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Confirmation of gluten-free status of wheatgrass (*Triticum aestivum*)

S.L. Adrianos[1], B. Mattioni[2] and M. Tilley[1*]

[1]USDA-ARS, Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, KS 66502, USA; [2]Laboratory of Cereals, Food Science and Technology Department, Federal University of Santa Catarina, Florianópolis, SC, Brazil; michael.tilley@ars.usda.gov

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RESEARCH ARTICLE

Abstract

Young wheat (*Triticum aestivum*) leaves contain concentrated nutrients that may be beneficial as a nutritional supplement to those with malabsorption disorders such as celiac disease (CD) if both gluten-free, and free of gluten contamination. The tender 8-10 day leaves are juiced and the juice is consumed, added to smoothies, or consumed as a purchased wheatgrass dietary supplement in tablet, capsule and liquid forms. CD and other gluten related disorders cause both malabsorption of nutrients and an abnormal immune reaction to gluten. The only effective therapy is a lifelong gluten-free diet. Codex Alimentarius sets the gluten threshold level at 20 mg/kg for gluten-free foods. This threshold has been adopted by many regulatory agencies such as European Commission and the US Food and Drug Administration. Consumption of wheatgrass extract has been reported to alleviate symptoms of ulcerative colitis and anaemia which are often symptoms of CD. This investigation utilised the R5 and G12 sandwich enzyme-linked immuno sorbent assays (ELISAs) to examine the potential gluten content of commercial and home-grown wheatgrass. Samples were spiked with commercial gliadin to assess recovery rates. Acceptable recovery rates ranging from 91 to 133% from gluten spiked samples were found. In all preparations the gluten content of wheatgrass leaf tissue was lower than the limit of detection and limit of quantification for both ELISAs as well as the thresholds defined by Codex Alimentarius standards. According to results presented here appropriately prepared wheatgrass contains no gluten and may have a role as a dietary supplement in a gluten-free diet.

Keywords: wheatgrass, gluten analysis, ELISA, toxic gliadin peptide

1. Introduction

Traditional medicine has long valued dietary supplementation with herbal or nutraceutical compounds. This practice is increasingly common to augment functional metabolites, e.g. anti-oxidants of a total wellness program. Sprouts of many plant species are consumed in various forms due to high concentration in high value nutrients. Wheatgrass, the leaves of common wheat (*Triticum aestivum* L.) are a plentiful and easy source for such supplements (Benincasa *et al.*, 2015). Wheatgrass is consumed as a juice of freshly harvested leaves or reconstituted from powdered leaves. To ensure freshness and purity many consumers choose to grow and process plants at home.

Although limited data exists regarding the efficacy of wheatgrass in the medical literature, clinical studies have shown positive effects alleviating symptoms of ulcerative colitis (Bar-Sela *et al.*, 2007; Ben-Arye *et al.*, 2002) and haematological toxicity related to chemotherapy, rheumatoid arthritis, haematological diseases, diabetes, obesity, and oxidative stress (Bar-Sela *et al.*, 2007). A recent review summarised the laboratory and clinical literature on wheatgrass as a therapeutic agent (Bar-Sela *et al.*, 2015).

Young wheatgrass leaves contain concentrated nutrients that may be beneficial as a nutritional supplement to those with malabsorption disorders such as celiac disease (CD) if both gluten-free, and free of gluten contamination. CD is a globally occurring genetic disease that affects up to 3 million Americans when exposed to gluten. CD causes both malabsorption of nutrients and an abnormal immune reaction to gluten and can vary in severity between patients. A study in a healthy adult population in the USA reported

a five-fold increase in the incidence of CD from 1999 to 2008 (Riddle *et al.*, 2012). In addition to CD, there are other gluten related disorders which constitute important cases of food intolerance (Catissi *et al.*, 2013; Sapone *et al.*, 2012). Among the gluten related disorders, wheat allergy (WA) is defined as an adverse immunologic reaction to gluten proteins. Depending on the route of the allergen exposure and the underlying immunologic mechanisms, WA are classified into: (1) classic food allergy affecting the skin, gastrointestinal tract or respiratory tract; (2) wheat dependent, exercise-induced anaphylaxis; (3) occupational asthma (baker's asthma)/rhinitis; and (4) contact urticaria. It is now becoming clear that besides CD and WA, there are cases of gluten reactions in which neither allergic nor autoimmune mechanisms can be identified which are generalised as gluten sensitivity (Catassi *et al.*, 2013; El-Salhy *et al.*, 2015). The remission of symptoms occurs naturally or with concomitant use of supplement and allopathic drugs (Dickey, 2007). In cases of CD and gluten sensitivity, the only effective therapy is to assume a gluten-free diet (Cosnes *et al.*, 2008).

