Extention of the Spectra of Plant Products for the Diet in Coeliac Disease

Jiří PETR[1], Ivan MICHALÍK[2], Helena TLASKALOVÁ[3], Ivana CAPOUCHOVÁ[1], Oldřich FAMĚRA[1], Dana URMINSKÁ[2], Ludmila TUČKOVÁ[3] and Henrieta KNOBLOCHOVÁ[2]

[1]Czech University of Agriculture, Prague, Czech Republic; [2]Slovak University of Agriculture, Nitra, Slovak Republic; [3]Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Abstract

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The authors studied an extension of the sources of plant products for the diet in coeliac disease. This disease is induced by the components of glutenin proteins. In a collection of crops, they examined the contents of the total and protein nitrogen, the composition of protein fractions, the electrophoretic composition of reserve gluten and prolamine proteins, and the immunological determination of the gliadin amount using ELISA test. By immunological tests, gliadin content below 10 mg per 100 g of sample was found in the following species: amaranth (*Amaranthus hypochondriacus* and *A. cruentus*) followed by quinoa (*Chenopodium quinoa*), sorghum species – grain sorghum and sweet sorghum (*Sorghum bicolor* and *S. saccharatum*), millet (*Panicum miliaceum*), foxtail millet (*Setaria italica* ssp. *maxima*), broadrood (*Digitaria sanguinalis*) and buckwheat (*Fagopyrum esculentum*). These species can be considered as suitable for the diet in coeliac disease. Below-limit values were found in triticale (*Triticosecale*) and some oats varieties; this, however, will need some other tests. The analysed samples differred by the contents of crude protein and fraction structures of the protein complex. In pseudocereals amaranth, quinoa and buckwheat, the proportion of the soluble fractions of albumin and globulin was 50–65%. In grain sorghum, their proportion was 20.5%, in sweet sorghum 7.8%. In millet, foxtail millet, and broadrood, their proportion amounted to 12–13%. The proportion of prolamines was higher in sweet sorghum than in grain sorghum. Pseudocereals and millet contained 3–6% of prolamines, Italian millet 38.7%, and broadrood 23.1%, respectively. The two latter species had, however, lower contents of glutenins. In the other species studied, the contents of glutenins ranged from 12 to 22%. Electrophoretic analysis PAGE of prolamine proteins or SDS-PAGE ISTA, developed for gluten proteins, confirmed the results of immunological tests on the suitability of quinoa, grain sorghum, sweet sorghum, buckwheat, amaranth, broadrood, millet and foxtail millet for the diet in coeliac disease. These species did not contain prolamins or the content of α-prolamins was negligible in the given samples. The tested species of wheat, triticale, and oats species were manifested as substandard or unhealthy for the diet.

Keywords: coeliac disease; gluten-free diet; amaranth; quinoa; sorghum; millet; foxtail millet; broadrood; buckwheat; fraction composition of proteins

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

Gluten is a group of reserve proteins of cereal grain which forms a coherent adhesive lattice after moistening. This is important for the preparation of

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leavened dough and baker's goods. It is composed of fractions differring in their solubility. Above all, it is the fraction of ethanol-soluble proteins, the so-called prolamins, and the fraction of proteins soluble in bases, the so-called glutelins. Gluten is partially contaminated also by cytoplasmic NaCl-soluble proteins, i.e. the fraction of albumins and globulins. However, the fraction of cytoplasmic proteins in whole-grain cereal flour forms about 30% of the total content of proteins. The second group of the so-called reserve proteins forms 60–70%. Just these hard-soluble gluten fractions, and above all prolamins of a low-molecular weight of about 30 kDa, are responsible for the increasing occurrence of the above-mentioned disease.

Considering the species and varieties of cereals, properties of gluten are different, too. With cereal species of the first group that includes wheat, rye, barley and oats, the mentioned intolerance to gluten proteins appears with all but some exceptions. It is the intolerance to prolamins and glutenins or their component, α-gliadin, respectively, and their fissile products. As little as 0.1 g of gliadin is a probably deleterious dose for a patient. There is also the question whether in the past, with the original forms of cereals, particularly with wheat, intolerance to gluten appeared in our predecessors, or whether or not the original wild and lower pale-wheat species contained the fractions mentioned.

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

The use of maize and rice is the most widespread in this direction. In the remaining species, the possibility of utilisation is presumed, but it has not been adequately tested. The greatest attention for the usage in the diet for coeliac patients should be paid to sorghum, 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 other cereal species. Sorghum, together with maize, belongs to the most productive cereals with the type of photosynthesis C-4 which also determines its high productive capacity.

Sorghum also shows a higher production of dry matter per unit of utilised nitrogen and it is marked by a better availability of water as given by a lower ratio of transpiration to photosynthesis. It is also the most resistant to drought and is tolerant to stress factors such as salinity and drought. Its yields are growing owing to the progress in the breeding of hybrid varieties. 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. It also offers greater possibilities of its utilisation in Europe for food purposes which has been allowed only in a limited degree till now. The assortment of food prepared from sorghum is very rich in the regions of the greatest spread of sorghum species.

