



Gluten contamination in the Canadian commercial oat supply

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A growing body of evidence suggests that a majority of people with celiac disease and on a gluten-free diet can safely consume pure oats in moderate amounts; however, previous studies have indicated that the commercial oat supply in other countries, and in Canada to some extent, is contaminated with other grains. This study has confirmed that the commercial oat supply in Canada is heavily contaminated with gluten from other grains. Approximately 88% of the oat samples ($n=133$) were contaminated above 20 mg kg^{-1} and there were no differences between the oat types tested. Only one gluten-free variety of oats was analysed and it consistently provided negative results in all analyses. It is difficult to determine where the contamination originates, but there are possibilities for cross-contamination in the field, in the transport of the grain, in the storage of the grain, and in the milling and packaging facilities. It is clear from this study that only those products that have been certified 'pure' oats would be appropriate for a gluten-free diet.

Keywords: immunoassays; allergens; cereals and grain

Introduction

Food allergies and intolerances are two sensitivities that, although they have distinct physiological pathways, require the strict avoidance of specific triggering substances. Wheat is one of these triggers and is among the eight most common food allergens identified by the *Codex Alimentarius* (Codex Alimentarius Commission 2010). Overall, food allergies affect approximately 5% of young children and 3–4% of adults (Sicherer and Sampson 2010). Prevalence data from adult and paediatric clinical populations suggest that wheat allergy can range from 2.5% in one American study to 25% of the allergenic population in one study from France (Hischenhuber et al. 2006). Adverse reactions to wheat can be severe, such as wheat-dependent exercise-induced anaphylaxis, but reactions can also include respiratory (e.g. bakers' asthma), contact (e.g. atopic eczema/dermatitis), or gastrointestinal problems such as irritable bowel syndrome and ulcerative colitis (Battais et al. 2005). Celiac disease is an inherited autoimmune disease that results in an intolerance not only to wheat, but also to other cereals such as barley and rye. It is estimated to affect approximately 0.5–1.0% of people in developed countries (Catassi and Fasano 2008) and its prevalence is on the rise (Rubio-Tapia and Murray 2010). Fortunately, withdrawal of the gluten-containing foods from the

diet can, in most cases, reverse this damage and a significant recovery of the intestinal mucosa is observed (Farrell and Kelly 2002).

Given the ubiquitous presence of these cereals in the Western diet (e.g. pasta, breakfast cereals, most breads, thickeners and stabilizers used in soups, processed meats, etc.), the strict avoidance of wheat and other sources of gluten is a lifetime challenge (Rashtak and Murray 2009). Individuals on a gluten-free diet need to replace these cereals with products derived from naturally gluten-free cereal grains (e.g. rice, corn, buckwheat, sorghum, etc.), but the recommended amounts of fibre, iron and calcium can be more difficult to obtain on such a diet and good planning is required (Thompson et al. 2005). Oats are known to be a source of both soluble and insoluble dietary fibre, B-complex vitamins (thiamin, niacin and riboflavin), iron and proteins (Thompson 2003; Haboubi et al. 2006). However, the safety of oats as part of a gluten-free diet has been an object of debate because there is evidence to indicate some individuals with celiac disease are intolerant to oats in addition to wheat, barley and rye (Silano et al. 2007). The complexity of celiac disease along with a certain homology in the protein fraction of oats with other gluten-containing cereals is likely to be the reason why some individuals react to oats. However, there is a

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significant difference between the proteins found in oats and the immuno-stimulatory protein sequences found in wheat, rye and barley which may explain why most people with celiac disease are able to tolerate these cereal grains (Pulido et al. 2009). In fact, there is a growing body of evidence to suggest that pure oats (uncontaminated with other gluten-containing cereal grains) are safe to consume in moderate amounts for the majority of people suffering from celiac disease and would be very important source of proteins, carbohydrates and fibre (Rashid et al. 2007).

