https://doi.org/10.17221/114/2018-CJFS

The gluten content in oat products available on the Czech market

Jana Rysová, Eva Mašková, Zuzana Šmídová*

Department of Research, Food Research Institute Prague, Czech Republic

**Corresponding author: j.rysova@vupp.cz*

Citation: Rysová J., Mašková E., Šmídová Z. (2019): The gluten content in oat products available on the Czech market. Czech J. Food Sci., 37: 345–350.

Abstract: The gluten content in oat products on the market in the Czech Republic was monitored. The ELISA kit Ridascreen gliadin from R-Biopharm based on the R5 antibody was used for the gliadin determination. Oat products (41 samples), including 21 samples of oat flakes, were tested. Only 6 samples were labelled as gluten-free and it was confirmed that these samples meet the criteria for gluten-free foods. The total of 29 oat products, of which 15 were samples of oat flakes, contained more than 20 mg/kg of gluten. This means that 71% of oat cereal products were contaminated with cereals toxic for people with celiac disease. It was shown that most oat products on the Czech market are not safe for people with gluten intolerance.

Keywords: ELISA; gluten; oat products

Celiac disease as autoimmune disease and other diseases related to gluten intolerance are currently on the rise. Therefore suitable raw materials for the preparation of gluten-free foods are sought in the present time. Oat is considered to be one of cereals potentially useful in gluten-free diet. It is a rich source of proteins, lipids, vitamins, minerals and fiber, *i.e.* oat products could nutritionally improve gluten-free diet, which may be deficient in fiber, iron and calcium. The gluten-free diet can also threaten with a lack of vitamins B1, B2 and folic acid (Pawlovska *et al.* 2012; Comino *et al*. 2015). The protein content of the oat is very high compared to other cereals, and reaches up to 17–24%. The major storage protein of oat is avenalin constituting up to 80% of oat proteins.

The carbohydrate fraction comprises a readily cleavable starch from 25% to 30% of amylose, β-glucans, pentosans, and monosaccharides. Furthermore, oat contains 6–10% of fat with a high content of unsaturated fatty acids including the essential fatty acids. Many lipophilic substances with significant antioxidant properties are bound to the fat component (Singh *et al*. 2013). Oat fiber and in particular β-glucans affect the function of the gastrointestinal tract by water absorption and prebiotic effect (Daou & Zhang 2012) and also contribute to lowering of blood cholesterol (EU Commission Regulation No. 1160/2011). Antioxidant potential of oat depends on the content of polyphenols, lipophilic substances such as tocopherols, sterols and tocotrienols, as well as flavonoid colorants (Chmelova *et al.* 2015) and avenanthramids (Koenig *et al.* 2014).

There are different opinions on the safety of oat inclusion into the gluten-free diet. Oat contains different ratio of particular protein fractions compared to conventional cereals. Prolamins represent 10–20% of

Supported by the Ministry of Education, Youth and Sport of the Czech Republic, Grant No. LM2018100 and Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0318.

https://doi.org/10.17221/114/2018-CJFS

oat proteins, wheat 40–50%, rye 30–50% and barley 35–45%, respectively. The amino acid sequence of oat prolamins comprises 35–50% of proline and glutamine, whereas wheat and barley prolamins contain 70% of these amino acids. This amino acid composition likely explains lower toxicity of oats for celiacs.

The extensive review and systematic analysis of studies on the safety of oat as a part of gluten-free diet was carried out (Farage *et al.* 2014; Pinto-Sanchez *et al.* 2017). In analysed scientific publications, 15 of 26 studies were carried out as *in vivo* studies in adults and children. The consumption of oat was going on for several months to years and the quantity of consumed oat ranged from 20 g to 100 g per day. The evaluation of these studies was based on the serological parameters, morphological changes of intestinal mucosa and on the level of anti-tTG antibodies.

In some other studies, however, the tolerance to oat was evaluated on the basis of subjective symptoms incidence after consumption. The results of these works are not uniform. Oats were assessed as safe in 16 studies and in 10 cases some cultivars were described as toxic or potentially toxic. Real *et al.* (2012) have found the direct correlation between the immunoreactivity of different oat varieties and the presence of more or less toxic peptides. This means that immunotoxicity of oat cultivars can vary within wide limits.

