



Gluten detection in foods available in the United States – A market survey



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ABSTRACT

Many gluten-free (GF) food choices are now available in supermarkets. However, the unintentional presence of gluten in these foods poses a serious health risk to wheat-allergic and celiac patients. Different GF labelled foods (275) and non-GF labelled foods, without wheat/rye/barley on the ingredient label (186), were analysed for gluten content by two different enzyme linked immunosorbent assay (ELISA) kits. Considering the gluten threshold of 20 ppm, GF labelled foods had 98.9% GF labelling compliance with 1.1% (3 out of 275) of foods being mislabelled/misbranded. Among the non-GF labelled foods, 19.4% (36 out of 186) of foods had >20 ppm of gluten, as measured by at least one ELISA kit, of which 19 foods had >100 ppm of gluten. The presence of oats in non-GF labelled foods was strongly correlated with a positive ELISA result. Gluten was also found in a significant number of foods with gluten/wheat-related advisory warnings.

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1. Introduction

Gluten-free (GF) foods and beverages are becoming increasingly popular specialty foods in the US market with retail sales amounting to almost \$1.6 billion in 2010 (Sapone et al., 2012). The growing consumer demand for GF foods has led to an increase in gluten-substituted food product development. In 2010, 10.8% of new foods and beverages contained GF health and nutrition-related claims, second only to high vitamin/mineral claims (12.2%) (Martinez, 2013). The rise in GF foods is partly due to increased awareness among individuals with celiac disease (CD), wheat allergy and gluten sensitivity, which requires these patients to follow a strict gluten-free diet. However, consumer perception of other benefits associated with a GF diet, such as digestive health, nutritive value, weight management, and high quality, also contributes significantly to increased sales of GF foods (Martinez, 2013).

Celiac disease is a cell-mediated autoimmune disease whereas wheat allergy is an immunoglobulin E (IgE)-mediated reaction. The symptoms of these disorders may vary, depending on individual sensitivity and disease severity. Celiac disease causes villous atrophy of the small intestine, resulting in various gastrointestinal and extraintestinal/systemic complications (Rallabhandi, 2012). Like other food allergies, depending on the severity, the symptoms of wheat allergy may range from mild itching to life-threatening

anaphylaxis. Since there is no cure available, avoidance of gluten/wheat in the diet is the best option for patients. Both CD and wheat allergy are caused by the ingestion of wheat proteins. Gluten is the group of proteins causing CD, whereas major wheat allergens belong to the albumin/globulin group of proteins. Gluten also causes certain forms of wheat allergy, such as wheat-dependent exercise-induced anaphylaxis, in sensitive individuals (Bouchez-Mahout et al., 2010; Matsuo, Kohno, Niihara, & Morita, 2005). It is not surprising that the proteins from rye and barley are also implicated in CD (Vader et al., 2002), since they belong to the same Triticeae family as wheat. Additionally, rye and barley have a high degree of protein homology and immunological cross-reactivity with wheat allergens (Palosuo, Alenius, Varjonen, Kalkkinen, & Reunala, 2001). Hence, gluten refers to a heterologous group of proteins composed of prolamin and glutelin fractions from wheat, rye and barley. The prolamin fraction is alcohol-soluble and is known as gliadin (wheat), secalin (rye) or hordein (barley), depending on the source grain.

Consumers rely on the food claims/labels to purchase foods with or without a specific ingredient. Gluten can be unintentionally introduced into food due to cross-contact of inherently GF grain with wheat, rye and/or barley during harvest, transport or storage. Cross-contact during food manufacturing, when using shared equipment, can also result in the presence of gluten in inherently GF foods. The accidental presence of gluten in food may be safe for most consumers, but can result in severe reactions in gluten-sensitive individuals. Although there is scarce

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information on gluten-specific food recalls, a recent study by Gendel and Zhu (2013) ranked wheat as the second major food allergen, following milk, responsible for food allergen recalls. Wheat is one of the main sources of gluten in foods. Food regulatory agencies have labelling mandates to authenticate GF claims. A limit of 20 ppm of gluten is the threshold for a food to be labelled as “gluten-free”, a level agreed upon by several food regulating agencies, including Codex Alimentarius, the European Union, and the US Food and Drug Administration. Various gluten detection methods have been developed in recent years, based on enzyme-linked immunosorbent assay (ELISA) (Moron et al., 2008; Skerritt & Hill, 1990; Valdes, Garcia, Llorente, & Mendez, 2003), lateral flow device/dipstick (Allred & Park, 2012), polymerised chain reaction (Dahinden, von Buren, & Luthy, 2001; Mujico, Lombardia, Carmen Mena, Mendez, & Albar, 2011; Sandberg, Lundberg, Ferm, & Yman, 2003) and mass spectrometry (Sealey-Voyksner, Khosla, Voyksner, & Jorgenson, 2010; Tanner, Colgrave, Blundell, Goswami, & Howitt, 2013). ELISA remains the most commonly used method for gluten quantitation and several ELISA kits, based on different polyclonal and monoclonal antibodies, are commercially available.