The possibility of using of sorghum for the diet in coeliac disease was derived from the common use of maize and rice of the same group of cereals. However, its suitability should be tested as concerns the composition of the protein complex and immunology for the elimination of the disease – gluten-sensitive enteropathy.

The contents of different substances are usually very different according to the site of cultivation and the agricultural practices. For example, the content of proteins is strongly affected by nitrogen fertilisation; it mainly increases the percentage of the prolamine fraction, called kephirine in sorghum (Sawhney & Naik 1969). Just the problem of the contents of different protein fractions and the components composition of reserve proteins to be used for the diet in coeliac disease seems to be one of the most important questions. From the results of the analyses of sorghum grain from the main regions of its cultivation, Jambunathan *et al*. (1984) indicated a low content of prolamines i.e. 25.2% of the total protein, further, 17.4% of albumin and globulin, and 39.7% of glutelin, the residue being 10.6%. The content of essential amino acids, particularly of lysine and tryptophan, is much valued in albumin and globulin fractions. On the other side, prolamine fraction is poor in lysine, arginine, histidine, and tryptophan. This fraction contains much proline, glutamine acid, and leucine that do not belong among essential amino acids (Abuja *et al*. 1970).

The above-mentioned results of the analyses for the contents of nutritive substances prove the suitability of the use of sorghum species for traditional human nutrition. For the purpose of this research given to the study of its use for the diet for coeliac patients, it should be tested whether the contents of nutritive and unhealthy substances are

suitable for these purposes when cultivated under the Czech conditions.

Similarly, millet and foxtail millet of the group of thermophilous cereals should be tested. Both these species as hulled millet have been used particularly by Slavonic nations since long ago. The preparation of meals is limited to mushy foods only a low content of prolamines, below the limit that allows its use for the diet, is reported for millet (Aubrecht *et al*. 1998).

In new cereal species such as the hybrid of wheat and rye – triticale – no immunological responses are known either.

A great attention should be devoted to oats. It has been discussed for a long time whether it meets the criteria for gluten-free diet. The present studies coincide in that the content of prolamins in oats is much lower compared to wheat, rye or barley (Moulton 1959; Thompson 1997). Oats contain approximately 10–15% of prolamins of the total content of proteins, while wheat contains 40–50%, rye 30–50%, and barley 35–45%, respektively. (Moulton 1959; Janatuinen *et al*. 1995; Thompson 1997). Kumar and Farthing (1995) point out that if avenins (oat prolamins) are responsible for the toxicity of oats in coeliac patients, much greater amount of oats should be consumed than of wheat, rye, and barley for the same "deleterious" effect to be manifested. Owing to the above-mentioned ideas, proteins of buckwheat grain are well digestible and are marked by a balanced proportion of essential amino acids (Karlubík *et al*. 1997).

Despite the fact that, as regards the diet for coeliac disease, oats are marked by a more favourable composition of protein fractions and a more valuable composition of amino acids for nutrition as compared with wheat, rye or barley, oat toxicity for coeliac patients is still a subject of discussions. Dicke *et al*. (1953) consider as necessary to eliminate completely wheat, rye, barley, and oats from the food for coeliac patients. Based on their tests, Baker and Read (1976) 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 feed of 50 to 70 g of oats did not find any deleterious effect on intestinal mucous membrane although they reported 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 greater oat feeds for two years, i.e. 100 g/day. The participants of the experiment could choose the form of oats consuming – as oatmeal, bread, biscuits, scones etc. The results of the study showed no negative impacts of a frequent consumption of greater amounts of oats either on biopsy or nutritional status or the level of antibodies in the monitored patients.

Rispin *et al*. (1992) reported that the introduction of oats into gluten-free diet had also other positive effects – insoluble fibre helps to control the activity of bowels, increases the sensation of fullness, and soluble fibre reduces the level of cholesterol. According to Ryan (1996), however, the contamination of oats by wheat during the harvest or processing may induce problems, therefore it is necessary to pay a maximal attention to these processes.

The so-called pseudocereals are a very prospective group of crops for coeliac diet that includes buckwheat, amaranth, quinoa, *Echinochloa frumentacea*, *Digitaria sanguinalis*. These are botanically completely different species compared to cereal grasses (*Poaceae*) in which unfavourable fractions and proteins did not appear.

The buckwheat *Fagopyrum esculentum* belongs to the family of *Polygonaceae*. It is native to southwestern China and the region south off the Himalayas. It came to Europe in the Middle Ages from Siberia through Russia and Ukraine. It was very popular with Slavonic nations. Recently, it has been spread as an alternative crop for its nutritive and health effects.

Amaranth, *Amaranthus* ssp. is an ancient crop of Indians from South America with a very high nutritive value. For these qualities, its use has been recently spread in many European countries.

Quinoa (*Chenopodium quinoa*) is of a similar origin like amaranth; it was distributed together with other crops introduced after the discovery of America. Now its cultivation is tested because of its valuable nutritional qualities.