Similar conclusions were reached by Silano *et al.* (2014), who pointed out the differences between oat varieties ability to induce changes in the intestinal mucosa and to cause subsequent inflammation. G12 monoclonal antibody against the 33-mer peptide was used for toxicity testing of oat cultivars (Comino *et al.* 2015; Comino *et al.* 2016). The test group of oats can be divided according to reactivity with G12 antibody into three categories – non-reactive, moderately reactive and highly reactive. Most clinical studies support the inclusion of non-toxic varieties of oat into a gluten-free diet, but the results of studies are difficult to compare because of the different methodologies and the use of various oat cultivars. These studies suggest a wide range of patients' sensitivity to avenin (Pulido *et al.* 2009; Lerner 2014; Aaltonen *et al.* 2017; Pinto-Sanchez *et al.* 2017). These authors also concluded that the inclusion of pure oat into a gluten-free diet would improve the nutritional value and palatability of this diet. Tapsas *et al.* (2014) performed a survey on the consumption of oat among children with celiac disease in Sweden, where 89.4% of children included oats into the diet, of which 81.9% consumed gluten-free oats. Oat consumption remained without consequences in most cases. Kaukinen *et al.* (2013) carried out a study with 106 individuals, 66% of which consumed about 20 g of oat per day and no changes of intestinal mucosa were observed.

In the long-term study (Cooper *et al.* 2013), 46 patients were consuming oat for one year as a part of a gluten-free diet. In 95% of patients no adverse histological changes of mucosa status or even mucosa improvement were reported. The influence of oat on the intestinal mucosa of pediatric patients was also examined by Sjöberg *et al.* (2014) by detecting the levels of mRNA related to 22 active proteins and by the activation of lymphocytes. The mucosa changes were found in children who did not tolerate oat. These studies indicate that the oat cultivars with immunogenic potential but at the same time less toxic or nontoxic varieties are included among cultivated oats.

The contamination of commercial oat products by other cereals is a common problem. Oat labelled as gluten-free must not contain more than 20 mg/kg gluten measured by ELISA method based on R5 or G12 antibodies. This oat must also be appropriately labelled. Many analyses have shown that most oat products on the market are contaminated with other cereals (De Souza *et al.* 2016; La Vieille *et al.* 2016). The possibility of oat contamination increases with a growing number of oat processing operations.

The aim of this study was to examine the contamination of oat products on the Czech market with gluten containing cereals toxic for celiac patients. The ELISA kit based on the R5 monoclonal antibody was therefore deliberately used for the gluten determination. R5 antibody does not react with the oat proteins. This antibody was raised against ω-secalin and its specificity is directed against repetitive epitopes QQPFP, QQQFP, LQPFP, and QLPFP contained in prolamins of wheat, barley, rye and their hybrids such as triticale or tritordeum (Kahlenberg *et al.* 2006).

MATERIAL AND METHODS

Samples of naked oats, oat flakes and other oat products were purchased at different stores in the Czech Republic – supermarkets, specialized health food stores and small-area stores, including convenience stores. The total number of samples was 42, of which 20 were oat flakes and 22 were other oat products. The description and origin of the samples are given in Tables 1 and 2. The Ridascreen gliadin ELISA kit (Art. No. R7001) and Cocktail Solution (Art. No. R7006) are produced by R-Biopharm AG (Germany) and supplied by JEMO Trading Ltd. (Slo-

https://doi.org/10.17221/114/2018-CJFS

vakia). The limit of detection of this ELISA kit is 1 mg of gluten per kg of sample. The limit of quantification (5 mg/kg of gluten) matches the value of the first non-zero calibrator in ELISA kit. Pure ethanol – 96% (No. 70390-11000; Penta, Czech Republic) and deionized water (Type I, ISO 3696) from devices Milli-Q Gradient (Merck, Germany).

The grinder Scarlett Silver Line (ARIMA Holding Corp., China), water bath EL-20R (Kavalier, Czech Republic), Galaxy Ministar Centrifuge C1413 (VWR, Republic of Korea) and laboratory shaker LT2 (Kavalier, Czech Republic) were used for sample preparation and Tecan Sunrise Remote Microplate Absorbance Reader (Switzerland) for absorbance measurement.

Sample preparation and gluten determination followed the procedure recommended by ELISA kit producer. After homogenization, 0.25 g of the sample was mixed with 2.5 ml of Cocktail solution. Samples were incubated for 40 min at 50°C with occasional shaking. Samples were cooled and after that 7.5 ml of 80% ethanol was added followed by shaking for 60 min and centrifugation for 10 min at 6000 rpm.