It is important to evaluate the gluten content in foods for labelling compliance and consumer safety. Past research has focussed on specific food categories for assessment of gluten content, such as single ingredient foods (Dostalek et al., 2009; Koerner et al., 2011, 2013; Thompson, Lee, & Grace, 2010) or cereal foods (Gelinas, McKinnon, Mena, & Mendez, 2008). There is scarce information on gluten levels in other complex foods. The objective of this study was to determine the gluten content in various foods labelled GF and those not labelled as GF, but without gluten-containing ingredients. Previous work has shown acceptable gluten recovery with R5 monoclonal-based ELISA (R-Biopharm) and a proprietary polyclonal-based ELISA (Morinaga) in spiked (Geng, Westphal, & Yeung, 2008; Sharma, 2012) as well as incurred (Sharma et al., 2013) food samples. Also, both ELISA methods have been validated by multi-laboratory evaluation and the R5-Mendez ELISA method has been suggested by Codex Alimentarius for determination of gluten in foods. Hence, these ELISA kits were used as the gluten detection methods in the current study.

2. Materials and methods

2.1. Materials

Food samples were purchased from local grocery stores. Two different ELISA kits were used in the study: wheat protein sandwich ELISA (181GD; Morinaga Institute of Biological Science, Inc., Yokohama, Japan) and R5 monoclonal-based RIDASCREEN® Gliadin sandwich ELISA (R7001; R-Biopharm AG, Darmstadt, Germany). The monoclonal antibody A1 and the goat anti-mouse peroxidase

conjugate were from Biomedal S.L. (Spain) and Sigma Chemical Co. (St. Louis, MO, USA), respectively.

2.2. Food samples

In total, 461 different food samples were divided into 2 groups: GF labelled foods (275) and non-GF labelled foods with no wheat/rye/barley on the ingredient label (186). Depending on the type of food samples, each group was further divided into various categories: (1) grains/seeds/nuts/legumes, (2) condiments/sauces, (3) curry/soup/soup mixes, (4) baking mixes, (5) baked foods, (6) pasta products, (7) breakfast cereals, (8) snack foods, (9) granola/bars/energy bars, (10) beverages/ice-creams/frozen desserts, (11) meat/meat substitutes/refrigerated or frozen foods, and (12) others. Homogeneous liquid and powdered solid foods were weighed and extracted without any further preparation. For all other foods, an appropriate quantity was ground in a blender (LB10, Waring Laboratory) into a homogeneous mixture. The food samples were stored at either 4 °C or –20 °C prior to further use.

2.3. Gluten measurement by ELISA

Two subsamples were taken (1 g each for Morinaga; 0.25 g for R-Biopharm) from each food sample. Further, each subsample was evaluated in duplicate for the gluten content according to the kit recommendations. The different characteristics of the kits used are shown in Table 1. The optical density in the ELISA plate wells was measured by a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). A 4-parameter fit, using the SoftMax Pro 5.4 software, was applied to plot the standard curve and calculate gluten concentration in food samples. The results were reported as averages of four readings. A gluten content of less than 5 ppm, as measured by both kits, was considered a food with no gluten.

2.4. Western blotting

Food samples (100 mg) were extracted in 1 ml of 50 mM Tris–HCl buffer, pH 7.5, containing 1% sodium dodecyl sulphate (SDS) and 2% β-mercaptoethanol (β-ME) for 1 h at room temperature followed by centrifugation at 10,000g for 15 min. About 200 µl of supernatant were mixed with 50 µl of SDS–PAGE sample buffer (50 mM Tris–HCl, pH 6.8, containing 1% SDS, 30% glycerol, 0.01% bromophenol blue, and 2% β-ME) and incubated for 10 min in a boiling water bath. Cereal grain flours extracted similarly were used as positive (wheat, rye and barley) and negative (oat and corn) controls. The protein estimation was performed using a 660 nm protein assay kit (Pierce, Rockford, IL, USA). Protein samples (20 µl), along with grain controls (2 µg protein) and protein molecular weight markers (Fisher Scientific, Pittsburgh, PA, USA),

Table 1
Different characteristics of ELISA kits used in the study.