In Germany and the Czech Republic, *Digitaria sanguinalis* has been cultivated for food purposes. It is husked and mashed like millet.

Echinochloa frumentacea has a similar utilisation, it is much cultivated in Orient and is cultivated also in Europe and in the USA like other thermophilous species. It is consumed husked or ground to flour.

This collection of crops was tested in cultivation in Central Bohemia and it was evaluated from the aspect of their possible use in the diet for coeliac patients.

MATERIAL AND METHODS

The aim of this research, i.e. the study of the spectra of the crops for the diet in coeliac disease, was to test whether in the cultivation under the conditions in the Czech Republic the contents of nutritive and unhealthy substances are suitable for these purposes. A strong effect of the cultivation conditions, the weather pattern, and agricultural practices on the quality of cereals, particularly on the content of proteins and their composition is well known. Therefore, the authors of this study focused their attention on a detailed study of the chemical composition including immunological testing of the suitability of these species for this diet.

The following species were studied:

Grain sorghum, 5 varieties and hybrids *Sorghum bicolor* (L.) Moench

Sweet sorghum, 5 varieties *Sorghum saccharatum* (L.) Moench

Millet, *Panicum miliaceum* L. – hulled grains

Foxtail millet, *Setaria italica* ssp. *maxima* L. – whole grains

Buckwheat, *Fagopyrum esculentum* Moench – millet grout

Amaranth, *Amaranthus hypochondriacus* and *Amaranthus cruentus*

Quinoa, *Chenopodium quinoa* Willd

Broadrood, *Digitaria sanguinalis* L.

The seeds of the above-mentioned species were sown in the last decade of April on two experimental sites of Central Bohemia (Experimental Station of the Czech University of Agriculture in Prague-Uhříněves and Experimental Station of the Czech University of Agriculture in Prague-Suchdol), in a sugar beet-growing region with the production potential of soils of about 80 points. The plants were harvested, the panicals were hot-air dried out and then thrashed with flails. After the harvest, seed samples were prepared for the analyses and the following substances were determined:

1. total nitrogen (after Kjeldahl) (all samples in three triplicated)

2. protein nitrogen (determined by the method after Berstein) (all samples in triplicates)

3. composition of protein fractions (discontinued fractionation after Osborn) (all samples in triplicates), modification by Michalík *et al*. (1994) and Michalík (2002)

4. electrophoretic composition of reserve proteins (SDS-PAGE ISTA)

5. electrophoretic composition of prolamine proteins (PAGE)

Samples were analysed by standard reference vertical discontinued electrophoresis in polyacrylamide gel in acid medium that was recommended by ISTA (Draper 1973 in the modifications after Horvát 1994). The device SE 600 Series Electrophoresis Unit Hoefer Pharmacia Biotech. Each sample in duplicates. Wheat varieties *Triticum aestivum* L. Chinese Spring and Marquis were used as standards

6. immunological determination of the gliadin content (ELISA-enzyme immunoassay, the kit Reidel)

The method resides in the interaction of specific antibodies against gliadin with gliadin present in the food sample (commercial kit RIEDEL-de Haën). 1 g of sample is extracted with 10 ml of 50% ethanol; after centrifugation, the diluted supernatant is applied on a small plate with an antibody. After the appropriate incubation and washing, an antibody marked by peroxidase is applied and this is followed by the reaction with the substrate and chromogene. On its termination, optical density is read. As prescribed by the presently valid Codex Alimentarius, the food that contains less than 10 mg of gliadin per 100 g is considered as gluten-free.

RESULTS AND DISCUSSION

The results of the determination of the total nitrogen and protein nitrogen (average for three determinations) are given in Table 1. The average content of proteins in grain and sweet sorghum were 9.8% (at N × 5.7) and 10.3 (at N × 6.0), respectively, under the conditions of cultivation in the fertile region of Central Bohemia. Hubbart *et al*. (1950) from the USA found 12.3% (N × 6.25), Rajki-Siklósi (1993) reported the content of proteins 10.6–10.7% in similar varieties from Hungary. In a wide range of varieties of the world collection, the average content of proteins was 11.4 (Jambunathan & Subramanian 1988). The variability of the content of proteins is caused mainly by the cultivation conditions, particularly by the level of nitrogen nutrition and the variety. The weather conditions, above all warmth and moisture, decide upon the utilisation of the primary photosynthetic products in the goal-directed synthesis of proteins and starch. For the given reason, the content of proteins in the same variety depends on the cultivation conditions. The content of proteins of 9.6% was found in hulled