Canada has established requirements for the production of uncontaminated oats including the growing, processing, testing and labelling stages of the product. It cannot be assumed that all varieties of oats sold in Canada are uncontaminated with other gluten sources because the infrastructure used for growing, transporting and milling of other grains may be used for oats and cross-contamination is likely to occur. There have been a few studies that have shown the likelihood and levels to which oat varieties are contaminated with other gluten-containing cereals, but most of these investigations were on oats sold in the United States and Europe and only two had limited information on varieties sold in Canada (Thompson 2004; Hernando et al. 2006, 2008; Gélinas et al. 2008). To obtain a better picture of gluten contamination within the oat varieties sold at Canadian retailers we collected a large sampling from across the country for a variety of different oat products.

Materials and methods

Food samples

Oat samples were collected from retail outlets in Newfoundland, Prince Edward Island, Quebec, Ontario and British Columbia at two different times in the year to ensure that at least two samples with distinct lot codes could be collected. Mixing four samples of each lot in a large bowl by hand produced a composite material, of which approximately 1 kg was finely ground to between 100 and 200 microns using a centrifugal mill (Retsch Mill ZM 200, Haan, Germany). The milled samples were then homogenized by mixing for 30 min with a 1000 W industrial stand mixer (Cuisinart, Model #SM-70C) to produce a finely ground homogenized composite oat material. A 200 g subsampling of this material was then stored for analysis. The centrifugal mill and the stand mixer components were disassembled between samples for cleaning with copious amounts of hot water and allowed to air dry. Samples collected in British Columbia were processed in a slightly different manner ($n = 35$ of 133). Composite samples of approximately 500 g were milled using a commercial blender

(Waring, Torrington, CT, USA) before homogenization with a bottle roller.

ELISA systems

Gluten analysis was performed in duplicate using the RIDASCREEN R-7001 gliadin ELISA (R-Biopharm Inc., Washington, MO, USA). This kit performs the quantitative analysis of prolamins from wheat (gliadins), barley (hordeins) and rye (secalins). The gliadin standards supplied with the kit are calibrated to the Prolamin Working Group reference material with a limit of detection of 3 mg of gluten per kg of sample and a quantification range for gluten from 5 to 80 mg kg⁻¹ (Van Eckert et al. 2006). The kit instructions were followed for this analysis and the procedure is only briefly described here. The nature of the oat flour samples did not require any special extraction solutions so the ethanol extraction process described in the kit insert was used for the analysis. Each sample of oats (1.0 g) was weighed into a 15 ml centrifuge tube, a 60% aqueous ethanol solution (10 ml) was added and the tubes were put on a vortex for 30 s. Samples were mixed for an additional 10 min on a shaker (VWR OS-500) followed by centrifugation at room temperature for 10 min at 2500 g (ThermoFisher Sorvall RT-1). A measured aliquot of the supernatant was removed; diluted 50 times and then 100 µl of this solution were used in the assay. Standards and samples were added in duplicate wells on the plate and allowed to incubate for 30 min at room temperature followed by three washing steps. Enzyme conjugate was added to each well and the plate was incubated for 30 min followed by an additional three washing steps. At this point substrate and chromogen were added to each well and allowed to react for 30 min followed by the addition of stop reagent. The absorbance was read at 450 nm and the data analysed to determine gluten concentration. The plate results are given in gliadin concentration and need to be adjusted for the dilution factor to obtain the gliadin concentration in solution, which is then converted to gluten content by multiplication by two. All samples that showed an absorbance value over the highest standard were further diluted until the results were within the calibration range.

An ELISA from Tepnel Biosystems (BioKits line of ELISA kits are now being distributed by Neogen, Lansing, MI, USA; <http://www.neogen.com>) was used to screen a subset of the original samples for barley contamination. This particular kit uses an antibody that recognizes ω -gliadin, which has a low cross-reactivity to barley hordeins (5%) compared with wheat gliadin (100%). The limit of detection for this assay is 1 mg kg⁻¹ of material and the quantification range is from 3 to 50 mg kg⁻¹ of gluten in a sample.