Characteristics	Morinaga	R-biopharm
Sample amount	1 g	0.25 g/0.25 ml
Extraction buffer	Sample extraction solution with 2% 2-mercaptoethanol	Cocktail solution followed by 80% aqueous ethanol (60% final concentration)
Final sample: buffer	1:20	1:40
Extraction temperature	Room temperature (RT)	Cocktail solution: 50 °C; after ethanol addition: RT
Extraction time	Overnight or at least 12 h	Cocktail solution: 40 min; after ethanol addition: 1 h
Antibody type	Anti-wheat protein polyclonal antibody	R5 monoclonal antibody
Limit of quantification	0.78 ng/ml (0.3 ppm) wheat protein	2.5 ppm gliadin (5 ppm gluten)
Specificity	Wheat: 100% Rye: 60.6% Barley: 41.6%	Not available

were loaded onto 4–12% Novex Bis-Tris gel (Invitrogen) and run at constant voltage (100 V) until the dye reached the gel bottom. The polypeptides separated on the gel were transferred to a 0.2 µm nitrocellulose membrane and visualised by Ponceau S stain. For Western blot, the stain on the membrane was washed with tris buffered saline containing 0.05% Tween 20 (TBS-T) and the membrane was incubated for 1 h at room temperature in blocking buffer (5% non-fat dried milk in TBS-T). After a brief wash in TBS-T, the membrane was incubated overnight at 4 °C in A1 anti-gliadin 33-mer antibody diluted 10,000× in blocking buffer. The membrane was then washed, thrice in TBS-T for 15 min each, and incubated with goat anti-mouse peroxidase conjugate antibody (10,000× in TBS-T) for 1 h at room temperature. The membrane was again washed thrice with TBS-T for 15 min each and developed using the CN/DAB substrate kit (Thermo Scientific, Rockford, IL, USA).

3. Results and discussion

3.1. GF labelled foods

In total, 275 GF-labelled foods were divided into 12 categories (Table 2). Among these categories, snack foods made up the great-

est number (20% of total GF labelled foods), followed by baked foods (10.5% of total GF labelled foods). Though not all GF labelled foods that were available in the market were surveyed, our selection of representative foods was consistent with the recent report by Martinez (2013), where snack foods (16.3%) were the major food category to bear no-gluten related claims in 2010, followed by bakery (15.1%), beverages (15.1%), sauces/dressings/condiments (12.6%) and chocolate/sugar/gum confectionary (6.4%). The gluten content of GF labelled foods, as measured by two different ELISA kits, is shown in Fig. 1. Out of 275 GF labelled foods, 10 foods (3.6%) had a gluten content >5 ppm by at least one ELISA kit. Of these, the R-Biopharm and Morinaga kits detected gluten in 8 and 9 foods, respectively. The range of gluten content detected by either of the ELISA kits was 5.8–554 ppm. The linear regression analysis of the gluten content measured by these two ELISA kits showed good fit ($R^2 = 0.9368$) but the Morinaga kit detected a higher gluten content than did the R-Biopharm kit (Fig. 2A). Using the slope of the linear equation in Fig. 2A, we observed that the gluten levels, as determined using the Morinaga kit, were 3.7 times that of the R-Biopharm kit. The difference in gluten levels between the two kits for one of the GF foods was significant (134 ppm by R-Biopharm and 554 ppm by Morinaga kit), causing the increased

Table 2
Food categories used for gluten quantitation using ELISA kits.