Table 1. The content of total and protein nitrogen and crude protein

Species, varieties	% of total N	Crude protein (%) (N × 5.7)	Crude protein (%) (N × 6.0)	% of protein N	% of pure proteins
Grain sorghum	1.720	9.808	10.32	1.564	90.5
Sweet sorghum	1.730	9.861	10.38	1.552	89.7
Proso millet hulled grain	1.683	9.593		1.473	87.5
Foxtail millet	2.020	11.647		1.849	91.5
Buckwheat hulled grain	1.122		6.732	0.982	87.5
Amaranth, *A. hypochondriacus*	2.685	15.304	16.110	2.034	75.7
Amaranth, *A. cruentus*	2.174	12.392	13.044	1.557	71.6
Quinoa, *Chenopodium quinoa*	1.613	9.194	9.678	1.375	85.2
Triticale	1.501	8.750+		1.333	88.8
Oats naked, *A. sativa* v. *nuda*	2.104	12.266+		1.859	88.3
Kamut, *Triiticum turgidum*	2.216	12.631		1.929	87.0
Broadrood, *Digitaria sanquinalis*	1.866	10.636	11.196	1.789	95.8

+ crude protein N × 5.83

millet, the majority of non-hulled grains of the Czech millet varieties showed the content over 10%. (The variety Hanácká Mana 10.06% and red millet Unikum 10.88%.) In the world assortment of millet, the content of proteins ranged between 11.3 and 12.7%. In the foxtail millet, the content of proteins was 11.6% but under intensive cultivation it was as much as 14.2% (Petr & Hradecká 1997). The content in the world collection was 11.2% (Hulse *et al*. 1980).

In buckwheat, the content of nitrogen substances was studied in buckwheat grout and the authors found (hulled buckwheat) the content of 6.7%. Savickij (1970) reported the content of 10%. In buckwheat achenes, 10.5 to 13.8% of proteins was reported (Joshi & Rana 1995), and Karlubík *et al*. (1997) reported that the content of proteins in buckwheat ranged from 10.9 to 13.0%. The high nutritive value of proteins in buckwheat grain, the sensory qualities, and the wide possibilities of its utilisation in the production of foods together with small demands for the cultivation conditions, the fact that buckwheat is a yielding honey-bearing plant and a suitable forecrop, indicate the possibilities of a marked distribution of buckwheat for cultivation.

Amaranth, which is quickly spreading now, seems to be a very hopeful crop for the diet in coeliac disease. In the species *Amaranthus hypochondriacus* as much as 16% of proteins is reported and this was confirmed by the authors' results. *Amaranthus cruentus* had, however, a lower content of proteins – 15.8%, and the processors from the Czech cultivation recorded such values.

Chemopodium quinoa is another utilised crop in which the authors found 9.19% of proteins which is lower in comparison with the literature data. Aufhammer *et al*. (1994) reported 16% under the German conditions (Stuttgart).

The contents of different fractions of proteins are very important objects of this research. The results were obtained by the method of discontinual fractionation and they allow to assess the analysed material in view of: 1. nutritive quality (percentage of cytoplasmic proteins), 2. technological quality (the content and percentage of reserve proteins). A survey of the contents and percentage of different fractions is presented in Table 2.

The presence of coeliac-active protein components can be assessed according to the proportion of prolamine proteins. The species and varieties in which the content of prolamine proteins is at the level of 4–8%, depending on the method used, can be considered as plant products suitable for the diet in coeliac disease.

In pseudocereals – buckwheat, amaranth, quinoa – the percentage of prolamine and glutelin is relatively low. Prolamins amount to 6.2%, glutelins to 18.7%, and insoluble residues to 25% in buckwheat. This coincides with the results obtained

Table 2. Protein fractions in species and varieties after prolamin extraction at 20°C and at 65°C

Species, varieties		Albumins + globulins	Prolamins	Glutelins	Rest
Grain sorghum at 20°C	Cont. (% N)	0.353	0.089	0.308	0.956
	percentage	20.5	5.2	17.9	55.5
Grain sorghum at 65°C	Cont. (% N)	0.353	0.605	0.214	0.541
	percentage	25.5	35.2	12.4	31.4
Sweet sorghum at 20°C	Cont. (% N)	0.135	0.128	0.410	1.106
	percentage	7.82	7.4	19.9	63.9
Sweet sorghum at 65°C	Cont. (% N)	0.135	0.768	0.287	0.534
	percentage	7.82	44.4	16.6	30.9
Buckwheat hulled grain	Cont. (% N)	0.561	0.070	0.210	0.281
	percentage	50.0	6.24	18.7	25.0
Quinoa	Cont. (% N)	1.038	0.076	0.297	0.196
	percentage	64.3	4.71	18.4	12.1
Amaranth, *A. hypochondriacus*	Cont. (% N)	1.589	0.079	0.604	0.515
	percentage	56.22	3.26	21.53	18.18
Amaranth, *A. cruentus*	Cont. (% N)	1.238	0.074	0.496	0.402
	percentage	55.3	3.31	22.19	17.93
Proso millet hulled grain	Cont. (% N)	0.219	0.112	0.213	1.136
	percentage	13.0	6.65	12.66	67.50
Foxtail millet	Cont. (% N)	0.257	0.780	0.187	0.769
	percentage	12.8	38.7	9.93	37.92
Broadrood, *Digitaria sanquinalis*	Cont. (% N)	0.224	0.432	0.191	1.010
	percentage	12.0	23.1	10.2	54.13

by Bonafaccia *et al*. (1994) where the content of prolamine was even below 1% and that of glutelin about 22%. The residue was 13.5–14.5%.