Results and discussion

There is scientific evidence to support the introduction of moderate amounts of pure oats in the gluten-free diet of individuals suffering from celiac disease (Pulido et al. 2009). Pure oats are recommended because it is believed, and has been shown to some extent, that the commercial oat supply is contaminated with other grains. In order to determine the extent of this contamination we analysed a large selection of packaged oat products that are readily available in the Canadian retail market. Figure 1 shows the gluten levels determined for the oat composites tested in this study using the RIDASCREEN® R-7001 sandwich ELISA (R5-ELISA). Of the 133 samples analysed only nine (6.8%) were found to contain levels of gluten below the 20 mg kg^{-1} limit proposed by the Codex Alimentarius Commission for naturally gluten-free foods (Catassi and Fasano 2008). Three of these samples had undetectable amounts of gluten, while the remaining samples were between 5 and 20 mg kg^{-1} . The remaining samples ($n = 124$, 93.2%) showed contamination levels above this limit and ranged from approximately 21 to 3800 mg kg^{-1} . Previous studies that have measured the gluten content in oats had a limited number of samples from the Canadian market, but showed similar contamination levels to this study. For example, one study found eight of 12 samples of oats purchased in Canada (67%) were contaminated (Gélinas et al. 2008) while another found that seven of ten samples purchased in Canada (70%) were

contaminated with gluten at levels higher than 20 mg kg^{-1} (Hernando et al. 2008).

Another aspect of this study that is of interest is the variance within different lots of commercial oats sold in Canada. Is it possible to find a brand of oats that is consistently low in gluten? As mentioned above there were three samples of oats that showed undetectable levels of gluten using the R5-ELISA. Two of these samples were different lots from the one company and the last sample was part of a set of six different lots of rolled oats from a different company. The former two lots of oats from one company tested negative for gluten while the six lots from the other company ranged from zero to 133 mg kg^{-1} of oats. This was not an isolated example and all oat types showed variance within the different lots sold in Canada. The variability between different types of oats available on the Canadian market was also investigated by determining the average gluten level for the different types of oats (Table 1). Analysis of variance (ANOVA) for the

Table 1. Gluten content as a function of type of oat product.

Type of oat	Range (mg kg^{-1})	Median (mg kg^{-1})	Mean (mg kg^{-1})
Steel-cut oats	55–1467	660	645 ± 512
Rolled/flaked/oatmeal	0–2485	81	316 ± 497
Quick/minute oats	13–3784	534	655 ± 694
Oat bran	37–3469	280	704 ± 862

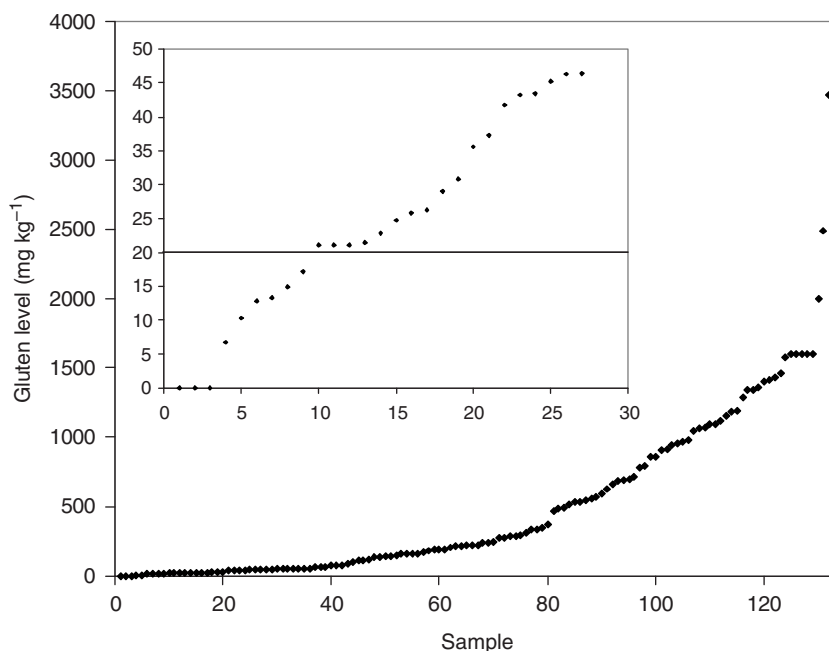


Figure 1. Survey results of the average gluten levels in a sampling of pre-packaged oats sold in Canadian retail stores using a commercial ELISA kit. The insert is an expanded region of the lower-level samples along with a line representing the 20 mg kg^{-1} level recommended by CODEX for gluten-free status.