Codex Alimentarius (2008) defines gluten as a protein fraction from wheat, rye, barley or their crossbred varieties and derivatives thereof, and some oat varieties (Silano *et al.*, 2014) to which some persons are intolerant, and that is insoluble in water and 0.5 M NaCl. Gluten is composed of prolamins that can be extracted by 40-70% ethanol, and alcohol-insoluble glutelins that can only be extracted under reducing and disaggregating conditions at elevated temperatures. To guarantee the safety of gluten-free products for CD patients, Codex (2008) sets the threshold level as 20 mg/kg gluten for gluten-free foods. This threshold has been adopted by many regulatory agencies such as European Commission (2009) and the US Department of Health and Human Services – Food and Drug Administration (FDA, 2013).

Specific and sensitive analytical methods are needed for food quality control. Immunologic methods are currently recommended for the quantitative and qualitative determination of gluten in foods (Codex, 2008, 2014). A recent review (Bugyi *et al.*, 2013) thoroughly compared the properties of the many gluten enzyme-linked immuno sorbent assays (ELISAs) currently marketed.

Sandwich (to analyse intact gluten) and competitive (to analyse partially hydrolysed gluten in fermented foods) ELISA formats based on the R5 monoclonal antibody (Valdes *et al.*, 2003) were successfully validated. The R5 antibody primarily recognises the epitope QQPFP, which is present in gliadins, secalins, and hordeins and occurs in many peptides that are toxic or immunogenic for CD patients (Osman *et al.*, 2001). The R5 sandwich ELISA method was endorsed as a type 1 method by the Codex Committee of Methods on Analysis and Sampling (Codex, 2014). The G12 monoclonal antibody recognises a 33-mer peptide from wheat gliadin that has been shown to be key contributor to gluten toxicity. An ELISA assay utilising the G12 antibody was able to detect levels of gluten as low as 1 mg/kg in (Halbmayr-Jech *et al.*, 2012) and has been certified by AOAC International as OMA 2014.03 (Halbmayr-Jech *et al.*, 2015).

With wheat being a major dietary protein source, interest in wheatgrass juicing, and increasing incidence of gluten intolerance, further research is needed to better understand the potential gluten content of wheatgrass.

2. Materials and methods

Samples

Commercial organic gluten-free certified wheatgrass powder was purchased from PINES International Inc. (Lawrence, KS, USA). A wheatgrass kit for home growing (WheatgrassKits.com, Salt Lake City, UT, USA) was purchased for this project. The kit contained hard red *Triticum aestivum* wheat seed, growing trays, growing media, and mineral supplements. The wheat was grown according to the kit instructions. Briefly, the seeds were soaked in water overnight, placed on wet media with minerals in the growing trays, and grown in an otherwise gluten-free environment under plant grow lamps. The lamps were illuminated 30 cm from the plants for 12 hours per day. When the plants were approximately 15 cm in height (8-10 days) the leaf tissue was harvested. The leaves were cut with scissors approximately 2.5 cm above the seed mat to ensure that no seeds were carried over with the harvested leaf tissue. The harvested plant tissue was immediately frozen with liquid nitrogen, stored at -80 °C, and lyophilised. The dried samples were homogenised in a Geno/Grinder 2000 (SPEX SamplePrep Inc., Metuchen, NJ, USA). New Geno/Grinder vials were used to ensure no cross contamination of samples for protein extraction.

Sample extraction and enzyme-linked immuno sorbent assay analysis

Two ELISA kits, the RIDASCREEN R5 gliadin ELISA (R-Biopharm Ag, Darmstadt, Germany), and the AgraQuant ELISA Gluten G12 (Romer Labs, Union, MO, USA) were used to quantify the presence of gluten in the leaf tissue. The assay contained gluten standards to construct the standard curves for each experiment. Maize starch was used as a negative gluten control and as a base to determine variation in gliadin recovery in the extracts. Wheat flour (cv. Jagger) with a protein content of 11.6% was used as a positive control. The five different samples assessed were wheatgrass (WG), maize starch (starch), wheat flour, WG + wheat flour, and starch + wheat flour.

Sample extractions were performed according to the manufacturer's protocol with the following modifications. Briefly, 250 mg WG or starch was placed in a 15 ml conical tube; the flour samples contained 10 mg wheat flour; the last set of samples contained 240 mg of WG or starch with the addition of 10 mg wheat flour. Extraction solution (2.5 ml) was added to each tube and vortexed until dispersed, incubated at 50 °C for 40 min., cooled, 7.5 ml of 80% ethanol added, and mixed by vortex. The samples were placed on a Labquake Shaker (Barnstead/Thermolyne, Dubuque, IA, USA) and rocked at full speed for 60 min. at room temperature. Samples were centrifuged for 10 minutes at 2,000×*g* and clear aqueous supernatant was transferred to new 2 ml tubes. The extracted samples were then diluted with the prepared sample dilution buffer an additional 1:10 and vortex mixed. Gluten-free samples were prepared in a gluten-free environment. Samples containing gluten were prepared in a separate lab. A gluten-free workspace was created by washing all surfaces with detergent and water, then rewashing with 70% ethanol. Gluten-free work areas were validated using the RIDA QUICK Gliadin swab test (R-Biopharm Ag, Darmstadt, Germany). If gluten was detected, surfaces were rewashed until no gluten was detected.