The content of prolamine and glutelin in hulled millet was also low but there is a problem here in a great amount of the insoluble residue that the authors continue to study in sorghum species. Dendy (1995) found in millet 31% of prolamins, 11% of globulin, 9% of albumin, and 8% of glutelin.

The below mentioned authors found in foxtail millet – 17.1% of albumin and globulin, 56.1% of prolamine, 8.9% of cross-liked prolamine, glutelin-like 9.2%, and glutelin 6.7%. The residue was only 2% (Monteiro *et al*. 1982).

No significant differences were found between the species *Amaranthus hypochondriacus* and *Amaranthus cruentus* in the percentages of different protein fractions. The values found coincide with those reported by Muchová *et al*. (2000) who found even higher contents of albumin and globulin, and a lower proportion of prolamins, the insoluble residue was also lower. The percentage of globulins was almost identical with that in the authors' experiments. It is remarkable that a relatively low percentage of pure proteins (72–76% of nitrogen substances, Table 1) is typical for amaranth. This percentage is lower by about 20% as compared with the grain of sorghum, millet, oats, triticale and quinoa. It follows from the above text that a high proportion of the not-protein nitrogen substances represents the fraction of the so-called nitrogen substances. This is an important fact where the determination of the nutritive quality of amaranth grain is concerned.

The solubility in 7 % ethanol of sorghum species was connected with problems at room temperature

Table 3. Quantitative evaluation of SDS-PAGE of electrophoretic analysis of reserve (gluten) proteins

Denotation of sample	Species, varieties	HMW (PI)[a]	LMW + gliadins (PI)[a]	Residual alb + glo (PI)[a]	HMW (%)[b]	LMW + gliadin (%)[b]	Residual alb + glo (%)[b]
S 1	*Triticum aestivum* L., Chinese Spring	139.66 (4)[c]	197.11 (9–15)[c]	76.09 (4–12)[c]	28.60	50.81	20.59
S 2	*Triticum aestivum* L., Marquis	136.54 (5)[c]	163.26 (6–15)[c]	72.91 (6–13)[c]	29.21	46.35	24.44
2.	Amaranth, *Amaranthus cruteanus* A 200	0.00 (0)[c]	253.24 (12–14)[c]	158.42 (12)[c]	0.00	54.16	45.84
3.	Amaranth, *A. hypochondriacus* Dakota	0.00 (0)[c]	299.15 (14)[c]	174.04 (11–14)[c]	0.00	59.38	40.62
4.	Amaranth, *A. hypochondriacus* hybr. K 432	0.00 (0)[c]	258.82 (15)[c]	162.43 (14–16)[c]	0.00	58.14	41.86
5.	Amaranth, *A. hypochondriacus* hybr. K 433	0.00 (0)[c]	298.52 (13–16)[c]	243.84 (13–14)[c]	0.00	48.61	51.39
6.	Amaranth, *A. hypochondriacus* Konyz	0.00 (0)[c]	247.01 (14)[c]	154.14 (11)[c]	0.00	57.03	42.97
7.	Amaranth, *A. cruentus* N 1008	0.00 (0)[c]	285.12 (13–14)[c]	192.73 (10–14)[c]	0.00	48.89	51.11
8.	Amaranth, *A. cruentus* YRR R150	0.00 (0)[c]	434.61 (18–19)[c]	217.51 (14)[c]	0.00	65.34	34.66
9 .	Sweet sorghum, *S. saccharatum* BAZ 1999 UH 6	51.54 (3)[c]	262.78 (12–15)[c]	166.90 (3–4)[c]	4.51	39.39	56.10
10.	Sweet sorghum, *S. saccharatum* SEVA UH 7	45.88 (2)[c]	307.97 (14–17)[c]	139.42 (7–11)[c]	5.57	53.68	40.75
11.	Sweet sorghum, *S. saccharatum* SEVA UH 9	53.47 (3)[c]	328.02 (16–18)[c]	214.79 (7–8)[c]	4.52	40.67	54.81
12.	Grain sorghum, *S. bicolor* GK Zsofia F_1 SU 4	64.22 (3)[c]	299.25 (13)[c]	241.71 (12)[c]	4.67	44.49	50.84
13.	Grain sorghum, *S. bicolor* GK Zsofia UH 2	3.44 (0–1)[c]	146.21 (13)[c]	124.93 (8)[c]	0.90	42.73	56.37
14.	Grain sorghum, *S. bicolor* tanin free UH 3	0.00 (0)[c]	125.43 (7–12)[c]	97.90 (7–8)[c]	0.00	49.32	50.68
15.	Grain sorghum, *S. bicolor* GK Zsofia F_1 UH 4	0.00 (0)[c]	195.46 (15–16)[c]	174.58 (8–9)[c]	0.00	26.10	73.90
31.	Buckwheat, *Fagopyrum esculentum* hulled grain	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]
36.	Quinoa, *Chemopodium quinoa*	71.18 (5)[c]	173.25 (11)[c]	162.09 (10)[c]	10.37	40.76	48.87
37.	Broadrood, *Digitaria sanquinalis* ssp. *sanquinalis*	88.37 (3)[c]	197.84 (13)[c]	133.71 (8)[c]	19.40	40.83	39.77
38.	Foxtail millet, *Setaria italica* ssp. *maxima* Ciernoklas	30.33 (3)[c]	264.96 (16)[c]	267.89 (11)[c]	3.10	33.74	63.16
38.	Foxtail millet, *Setaria italica* ssp. *maxima* Kitaj	47.10 (6)[c]	303.14 (15)[c]	321.85 (12)[c]	2.69	28.69	68.62
39.	Foxtail millet, *Setaria italica* ssp. *maxima cumiza* UH	31.03 (3)[c]	279.50 (17)[c]	321.71 (10)[c]	1.66	26.78	71.56
32.	Foxtail millet, *Panicum miliaceum hulled* grain	47.05 (2)	344.44 (15)	196.79 (10)	5.51	52.08	42.41