RESULTS AND DISCUSSION

The samples of oats and oat products with a minimum of additional components in order to avoid contamination of gluten from other raw materials were selected for gluten determination. The analyzed samples included therefore oat grains and especially oat flakes, flour and bran. The porridge and cereal bars based on oat-

Table 1. Gluten content in samples (n = 20) of oat flakes

Sample	Producer/ country of origin	Distribution/sale	Precautionary labelling	Gluten content (mg/kg)	C.v. (%)
Oat flakes	Bílý Mlýn Kepka	Penam	gluten containing cereals	38.75 ± 2.60	6.71
Oat flakes	Albert	Albert	gluten containing cereals	50.34 ± 0.55	1.10
Oat flakes fine with fiber	EMCO	Globus	product contains gluten	> 80	
Fine whole oat flakes	Fortin Muhlewerke	Lidl	no	22.77 ± 0.27	1.20
Good life oat superior flakes	Czech Republic	Bonavita	product contains gluten	> 80	
Oat flakes Bio	Czech Republic	Country Life	product contains gluten	> 80	
Alnatura oat flakes fine Bio	EU agricultural production	Globus	no	> 80	
Oat flakes coarse	Czech Republic	Kaufland	product contains gluten	> 80	
Oat flakes fine	EMCO – Vřesce	convenience store	product contains gluten	> 80	
Oat flakes fine with sprouts	Morn Flake Oats limited, England	Natural Jihlava JK	no	> 80	
DM oat flakes Bio	Germany	DM drogerie markt	no	7.1 ± 0.95	13.43
Oat flakes	Slovak Republic	NatureLand, Slovakia	product contains gluten	< 5	
Oat flakes coarse	Germany	Tesco stores	no	> 80	
Oat flakes	Bílý Mlýn Kepka	BILLA	no	> 80	
Oat flakes Bio	PRO BIO	BILLA	no	> 80	
100% pure oat flakes gluten free	UK – Tilquihilie	Country life	gluten-free	< 5	
Oat flakes	J. Voženílek	convenience store	product contains gluten	6.55 ± 0.31	4.71
Czech oat flakes Bio	Bio nebio	health food store	no	> 80	
Oat flakes gluten-free	Spielberger GmbH	health food store	gluten-free	< 5	
Fine oat flakes with sprouts	Morning Foods, England	J.O.D. Dvořákovi	no	> 80	

Values are presented as a mean of x repetitions (n = 2) ± mean s.d.; c.v. – coeficient of variation

https://doi.org/10.17221/114/2018-CJFS

meal or flakes were also analyzed. Results of these analyses represent the average value of two parallel determinations and are shown in Tables 1 and 2.

In this study, 41 oat products were analysed, of which 21 were the samples of oat flakes. Three samples of oat flakes were labelled as gluten-free and our results confirmed this fact. Another three samples of oat flakes had low gluten content and two of these samples were labelled by precautionary labelling 'contains gluten'. Other oat flakes samples contained more gluten than the limit for the gluten-free products – 20 mg/kg of sample (Implementing Regulation 828/2014/EU). A high percentage of contaminated oat flake samples made us to test the naked oats in retail packaging and also to test other oat products. In both samples of naked oat high gluten content was detected, in one oat sample the gluten content exceeded the calibration range of the ELISA kit. This fact confirms the contamination of raw material for oat products preparation.

In order to verify the surface contamination, the oat with gluten content over 80 mg/kg was twice washed with 60% ethanol, dried and re-analyzed. The result of the repeated analysis was 61.5 mg/kg of gluten in the sample. The coefficients of variation of this determination are shown in Table 2. The difference in gluten content between 'natural' oat and washed oat is statistically significant. This result supports the theory about the surface contamination of oats.

Table 2. Gluten content in samples (n = 22) of oat products

Sample	Producer/ country of origin	Distribution/sale	Precautionary labelling	Gluten content (mg/kg)	C.v. (%)
Instant oatmeal, wholemeal	ProBio	ProBio	no	> 80	
Oat drink dried natural	Topnatur	health food store	no	45.29 ± 2.79	6.17
Oat flour wholemeal	Natural Jihlava	health food store	no	> 80	
Sprouted oat	Iswari	health food store	gluten-free	< 5	
Naked oat	Zdraví z přírody	health food store	no	> 80	
Naked oat washed in 60% ethanol	laboratory sample	laboratory sample	no	61.45 ± 1.04	1.70
Oat alternative of cream	Country life	health food store	gluten	16.17 ± 0.10	0.63
Oat bran	Natural Jihlava JK	health food store	no	> 80	
Oat alternative of cream	DM bio	DM bio	oat products containing gluten	> 80	
Oat drink natur	DM bio	DM bio	product contains gluten	37.18 ± 0.73	1.96
Vanilla porridge	Semix Pluso	health food store	no	> 80	
Porridge	Semix Pluso	health food store	no	> 80	
Puffed oat flakes with honey Bio	Germany	Allexx spol. s r.o., SK	gluten-free	< 5	
Naked oat Bio	Country Life		no	24.1 ± 0.40	1.65
Wholemeal oat flour	Natural Jihlava		no	> 80	
Oat Flapjack with pecan nuts, gluten-free	Wholebake, England	Country life	gluten-free	< 5	
Oat Flapjack with cranberry, gluten-free	Wholebake, England	Country life	gluten-free	< 5	
Nutty Flapjack	RUPA	Albert	no	12.11 ± 1.06	8.77
Natural Flapjack	RUPA	Albert	no	< 5	
Raspberry porridge	Albert	Albert	cereals containing gluten	> 80	
Oat bran Natur	Austria	Country life	no	> 80	
Alnatura oat bran with sprouts	Alnatura	Globus	no	> 80	