different oat samples confirms there is no significant difference between the oat types ($\rho=0.06$). Interestingly, analysis of the data obtained for regular and organic oats shows that there is a significant difference ($\rho < 0.0001$) between organic oats (mean = 240 mg kg⁻¹, $n=31$) and regular oats (mean = 616 mg kg⁻¹, $n=102$), but this crop is still significantly contaminated with gluten.

In order to determine the type of grain that has contaminated the oat samples a selection of oat types purchased were analysed again using an ELISA method that is insensitive to barley hordeins. The R5-ELISA responds to a peptide sequence that is present in gliadin and then reports the amount of gluten by referencing to a gliadin material based on multiple wheat cultivars (Van Eckert et al. 2006). This assay also responds to barley and rye because the same peptide sequence is present in hordeins and secalins; however, the gluten assay that responds to the ω -gliadin fraction of wheat has a low cross-reactivity (5%) towards barley hordeins. A high response from the R5-ELISA and a low response from the ω -ELISA is an estimate of the type of gluten contamination (Gélinas et al. 2008). For example, the level of gluten for sample 63 (Figure 2) using the R5-ELISA was determined to be 216 mg kg⁻¹ while the level for the same sample using the ω -gliadin ELISA was only 10 mg kg⁻¹. The response from the ω -ELISA is approximately 5% of the R5-ELISA and suggests that barley is the source of the gluten contamination for this sample. In another example, sample 36 reported 59 mg kg⁻¹ using the R5-ELISA and 16 mg kg⁻¹ using the ω -ELISA, suggesting the gluten contamination is likely a mixture of wheat and barley or possibly differences in the ω -gliadin content of sample. The data in Figure 2 indicate that the majority of samples (13 of 14) in the subset that tested positive for gluten were contaminated at some level with barley. Although individuals with gluten sensitivities also react

to the hordeins in barley, the actual level of gluten in the sample, measured by the R5-ELISA, can be overestimated due to the differences between barley and wheat, the latter of which has been used to calibrate the method (Kanerva et al. 2006). Canadian regulations do not require allergen labelling of grains like oats that are destined for further processing and allow the presence of a certain percentage of foreign components, which may include gluten-containing cereals (Canadian Grain Regulations 2008). Packagers of oats can voluntarily provide precautionary statements to warn allergic or intolerant consumers about the potential risk of cross-contamination. The results of this study have shown that precautionary labelling practices of commercial oats vary greatly (Table 2). For example, the oat products tested either had no precautionary statement pertaining to wheat or gluten ($n=65$) or presented various statements ranging from, 'may contain traces of wheat' ($n=43$) to less clear statements such as 'good manufacturing practices have been used to segregate ingredients in a facility

Table 2. Number of occurrences for different precautionary statements.

Precautionary statement	Number of occurrences
No warning	65
Wheat-free claim	2
May contain wheat or traces of wheat	43
May contain other allergens ^a	9
Manufactured in a facility that also uses wheat, etc. ^b	11
Wheat starch in the ingredient list	3

Notes: ^aExample: 'May contain traces of nuts and milk ingredients.'

^bThis also includes the following statement: 'Good manufacturing practices have been used to segregate ingredients in a facility that also processes peanuts, tree nuts, milk, eggs, wheat, sulfites and soy ingredients.'