[a]pixet intensity; [b]relative per cent; [c]number of bands; [d]method in not useful

Table 4. Quantitative evaluation A-PAGE of electrophoretic analysis of prolamine proteins

Denotation of sample	Sample	ω–gliadin (PI)[a]	β- + γ-gliadin (PI)[a]	α-gliadin (PI)[a]	ω-gliadin relative (%)[b]	β- + γ-gliadin (%)[b]	α-gliadin (%)[b]
S 1	*Triticum aestivum* L., Chinese Spring	199.22 (7–14)[c]	183.33 (5–12)[c]	187.42 (6–10)[c]	30.24	35.01	34.75
S 2	*Triticum aestivum* L., Marquis	158.07 (6–16)[c]	197.37 (8–15)[c]	137.73 (4–14)[c]	25.73	42.00	32.27
2.	Amaranth, *Amaranthus cruentus*, A200	0.00 (0)[c]	0.00 (0)[c]	73.76 (3)[c]	0.00	0.00	100.00
3.	Amaranth, *A. hypochondriacus*, Dakota	0.00 (0)[c]	0.00 (0)[c]	19.96 (1)[c]	0.00	0.00	100.00
4.	Amaranth, *A. hypochondriacus*, hybrid K 432	0.00 (0)[c]	0.00 (0)[c]	18.64 (1)[c]	0.00	0.00	100.00
5.	Amaranth, *A. hypochondriacus*, hybrid. K 433	0.00 (0)[c]	0.00 (0)[c]	33.45 (2–3)[c]	0.00	0.00	100.00
6.	Amaranth, *A. hypochondriacus*, Konyz	0.00 (0)[c]	0.00 (0)[c]	30.00 (1)[c]	0.00	0.00	100.00
7.	Amaranth, *A. cruentus*, N 1008	0.00 (0)[c]	0.00 (0)[c]	33.45 (1–2)[c]	0.00	0.00	100.00
8.	Amaranth, *A. cruentus*, YRR R 150	0.00 (0)[c]	0.00 (0)[c]	8.00 (1)[c]	0.00	0.00	100.00
9.	Sweet sorghum, *S. saccharatum*, BAZ 1999 UH 6	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
10.	Sweet sorghum, *S. saccharatum*, SEVA UH 7	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
11.	Sweet sorghum, *S. saccharatum*, SEVA UH 9	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
12.	Grain sorghum, *S. bicolor*, GK Zsofia F1 SU 4	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
13.	Grain sorghum, *S. bicolor*, GK Zsofia UH 2	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
14.	Grain sorghum, *S. bicolor*, tanin free UH 3	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
15.	Grain sorghum, *S. bicolor*, GK Zsofia UH 4	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
31.	Buckwheat, *Fagopyrum esculentum*, hulled grain	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
36.	Quinoa, *Chenopodium quinoa*	0.00 (0)	0.00 (0)	0.00 (0)	0.00	0.00	0.00
37.	Broadrood, *Digitaria sanguinalis* ssp. *sanguinalis*	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
38.	Foxtail millet, *Setaria italica* ssp. *maxima*, Ciernoklas	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
39.	Foxtail millet, *Setaria italica* ssp. *maxima*, Kitaj	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
40.	Foxtail millet, *Setaria italica* ssp. *maxima*, cumiza UH	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
32.	Foxtail millet, *Panicum miliaceum*, hulled grain	0.00 (0)[c]	0.00 (0)[c]	0.00 (0)[c]	0.00	0.00	0.00
34.	Wheat engrain, *Triticum monococcum*	63.00 (6)[c]	197.61 (7)[c]	303.41 (12–13)[c]	8.20	34.44	57.36
33.	Wheat emmer, *Tr. dicoccum*	68.76 (6)[c]	172.22 (6–7)[c]	140.77 (7–11)[c]	9.88	62.88	27.24
35.	Wheat spelt, *Tr. spelta*	80.77 (8–9)[c]	192.16 (8–9)[c]	177.86 (10–14)[c]	11.23	48.11	40.66
16.	Kamut, *Tr. turgidum*	71.76(5–7)	167.19(7–10)	215.33 (7–10)	12.58	41.86	45.56
41.	Triticale, *Triticosecale Presto*	117.65 (7–9)[c]	208.20 (8)[c]	77.93 (6)[c]	22.07	57.17	20.76
18.	Oats, *Avena sativa* L., Ankara 76	0.00 (0)	114.61 (3)	342.24 (5)	0.00	13.35	86.65
29.	Oats naked, *Avena sativa* var. *nuda*	0.00 (0)	71.59 (1)	301.81 (10)	0.00	21.31	78.69