Values are presented as a mean of x repetitions (n = 2) ± mean s.d.; c.v. – coeficient of variation

https://doi.org/10.17221/114/2018-CJFS

Furthermore, a group of products was analyzed, including flour, oat bran and porridge. All of these products have been assessed as contaminated. The gluten content above the limit of 20 mg/kg of food was found also in oat beverages and oat alternative of cream. On the contrary, the bars like 'Flap Jack' containing oat were evaluated as gluten-free.

Hernando *et al.* (2008) investigated the contamination of 134 oats from the genetic resources of Europe, USA and Canada, and commercial products by sandwich ELISA R5 antibody, Western blotting, MALDI TOF and Real Time PCR. Twenty-five oat samples contained gluten in the amount near the limit of detection, but 109 samples were contaminated. The contamination of oat products with gluten poses also problem in the United States (Thompson 2004). Due to the inconsistent results and the risk of contamination left the international Association of European Coeliac Societies (AOECS) the decision about the inclusion of oat in gluten-free diet at the national level (Standard for gluten-free foods from 2015).

CONCLUSION

Our study confirmed that most oat products sold on the Czech market are not safe for people with gluten intolerance and therefore these products can not be included in the gluten-free diet. One positive finding is the fact that products labelled as gluten-free were confirmed to be really gluten-free. Other analyzed products were often labelled with precautionary labelling such as 'contains gluten', 'may contain gluten' or the oat content as allergen was graphically highlighted in the product composition stated on the packaging. Manufacturers therefore do not violate any legislative rule in this case. Patients on a gluten-free diet who would be interested in oat inclusion into their diet, have to choose oat products labelled as gluten-free and accept their relatively high cost.

References

Aaltonen K., Laurikka P., Huhtala H., Maki M., Kaukinen K., Kurppa K. (2017): The long-term consumption of oats in celiac disease patients is safe: A large cross-sectional study. Nutrients, 9: 611. doi: 10.3390/nu9060611.

Comino I., de Lourdes Moreno M., Sousa C. (2015): Role of oats in celiac disease. World Journal of Gastroenterology, 21: 11825–11831.

Comino I., Bernardo D., Bancel E., de Lourdes-Moreno M., Sanchez B., Barro F., Suligoj T., Ciclitira P.J., Cebolla A., Knight S.C., Branlard G., Sousa C. (2016): Identification and molecular characterization of oat peptides implicated on coeliac immune response. Food & Nutrition Research, 60: 30324. doi: 10.3402/fnr.v60.30324

Cooper S.E.J., Kennedy N.P., Mohamed B.M., Abuzakouk M., Dunne J., Byrne G., McDonald G., Davies A., Edwards C., Kelly J., Feighery C.F. (2013): Immunological indicators of coeliac disease activity are not altered by long-term oats challenge. Clinical & Experimental Immunology, 171: 313–318.

Daou S., Zhang H. (2012): Oat Beta-Glucan: Its role in health promotion and prevention of diseases.

Comprehensive Reviews in Food Science and Food Safety, 11: 355–365.

De Souza M.C.P., Deschenes M.E., Laurencelle S., Roy C.C., Djilali-Saiah I. (2016): Pure oats as part of the Canadian gluten-free diet in celiac disease: The need to revisit the issue. Canadian Journal of Gastroenterology and Hepatology, Article ID 1576360, 8 pages. doi.org/10.1155/2016/1576360.

Farage P., Boas G.V., Gandolfi L., Oratesi R., Puppin Zandonadi R. (2014): Is the consumption of oats safe for celiac disease patients? A review of literature. Journal of Food and Nutritional Disorders, 3. doi.org/10.4172/2324-9323.1000138.