Category	Select examples	Number of food samples					
		Total	Contains oat	Gluten advisory	Gluten content (ppm) ^a		
					>5	>20	>100
<i>GF labelled foods</i>							
Grains/seeds/nuts/legumes	Quinoa, beef, millet, flaxseed, mixed nuts, sorghum/rice/amaranth/corn/garbanzo bean/buckwheat/almond/coconut/oat flour	17	1	1	1	0	0
Condiments/sauces	Vinegar, ketchup, soy sauce, salad dressing, mayonnaise, chilli season kit, relish, molasses, seasoning mix	22	0	4	1	1	0
Curry/soup/soup mixes	Noodle, vegetable/black bean/tomato/mushroom/tortilla/lentil soup, chilli, Indian curry, Thai curry	21	0	5	0	0	0
Baking mixes	Cake/cookie/pancake/bread/brownie mix, baking flour, all-purpose flour	20	0	0	0	0	0
Baked foods	Tortilla, muffin, breadcrumb, pita/sandwich bread, loaf, rolls, waffles, pancake, cheese pizza, pizza crust	29	0	2	0	0	0
Pasta products	Macaroni, brown rice/soybean/black bean spaghetti, rice/multigrain/corn pasta	16	0	0	1	0	0
Breakfast cereals	Buckwheat/flax/rice/corn cereal, maple crunch, oatmeal, steel cut/rolled oats	22	4	1	3	1	0
Snack foods	Rice cakes, rice/multi-seed crackers, tortilla/plantain/rice/lentil chips, quinoa/peanut butter/cinnamon/chocolate chip cookies, rice/ginger snaps, corn puffs	55	1	10	2	0	0
Granola/bars/energy bars	Fruit and nut/cranberry/raisin granola, fruit and nut/rice cereal/mixed nuts bar, granola cereal	18	4	0	0	0	0
Beverages/ice-creams/frozen desserts	Sunflower/soy/rice/almond/coconut/flax milk, coffee creamer, sherbet, coconut milk frozen dessert, vanilla ice-cream	15	0	1	0	0	0
Meat/meat substitute/ refrigerated or frozen foods	Butternut squash risotto/souffle, enchilada, burrito, teriyaki chicken bowl, polenta provencale, roasted turkey/beef, veggie burger, Italian dry salame	23	1	5	2	1	1
Others	Yeast, corn/tapioca starch, xanthan gum, hummus, soy nut/peanut butter, couscous, polenta, dried cranberries	17	0	0	0	0	0
Total		275	11	29	10	3	1
<i>Non-GF labelled foods (no wheat/rye/barley on the ingredient label)</i>							
Grains/seeds/nuts/legumes	Corn, rice, quinoa, mustard/caraway/sesame seed, mixed nuts, cashew, almond, rice/soy/oat flour, flax/corn meal	30	2	7	7	6	2
Condiments/sauces	Yellow mustard, vinegar, ketchup, corn syrup, soy sauce, blue cheese dressing	18	0	1	0	0	0
Curry/soup/soup mixes	Potato leek/split pea/lentil/tomato/black bean/white bean soup, vegetarian chilli, chilli mix, clam chowder	15	0	1	1	0	0
Breakfast cereals	Corn/oat bran flakes, rice crisps/puffs, oatmeal, toasted/steel cut oats	36	26	17	25	22	14
Snack foods	Corn/tortilla/vegetable/potato/lentil chips, rice crackers/cakes, soy crisps, cheese puffs, oatmeal cookie	20	1	8	2	2	0
Granola/bars/energy bars	Trail mix/granola/nutrition/protein bar, fruit and nut/oats and honey/apple blueberry granola	17	13	9	9	4	3
Beverages/ice creams/frozen desserts	Rice/oat beverage, soymilk, almond milk, coffee/soy creamer, vanilla ice cream, frozen yogurt, sorbet	16	1	1	1	0	0
Meat/meat substitute/ refrigerated or frozen foods	Tofu, roasted turkey/beef, veggie burger, chicken and rice, enchilada, beef/chicken franks, sausage	19	1	3	1	0	0
Others	Corn starch, yeast, hummus, peanut butter, dried cranberries, potato pancake mix	15	0	6	2	2	0
Total		186	44	53	48	36	19

^a Gluten content reported by at least one kit. The samples with gluten >20 ppm are also included in >5 ppm column, while the samples with gluten >100 ppm are included in >5 and >20 ppm columns.