[a]pixet intensity; [b]relative per cent; [c]number of bands; [d]method in not useful

Table 5. Immunology ELISA test – amount of gluten in plant species and varieties

Denotation of sample	Species, varieties	Amount of gluten (mg/100 g of sample)
2.	Amaranth, *Amaranthus cruteanus,* A200	2.4
3.	Amaranth, *A. hypochondriacus,* Dakota	7.7
4.	Amaranth, *A. hypochondriacus,* hybrid K 433	9.2
7.	Amaranth, *A. cruentus,* N 1008	8.4
8.	Amaranth, *A. cruentus,* YRR R 150	2.4
9.	Sweet sorghum, *S. saccharatum,* BAZ 1999 UH 6	3.4
10.	Sweet sorghum, *S. saccharatum,* SEVA UH 7	2.6
11.	Sweet sorghum, *S. saccharatum,* SEVA UH 9	3.2
12.	Grain sorghum, *S. bicolor* GK, Zsofia F_1 SU 4	2.1
13.	Grain sorghum, *S. bicolor,* GK Zsofia UH 2	3.5
14.	Grain sorghum, *S. bicolor,* tanin free UH 3	2.1
15.	Grain sorghum, *S. bicolor,* GK Zsofia UH 4	< std
31.	Buckwheat, *Fagopyrum esculentum,* hulled grain	< std
36.	Quinoa, *Chenopodium quinoa*	1.8
37.	Broadrood, *Digitaria sanquinalis* ssp. *sanquinalis*	1.2
40.	Foxtail millet, *Setaria italica* ssp. *maxima,* cumiza UH	4.3
32.	Foxtail millet, *Panicum miliaceum,* hulled grain	< std.
34.	Wheat engrain, *Triticum monococcum*	>> std
33.	Wheat emmer, *Tr. dicoccum*	>> std
35.	Wheat spelt, *Tr. spelta*	>> std
16.	Wheat turgid, *Tr. turgidum,* Kamut	< std
41.	Triticale, *Triticosecale,* Presto	< std
18.	Oats, *Avena sativa* L., Ankara 76	240.6
29.	Oats naked, *Avena sativa* var. *nuda*	51.5

< std – lower than standard (limit value is up to 10 mg/100 g DM)

because there resulted a great amount of insoluble residue. The authors Jones and Beckwilth (1970), Haikerwall and Mathieson (1971) and others pointed this out. Therefore, we present here also the values of fractionation at room temperature and at 65°C. The results in Table 2 prove the effect of temperature on the solubility of prolamine protein. The percentage of prolamine reaches then 35–44%, and the solubility of the extracted fractions amounts to about 70%.

Grain sorghum has somewhat lower contents of prolamines and glutelins than sweet sorghum. As reported by Jambunathan and Subramainian (1984), sorghum contained on average 17.4% of albumins and globulins, 6.4% of prolamines and 18.8% of cross-linked prolamine to it 25.2% in total glians, 35.7% of glutelin and 4.0% of glutelin-like and 10.6% of residues.

Exact and standard results can be obtained mainly by electrophoresis; i.e. PAGE of prolamine proteins or SDS-PAGE ISTA developed for gluten proteins. This is based on the fact that proteins represented by the fraction of prolamine proteins of molecular weight of about 30 kDa are coeliac-active.

The qualitative evaluation of the electrophoretic analysis of reserve proteins (Table 3) showed that the proportion of reserve glutenin proteins of higher molecular weight, i.e. HMW-subunits, does not exceed in sorghum species 5.57%. In millet, the percentage was similar, only 5.5%, in foxtail millet the percentage was even lower, only 1.66–3.10%. In broadrood that was consumed like

millet and foxtail millet, the content was 19.4%. This HMW fraction was not found in the species studied and in amaranth species but in quinoa its content was 10.37%. These findings confirm that flour from amaranth does not suit the criteria of baking quality. Amaranth can be used in bread production only as an additive to wheat or rye flour, respectively.

Gluten and prolamine proteins in buckwheat should not be determined by this method. Low-molecular glutenin subunits (LMW) and monomeric gliadins represented in amaranth 48.6–65.3%, in sorghum species 26.1–53.7%, in quinoa and broadrood 39.8%, foxtail millet had the highest content, i.e. 63.2–71.6%, and millet 42.4%.