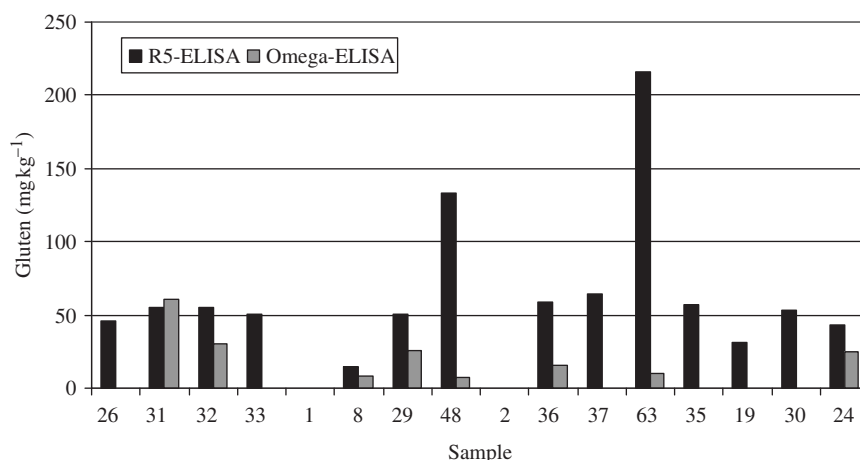


Figure 2. Results for the determination of gluten source for a subset of samples using two different ELISA kits.

that also processes peanuts, tree nuts, milk, eggs, wheat, sulfites and soy ingredients' ($n = 11$). The latter statement is confusing and contains some uncertainty for many individuals with food allergies (Verrill and Choiniere 2009) and may be a reason why precautionary statements are increasingly being ignored by allergic consumers (Sheth et al. 2010). A concern to no precautionary statement, or an ambiguous one, is a precautionary statement that provides an incomplete list of allergens potentially present through unavoidable cross-contamination. For example, some of the oat samples contained statements indicating the potential presence of other allergens including peanut, tree nuts, sesame and soy, but not for wheat or gluten. Given the increased knowledge of potential cross-contamination a consumer will likely know that these products will still be contaminated with gluten. The only sample that was gluten free in both lots that were tested actually had a wheat-free claim on the package and represents a pure oat product. In regard to oat varieties in Canada it would be suggested that all warnings should be adhered to and avoidance of all commercial oat varieties other than pure oats be followed. Although there were some varieties of pre-packaged oats that were low in gluten the vast majority would not be safe for the allergenic or intolerant consumers.

Conclusions

This study was done to determine the extent of the contamination of the commercial oat supply in Canada. Oat samples were collected from retail stores across the country and at different times in the year to ensure two different lots were obtained for each product. Taking into consideration the lot-to-lot variability, approximately 88% of the products tested were contaminated above the Codex-recommended gluten-free level and ranged from 21 to 3800 mg kg⁻¹ of oats. There was little difference in the level of gluten between the processed types of oats, but the organic varieties (mean = 240 mg kg⁻¹) did have a statistically significant lower level of gluten compared with the regular variety of oats (mean = 616 mg kg⁻¹). Although the organic varieties of commercial oats contain lower levels of gluten they still would not be considered gluten-free or safe. Further analysis on a portion of the samples collected using a method that is known to have a low sensitivity to barley hordeins indicated that many of these samples were likely contaminated with barley. Only one sample of oats consistently produced negative results using both ELISA methods and this sample contained a wheat-free claim on the package. The pure variety of oats, those that have been determined experimentally to be gluten-free, are the only variety that are recommended for someone following a gluten-restrictive diet. This study also showed that voluntary

precautionary labelling was used on roughly half of the products tested and in some cases the statement was inadequate. Therefore, educational initiatives are important in communicating the risk of gluten contamination in naturally gluten-free cereal grains such as (regular) commercial oats. Initiatives are underway to collect data on the gluten contamination of other naturally gluten-free cereal grains in Canada, which may have an important impact on the consumption patterns of individuals with celiac disease or wheat allergy in Canada.