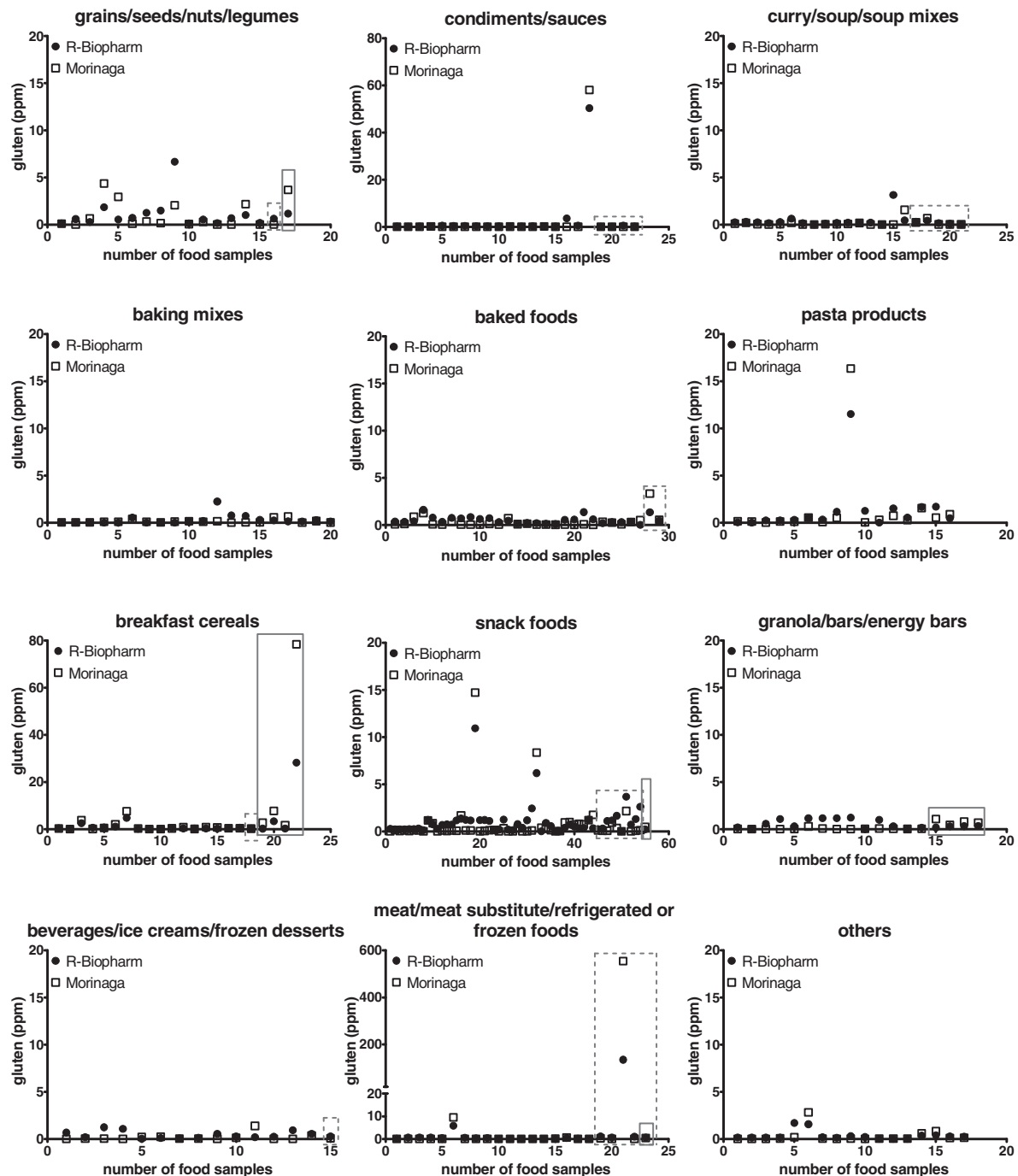


Fig. 1. Gluten content in food labelled “gluten-free”. Within the graphs, the box with broken-lines indicates foods with a wheat/gluten advisory statement and the box with solid-lines indicates foods with oats as an ingredient.

slope of linear equation from 1.5 to 3.7 in Fig. 2A. Detection of higher gluten levels by the Morinaga kit has been reported earlier (Geng et al., 2008; Sharma, 2012) and may be due to the difference in target analyte. The Morinaga kit detects wheat proteins whereas the R-Biopharm kit detects gluten. The assay variables listed in Table 1, along with different calibration standards, may be responsible for the difference in gluten quantitation observed between the two ELISA kits. More studies are warranted to investigate the relationship between different ELISA kits with respect to the actual gluten content in foods.

As mentioned earlier, the Codex Alimentarius, European Union, and the US Food and Drug Administration have established a 20 ppm gluten content as the limit for foods labelled GF. Using this

definition, we found that 1.1% (3 out of 275) of tested foods labelled GF were mislabelled/misbranded, as they had >20 ppm of gluten. Both ELISA kits showed 3 foods with >20 ppm of gluten, of which one food had a gluten content >100 ppm. Gelinas et al. (2008) reported 9% of GF cereal foods (7 out of 77) available in Canada to contain >20 ppm of gluten, as measured by the R5-ELISA (R-Biopharm). Investigation of gluten content, by R5 ELISA, in 205 commercially available GF products from 4 European countries (Italy, Spain, Germany and Norway) revealed that 94% and 99.5% had gluten concentrations below 5 and 20 ppm, respectively (Gibert et al., 2013). More recently, Thompson and Grace (2013) evaluated 112 different GF labelled foods sold in the US, using R5 ELISA, of which 4 products (3.6%) showed ≥ 20 ppm of gluten.

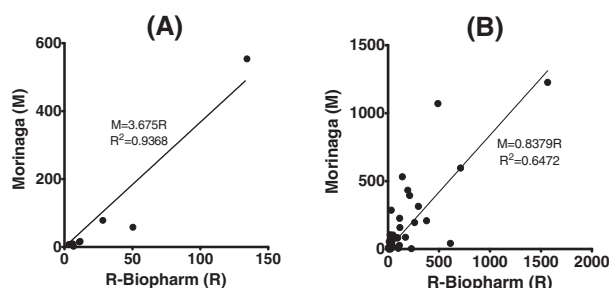


Fig. 2. Comparison of measured gluten content, using different ELISA kits with GF labelled foods (A) and non-GF labelled foods with no wheat/rye/barley on the ingredient label (B). The foods with gluten content >5 ppm by at least one ELISA kit were considered for the graph and regression analysis. The intercept is set to zero.

Our findings for gluten content in GF labelled foods available in the US are consistent with recent reports, suggesting an approximate GF labelling compliance of 98.9%.

Most of the foods labelled GF did not contain oats as an ingredient. Use of certified GF oats in food production may increase the product manufacturing cost. However, 11 GF labelled foods (4%) had oats as one of the ingredient, of which 36.4% (4 out of 11) belong to each of the breakfast cereals and granola/bars/energy bars categories. Among the 11 oat-containing GF-labelled foods, 2 breakfast cereals had a gluten content >5 ppm by at least one ELISA

kit, of which one food was >20 ppm (Fig. 1). None of the other oat-containing food categories had any detectable levels of gluten. It is not known if the oats were certified GF or tested for gluten content before being used in GF labelled food production. Based on ELISA analysis of the finished product, it is difficult to predict if the source of gluten is commercial oats that is contaminated with wheat, rye or barley, or cross-contact with these grains during food production. Good manufacturing practices and quality control, such as initial gluten testing of raw materials, can help prevent misbranding of foods labelled GF.

