



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-Mahiout 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

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