Hernando A., Mujico J.R., Mena M.C., Lombardía M., Méndez E. (2008): Measurement of wheat gluten and barley hordeins in contaminated oats from Europe, the United States and Canada by Sandwich R5 ELISA. European Journal of Gastroenterology and Hepatology, 20: 545–554.

Chmelová D., Ondrejovič M., Havrlentová M., Hozlár P. (2015): Antioxidant activity in naked and hulled oat (*Avena sativa* l.) varieties. Journal of Microbiology, Biotechnology and Food Sciences, 4: 63–65.

Kahlenberg F., Sanchez D., Lachman I., Tuckova L., Tlaskalova H., Mendez E., Mothes T. (2006): Monoclonal antibody R5 for detection of putatively coeliac-toxic gliadin peptide.

European Food Research and Technology, 222: 78–82.

Kaukinen K., Collin P., Huhtal, H., Mäki M. (2013): Long-term consumption of oats in adult celiac disease patients. Nutrients, 5: 4380–4389.

Koenig R., Dickman J. R., Kang Ch., Chu Y. F., Li L. (2014): Avenanthramide supplementation attenuates exercise-induced inflammation in postmenopausal women. Nutrition Journal, 13: 21. doi: 10.1186/1475-2891-13-21.

Lerner A. (2014): The enigma of oats in nutritional therapy for celiac disease. International Journal of Celiac Disease, 2: 110–114.

La Vieille S., Pulido O. M., Abbot M., Koerner T., Godefroy S. (2016): Celiac disease and gluten-free oats: A Canadian position based on a literature review., Article ID 1870305, 10 pages. doi: 10.1155/2016/1870305.

https://doi.org/10.17221/114/2018-CJFS

Pawlovska P., Diowksz A., Kordialik-Bogacka E. (2012): State-of-the-art incorporation of oat into a gluten-free diet. Food Reviews International, 8: 330–342.

Pinto-Sanchez M. I., Causada-Calo N., Bercik P., Ford, A.C., Murray J.A., Armstrong D., Semrad C., Kupfer, S.S., Alaedini, A., Moayyedi, P., Leffler, D.A., Verdú, E.F., Green, P. (2017): Safety of adding oats to a gluten-free diet for patients with celiac disease: Systematic review and meta-analysis of clinical and observational studies. Gastroenterology, 153: 395–409.

Pulido O. M., Gillespie Z., Zarkadas M., Dubois S., Vavasour E., Rashid M., Switze, C., Godefroy S. B. (2009): Introduction of oats in the diet of individuals with celiac disease: a systematic review. Advances in Food and Nutrition Research, 7: 235–285.

Real A., Comino I., de Lorenzo l., Merchan F., Gil-Humanes J., Gimenez M., Lopez-Casado M. A., Torres M. I., Cebolla A., Sousa C., Barr, F., Pisto, F. (2012): Molecular and immunological characterization of gluten proteins isolated from oat cultivars that differ in toxicity for celiac disease. Plos ONE, 7: e48365. doi: 10.1371/journal.pone.0048365.

Silano M., Pozo E. P., Uberti F., Manferdelli S., Del Pinto T., Felli C., Budelli A., Vincentini O., Restani P. (2014): Diversity of oat varieties in eliciting the early inflammatory events in celiac disease. European Journal of Nutrition, 53: 1177–1186.

Singh R., De S., Belkheir A. (2013): Avena sativa (Oat), a potencial nutraceutical and therapeutic agent. An overview. Critical Reviews in Food Science and Nutrition, 53: 126–144.

Sjöberg V., Hollen E., Pietz G., Magnusson K. E., Magnusson K. F., Sundström M., Holmgren Peterson K., Sundström O., Hernell O., Hammarström S., Högberg L., Hammarström M. L. (2014): Noncontaminated dietary oats may hamper normalization of the intestinal immune status in childhood celiac disease. Clinical and Translational Gastroenterology, 5: e58. doi: 10.1038/ctg.2014.9.

Tapsas D., Fälth-Magnusson K., Högberh L., Hammersjö J. A., Hollén E. (2014): Swedish children with celiac disease comply well with a gluten-free diet, and most include oats without reporting any adverse effects: a long-term follow-up study. Nutrition research, 34: 436–441.

Thompson T. (2004): Gluten contamination of commercial oat products in the United States. New England Journal of Medicine, 351: 2021–2022.

Received: 2018–04–27
Accepted after corrections: 2019–07–23
Published online: 2019–10–25