálně aktivní bílkovinné komponenty jsou přítomné v prolaminové frakci. V hodnoceném souboru druhů a odrůd ovsa vypěstovaných v podmínkách středních Čech dosahoval podíl prolaminů (stanovení diskontinuální frakcionací podle Osborna) v roce 2001 v průměru 17,68 %, v roce 2002 15,36 % z celkového proteinu. Podíl albuminů a globulinů činil v roce 2001 v průměru 36,97 %, v roce 2002 41,04 % z celkového proteinu, podíl glutelinů byl v roce 2001 v průměru 37,61 %, v roce 2002 34,10 % z celkového proteinu a zbytek v roce 2001 v průměru souboru 7,55 %, v roce 2002 8,70 %. Elektroforetická analýza zásobních bílkovin (SDS-PAGE ISTA) ukázala zastoupení LMW + prolaminů v roce 2001 v rozmezí 56–77 %, v roce 2002 v rozmezí 52–73 %. Výsledky A-PAGE elektroforetické analýzy prolaminových bílkovin potvrdily přítomnost α-prolaminů, které se podílely na celkovém obsahu prolaminů v roce 2001 v rozmezí 50–88 %, v roce 2002 v rozmezí 77–100 % a β- + γ-prolaminů v roce 2001 v rozmezí 11–49 %, v roce 2002 0–22 %. To jsou hodnoty, které lze považovat z hlediska celiakie za rizikové. Výsledky imunologického stanovení množství prolaminů v zrnu ovsa pomocí ELISA ukázaly výrazné rozdíly mezi jednotlivými hodnocenými odrůdami i pokusnými ročníky. V roce 2001 byly hodnoty 7 vzorků ovsa ze 13 hodnocených a v roce 2002 hodnoty 10 vzorků ze 12 hodnocených pod hranicí limitu pro bezlepkovou dietu (10 mg prolaminů (gliadinů)/100 g sušiny vzorku), naproti tomu zbývající odrůdy limit někdy i výrazně

překročily. Z výsledků vyplynula značná odrůdová variabilita ve skladbě bílkovinného komplexu a ve výsledcích imunologického hodnocení. Výrazný byl i vliv ročníku na výsledky všech prováděných analýz. Proto lze na základě těchto výsledků považovat využití ovsa v dietě pro celiakii za rizikové.

Klíčová slova: oves; druhy; odrůdy; frakce bílkovin; imunologické testování; celiakie; bezlepková dieta

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