The content of the α-prolamine proteins fraction will be critical for the possibility of the utilisation for the diet (Table 4). In view of an objective interpretation of the results presented in Table 4, it can be said that prolamine fractions have not been detected in sorghum samples (samples 9–15) not because of their absence in grain but due to the methodological peculiarity of extraction of these proteins (Jones & Beckwilth 1970; Haikerwall & Mathieson 1971). The authors' results presented in Table 2 confirm the opinions of more authors on a poor solubility of reserve proteins of sorghum.

The results of these analyses have confirmed that thermophilous cereals, such as sorghum species, millet and foxtail millet as well as broadrood, are suitable for the diet. Out of pseudocereals, it is buckwheat, quinoa and amaranth, where the contents of gliadins are very low or were not found. For comparison, the authors have included in the table the data on original forms of wheat einkorn, engrain, *Triticum monococcum* L., emmer, *Triticum dicoccum* L., spelt *Triticum spelta* L., Kamut, *Tr. turgidum* L., standards *Tr. aestivum* L. and hybrids of wheat and rye triticale, *Triticosecale* Wittmack, and oats, *Avena sativa* L. that are unhealthy for the diet in coeliac disease. These results enable to characterise the analysed samples in view of their unhealthy effect in the nutrition of patients suffering from coeliac disease. The definitive standpoint may be offered by study of the presence of coliac-active gluten proteins by the ELISA method, and by means of the test on the basis of monoclonal antibodies. The results of these tests are in Table 5.

Both amaranth species, varieties and hybrids of sweet sorghum, buckwheat, quinoa, broadrood, millet and foxtail millet manifested themselves in these immunological tests as suitable for the diet in coeliac disease.

Original diploid, tetraploid and hexaploid wheat species highly exceeded the limit amounts of gliadin. Turgid wheat (*Triticum turgidum*) represented by an ancient Egyptian wheat Kamut and also the hybrid of wheat and rye-triticale, had lower gliadin contents than is the limit. This could be caused by the fact that they were cultivated ecologically, without fertilisation and pesticides. Above-limit content of gliadins was found in Kamut under an intensive cultivation. The authors of this article will study triticale and some oat species and varieties in the future again.

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Souhrn

Petr J., Michalík I., Tlaskalová H., Capouchová I., Faměra O., Urminská D., Tučková L., Knoblochová H. (2003): **Rozšíření spektra rostlinných produktů pro dietu při celiakii**. Czech J. Food Sci., **21**: 59–70.

Sledovali jsme rozšíření zdrojů rostlinných produktů pro dietu při celiakii. Toto onemocnění způsobují složky bílkovin nerozpustných frakcí prolaminu a gluteninů. V souboru plodin jsme sledovali obsah celkového a bílkovinného dusíku, skladbu frakcí bílkovin, elektroforetickou skladbu zásobních glutenových a prolaminových bílkovin a imunologické stanovení množství gliadinu ELISA testem. Imunologické testy prokázaly, že obsah gliadinu pod 10 mg na 100 g vzorku měly druhy: laskavce (*Amaranthus hypochondriacus* a *A. cruentus*) a dále merlíku čilského

– quinoi (*Chenopodium quinia*), čiroků zrnového a cukrového (*Sorghum bicolor* a *S. saccharatum*), prosa (*Panicum miliaceum*), béru vlašského – čumizy (*Setaria italica* ssp. *maxima*), rosičky krvavé (*Digitaria sanquinalis*) a pohanky (*Fagopyrum esculentum*). Tyto druhy lze považovat za vhodné pro dietu při celiakii. Podlimitní hodnoty se našly u tritikale (*Triticosecale*) a některých odrůd ovsa, což bude třeba znovu prověřit. U psedocereálií (amarantu, quinoi a pohanky) bylo zastoupení rozpustných frakcí albuminu a globulinu 50–65 %. U zrnového čiroku bylo jejich zastoupení 20,5 %, u čiroku cukrového 7,8 %, u prosa, béru a rosičky 12–13 %. Zastoupení prolaminů bylo vyšší u čiroku cukrového než zrnového. Pseudocereálie a proso měly nízký obsah prolaminů (3–6 %), ale bér 38,7 % a rosička 23,1 %. Tyto dva druhy měly však nižší obsah glutelinů. Ostatní sledované druhy měly obsah glutelinů v rozsahu 12–22 %. Elektroforetická analýza PAGE prolaminových bílkovin nebo SDS-PAGE ISTA vyvinutá pro glutenové bílkoviny potvrdila výsledky imonologických testů o vhodnosti uvedených druhů pro dietu při celiakii. Tyto druhy neobsahují prolaminy, resp. v uvedených vzorcích byl obsah α-prolaminů zanedbatelný.

Klíčová slova: celiakie; bezlepková dieta; laskavec; merlík čilský; čirok; proso seté; bér vlašský; rosička krvavá; pohanka setá; frakce proteinů

Corresponding author:

Prof. Ing. Jiří Petr, DrSc., Česká zemědělská univerzita v Praze, Agronomická fakulta, katedra rostlinné produkce, 165 21 Praha 6-Suchdol, Česká republika
tel.: + 420 224 382 546, fax: + 420 224 382 535, e-mail: jpetr@af.czu.cz