Twenty-nine GF labelled foods (10.5%) had gluten/wheat related advisory labelling, such as “produced/manufactured in a facility that also processes/uses wheat”, “made on equipment shared with wheat”, and “facility not dedicated gluten-free”. Only one food (meat/meat substitute/refrigerated or frozen food) out of these 29 foods contained a gluten level >20 ppm (Fig. 1). Since all foods in this group bear a GF claim, any gluten-related advisory label on these food packages may be confusing for consumers. A recent study found that non-celiac gluten-sensitive individuals rely more on GF claims as compared to celiac patients, who may rely more on the ingredient list to find GF foods (Verrill, Zhang, & Kane, 2013). The authors further reported a positive association between GF claims on the packaged foods and difficulty following a GF diet, which may be due to a misunderstanding or mistrust of the claim.

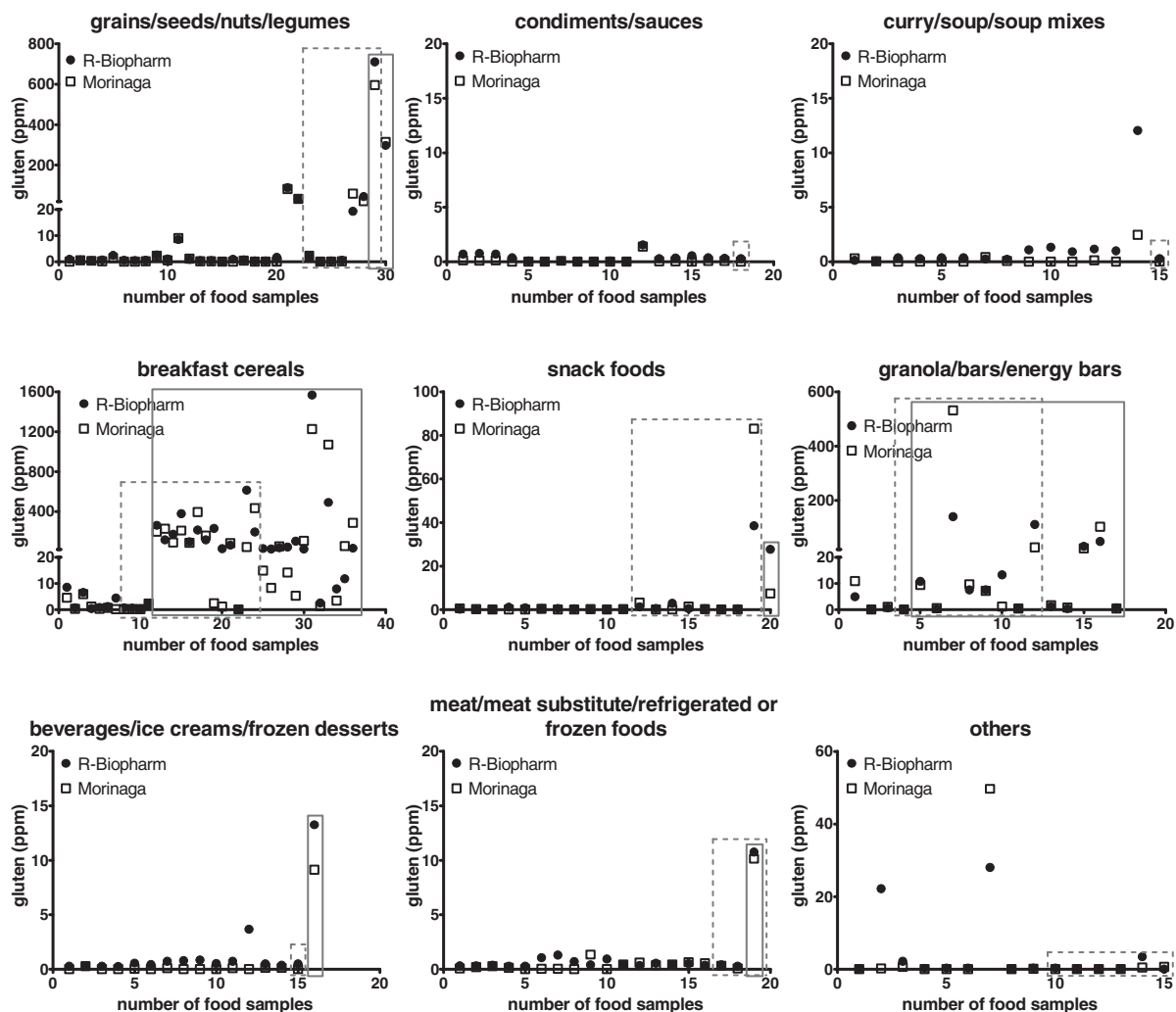


Fig. 3. Gluten content in food NOT labelled GF and that does not contain gluten/wheat/rye/barley as an ingredient on the label. Within the graphs, the box with broken-lines indicates foods with a wheat/gluten advisory statement and the box with solid-lines indicates foods with oats as an ingredient.