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References

- Battais F, Mothes T, Moneret-Vautrin DA, Pineau F, Kanny G, Popineau Y, Bodinier M, Denery-Papini S. 2005. Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat. *Eur J Allergy Clin Immun.* 60:815–821.
- Canadian Grain Regulations. 2008. Canadian grain regulations 1 August 2008, Minister of Justice; [cited 2010 Sept 15]. Available from: <http://www.grainscanada.gc.ca/legislation-legislation/regulation-reglement/2008/sch-ann-3-2008-eng.pdf/>
- Catassi C, Fasano A. 2008. Celiac disease. *Curr Opin Gastroen.* 24:687–691.
- Codex Alimentarius Commission. 2010. CODEX STAN 1-1985. Codex Alimentarius Commission; [cited 2010 Sept 15]. Available from: http://www.codexalimentarius.net/download/standards/32/CXS_001e.pdf/
- Farrell RJ, Kelly CP. 2002. Celiac sprue. *N Engl J Med.* 346:180–188.
- Gélinas P, McKinnon CM, Mena MC, Méndez E. 2008. Gluten contamination of cereal foods in Canada. *Int J Food Sci Tech.* 43:1245–1252.
- Haboubi NY, Taylor S, Jones S. 2006. Coeliac disease and oats: a systematic review. *Postgrad Med J.* 82:672–678.
- Hernando A, Mujico JR, Juanas D, Méndez E. 2006. Confirmation of the cereal type in oat products highly contaminated with gluten. *J Am Diet Assoc.* 106:665.
- Hernando A, Mujico JR, Mena MC, Lombardia 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. *Eur J Gastroenterol Hepatol.* 20:545–554.
- Hischenhuber C, Crevel R, Jarry B, Mäkis M, Moneret-Vautrin DA, Romano A, Troncone R, Ward R. 2006. Review article: Safe amounts of gluten for patients with wheat allergy or coeliac disease. *Aliment Pharmacol Ther.* 23:559–575.
- Kanerva PM, Sontag-Strohm TS, Ryöppy PH, Alho-Lehto P, Salovaara HO. 2006. Analysis of barley

- contamination in oats using R5 and ω -gliadin antibodies. *J Cereal Sci.* 44:347–352.
- Pulido OM, Gillespie Z, Zarkadas M, Dubois S, Vavasour E, Rashid M, Switzer C, Godefroy SB. 2009. Chapter 6 Introduction of oats in the diet of individuals with celiac disease. A systematic review. *Adv Food Nutr Res.* 57:235–285.
- Rashid M, Butzner D, Burrows V, Zarkadas M, Case S, Molloy M, Warren R, Pulido O, Switzer C. 2007. Consumption of pure oats by individuals with celiac disease: A position statement by the Canadian Celiac Association. *Can J Gastroenterol.* 21:649–651.
- Rashtak S, Murray JA. 2009. Celiac disease in the elderly. *Gastroenterol Clin N Am.* 38:433–446.
- Rubio-Tapia A, Murray JA. 2010. Celiac disease. *Curr Opin Gastroen.* 26:116–122.
- Sheth SS, Waserman S, Kagan R, Alizadehfar R, Primeau MN, Elliot S, St. Pierre Y, Wickett R, Joseph L, Harada L, et al. 2010. Role of food labels in accidental exposures in food-allergic individuals in Canada. *Ann Allergy Asthma Immunol.* 104:60–65.
- Sicherer SH, Sampson HA. 2010. Food allergy. *J Allergy Clin Immunol.* 125:S116–S125.
- Silano M, Benedetto RD, Maialetti F, De Vincenzi A, Calcaterra R, Cornell HJ, De Vincenzi M. 2007. Avenins from different cultivars of oats elicit response by coeliac peripheral lymphocytes. *Scand J Gastroenterol.* 42:1302–1305.
- Thompson T. 2003. Oats and the gluten-free diet. *J Am Diet Assoc.* 103:376–379.
- Thompson T. 2004. Gluten contamination of commercial oat products in the United States [7]. *N Engl J Med.* 351:2021–2022.
- Thompson T, Dennis M, Higgins LA, Lee AR, Sharrett MK. 2005. Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *J Hum Nutr Diet.* 18:163–169.
- Van Eckert R, Berghofer E, Ciclitira PJ, Chirido F, Denery-Papini S, Ellis HJ, Ferranti P, Goodwin P, Immer U, Mamone G, et al. 2006. Towards a new gliadin reference material-isolation and characterisation. *J Cereal Sci.* 43:331–341.
- Verrill L, Choiniere CJ. 2009. Are food allergen advisory statements really warnings? Variation in consumer preferences and consumption decisions. *J Food Prod Mark.* 15:139–151.