3.2. Non-GF labelled foods (no wheat/rye/barley on the ingredient label)

Unlike GF labelled foods, certain food categories (such as baking mixes, baked foods, and pasta products) were not found for non-GF labelled foods (which refer to foods with no wheat/rye/barley on the ingredient label for this study). As a result, the non-GF labelled foods were divided into 9 categories (Table 2). Out of a total of 186 non-GF labelled foods, breakfast cereals (19.4%) was the largest category, followed by grains/seeds/nuts/legumes (16.1%) and snack foods (10.8%). Gluten (>5 ppm) was detected in 48 foods (25.8%) by at least one ELISA kit, of which the R-Biopharm and Morinaga kits detected gluten in 47 and 41 foods, respectively. The range of measured gluten content by either of the kits was 5.3–1566 ppm. The gluten contents found in non-GF foods by different ELISA kits are shown in Fig. 3. More than half of the gluten-containing foods were breakfast cereals (25 foods), followed by granola/bars/energy bars (9 foods) and grains/seeds/nuts/legumes (7 foods). Condiments/sauces were the only category with no gluten. The linear equation relationship between the gluten measured by both kits for non-GF foods did not show an over-estimation by the Morinaga kit compared to the R-Biopharm kit, as was observed for GF foods (slope = 0.84, $R^2 = 0.6472$; Fig. 2B). The unknown source of gluten makes it difficult to analyse the results obtained from the two ELISA kits. The gluten estimations in most previous studies involved gluten (from wheat) or wheat spiked/incurred foods whereas, in a practical scenario, the source of gluten in commercial food samples may be wheat, rye, barley or their combination. If the gluten source is barley or rye, the ELISA results may not favour the estimations by the Morinaga kit since the specificity of the antibody used is lower for rye and barley. On the other hand, the R-Biopharm kit may over-estimate gluten in barley-containing foods, as the R5 antibody exhibits relatively higher sensitivity toward barley hordeins (Kanerva, Sontag-Strohm, Ryöppy, Alho-Lehto, & Salovaara, 2006; Tanner, Blundell, Colgrave, & Howitt, 2013). More studies are warranted to evaluate the effect of source grain (wheat, rye or barley) on the gluten quantitation by immunoassay.

Thirty-six foods (19.4%) had gluten contents >20 ppm as measured by at least one ELISA kit, of which 35 and 28 foods had >20 ppm gluten by R-Biopharm and Morinaga kit, respectively. Moreover, 19 foods had a gluten content >100 ppm by at least one ELISA kit. Western blot of foods with >100 ppm gluten (1 GF labelled and 19 non-GF labelled) using monoclonal antibody A1, confirmed gluten in these foods (Fig. 4). Foods exhibited gluten reactivity with A1 in the polypeptide range 34–95 kDa. The strength of immunoreactivity correlated with the level of gluten for most foods. Since these foods belong to various food categories

that have been processed differently, the gluten extraction efficiency may vary, thus affecting the immunoreactivity. Although there are overlapping A1-immunoreactive polypeptides among wheat, rye and barley proteins, the distinct wheat profile (~35–50 kDa) observed in the Western blot suggests wheat as one of the major sources of gluten. However, a blend of gluten source (such as wheat and barley mixture) has been reported as the probable source of gluten in many cereal foods (Gelinas et al., 2008).

Previous reports have estimated gluten contents in specific food categories. A study from Finland evaluated 59 naturally GF products (27 flours and 13 baked) by R5-ELISA and observed >20 ppm of gluten in 22% of foods (Collin, Thorell, Kaukinen, & Maki, 2004). Gelinas et al. (2008) reported >20 ppm gluten in 22.5% non-GF cereal foods (16 out of 71) purchased in Canada. A pilot study to evaluate the gluten content in 22 inherently GF (not labelled GF) single ingredient foods (grains, seeds and flours), purchased in the US, revealed that 32% of the samples contained ≥20 ppm gluten (Thompson et al., 2010). A similar category in our study (grains/seeds/nuts/legumes) showed >20 ppm gluten in 20% of samples (6 out of 30). The results indicate that, depending on the food category, gluten is detected in a greater percentage of non-GF labelled foods at levels that may pose a health risk to celiac patients.

Several previous studies have reported the presence of gluten in commercial oats (Hernando, Mujico, Mena, Lombardia, & Mendez, 2008; Koerner et al., 2011; Thompson, 2004). However, potential immunotoxicity and variable immunoreactivity between different oat varieties cannot be ignored (Ballabio et al., 2011; Comino et al., 2011). When used as an ingredient in food preparation, the presence of oat can result in a positive gluten result. Oat was found as an ingredient in 44 out of 186 non-GF foods analysed for gluten. Similar to GF foods, most oat-containing foods were found among the breakfast cereal (26 out of 44) and granola/bars/energy bars (13 out of 44) categories (Fig. 3). Out of 44 oat-containing non-GF foods, 36 (81.8%) and 29 (65.9%) foods had >5 and >20 ppm gluten, respectively, as measured by at least one ELISA kit. Also, all breakfast cereals (22 foods) and granola/bars/energy bar (4 foods) with >20 ppm gluten had oats as one of the ingredients. These results, in addition to the literature cited above, suggest that foods containing oats and not labelled GF, have a high probability of containing gluten and should be consumed with caution by gluten-sensitive individuals. The gluten concentration in such foods will depend on the amount of oats used as an ingredient and the level of gluten (from wheat, rye and/or barley) in those oats. Although other factors, such as toxicity of oats themselves, or cross-contact during manufacture, cannot be ignored, the presence of gluten from wheat/rye/barley in commercial oats appears to be the major

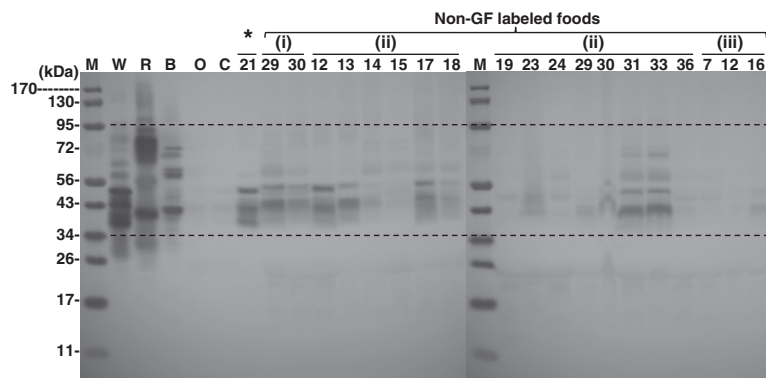


Fig. 4. Western blot of foods with >100 ppm of gluten using A1 antibody. M, protein marker; W, wheat; R, rye; B, barley; O, oat; C, corn; the numbers on top of each lane correspond to the number on x-axis within a specified food category in Figs. 1 and 3 for GF labelled and non-GF labelled foods, respectively. *GF labelled food-meat/meat substitute/refrigerated or frozen foods category; (i) grains/seeds/nuts/legumes; (ii) breakfast cereals; (iii) granola/bars/energy bars. The gluten reactive polypeptides are displayed between the dotted lines.

cause of the high gluten content (>20 ppm) observed in these foods.

Gluten/wheat-related advisory labels were found in 28.5% (53 out of 186) of the non-GF labelled foods. Granola/bars/energy bars had the highest percentage of foods with a gluten-related advisory label (52.9%; 9 out of 17), followed by breakfast cereals (47.2%; 17 out of 36) and snack foods (40%; 8 out of 20). Out of 36 non-GF foods with >20 ppm gluten by at least one ELISA kit, 18 foods did not contain a gluten/wheat-related advisory label. Of these, 4 foods did not have oats as an ingredient, either. Among the foods with a gluten/wheat advisory label, 43.4% (23 out of 53) contained gluten, as measured by at least one ELISA kit, of which 18 foods measured >20 ppm of gluten. Out of these 18 foods, 12 foods belonged to the breakfast cereal category, 3 to grains/seeds/nuts/legumes, 1 to snack foods, and 2 to granola/bars/energy bars. The presence of gluten in a significant number of foods with a gluten/wheat advisory shows that these warnings should be taken seriously and high risk of physiological response may be associated with consumption of these foods by sensitive individuals.

4. Conclusions

In summary, 3.6% of GF labelled foods had a gluten content ranging from 5.8 to 554 ppm, of which 1.1% foods were above the regulatory threshold of 20 ppm of gluten. However, depending on the food category of the non-GF labelled foods, gluten was detected in 25.8% of foods, at levels ranging from 5.3 to 1566 ppm, with 19.4% of foods having a measured gluten content of greater than 20 ppm. The presence of oats in a majority of the foods with high measured gluten levels suggests that oats are a potential source of gluten-containing grains and should be analysed for gluten content before being used as an ingredient in food production. Overall, the results exhibit 98.9% GF labelling compliance for GF labelled foods. However, non-GF labelled foods, containing oats as one of the ingredients, or foods with gluten/wheat advisory statements, may be a safety concern for consumers with celiac disease or wheat allergy.

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