The ELISAs were performed according to the manufacturer's instructions with each standard and sample performed in replicates. Briefly, 100 µl of each sample or standard was placed in the antibody coated microwell of the well holder plate and incubated at room temperature for 20 min. The samples were discarded, the wells washed 5 times with wash buffer, and tapped on absorbent blotting towels. To each well, 100 µl of conjugate was added, incubated at room temperature for 20 min., emptied, washed 5 times, and blotted dry. Next, 100 µl of substrate was added to each well and incubated in the dark at room temperature for 20 min. Finally, 100 µl of Stop solution was added to each well and then the plate sample wells were read on an ELx808 Ultra Microplate Reader (Bio-Tek Instruments Inc., Winooski, VT, USA) at 450 nm (OD450). The raw absorbance data was collected and entered into a dynamic spreadsheet (Romer Labs) for calculating concentrations. The graph was prepared using Microsoft Excel (Redmond, WA, USA).

Spiking and recovery

To determine potential inhibition of gliadin protein extraction by wheatgrass, duplicate samples were prepared, spiked with the 200 mg/kg standard 1:1 with either the WG extracts or maize starch extracts as a control. These samples were then prepared for the ELISA with the required 1:10 sample diluent required for the kit. Spiking solid to solid samples presents potential flaws in ensuring the homogeneity of the mixture and, if small samples are assessed the inherent difficulty in reproducibly weighing minute amounts of the spike analyte which according to Abbott *et al.* (2010) should be at relatively low levels (e.g. 0.5-5 mg/kg). In this case a liquid spike of gliadin was produced and assessed as described by Chu and Wen (2013). A spiking solution was prepared from commercial gliadin Sigma G-3375 (Sigma-Aldrich, St. Louis, MO, USA) a concentration of 1 mg/ml in 60% (v/v) ethanol. Extraction was performed using 10 ml of 60% ethanol containing various amounts of gliadin (1, 10 and 100 mg/ml) diluted from the 1 mg/ml stock solution. After extraction, the total content of gliadin in the 60% ethanol was determined. The recovery rate was calculated as follows:

$$\text{Recovery (\%)} = \left(\frac{C\text{-}C_o}{C_1}\right) \times 100$$

C denotes the detection value of the gliadin in the spiked sample; C_o represents the measured value of the original sample; and C_1 refers to the known spiked concentration of the gliadin in the same sample.

3. Results and discussion

Gluten level in wheatgrass and gluten spiked assay – recovery rate

To test for constituents that may inhibit the detection of gluten in the wheatgrass protein extracts, maize starch protein extracts that contained no gluten were used in parallel with wheatgrass protein extracts. Each duplicate sample was spiked with an equal volume of the 200 mg/kg of the supplied kit standard 1:1 and then diluted according to protocol 1:10 in the ELISA diluent. No inhibition of the ELISA was found in wheatgrass extracts (Figure 1).

Commercial and home-grown wheatgrass showed less than detection limits of gluten (>2 mg/kg) using both the R-Biopharm R5 and the AgraQuant Gluten G12 ELISAs.

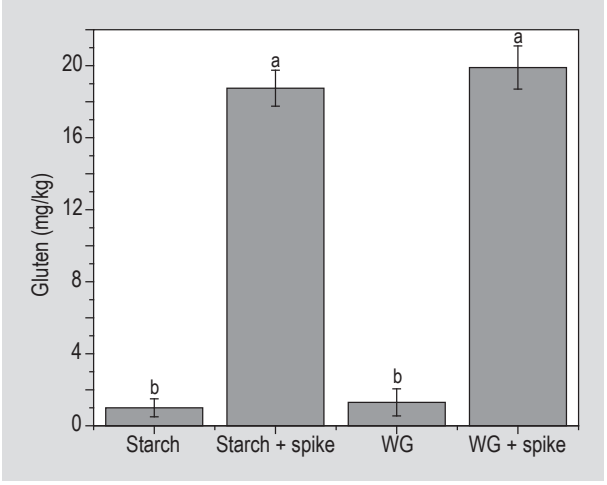

Figure 1. Gluten measured in wheatgrass (WG) and maize starch spiked with 20 mg/kg gluten standard. Means with the same letter are not significantly different (*P*<0.05).

The AgraQuant ELISA is one of the most sensitive assays available at this time with a detection limit of 2-200 mg/kg and a quantification limit of 4-200 mg/kg. No differences (P<0.05) were observed between commercial or home-grown wheatgrass samples.

Celiacs and other gluten sensitivity patients mandated to a gluten-free diet must avoid foods made from or containing ingredients of wheat origin. However this strictly applies to the seed or wheat flour. According to results presented here wheatgrass contains no gluten and is safe for consumption. Consumers should be cognisant that allergenic/toxic peptides can remain in the remnant seed of the sprout in excess of seven days. Michalcova *et al.* (2012) has shown that wheat seedlings rapidly activate peptidases and begin to degrade storage proteins. Likewise, Koehler *et al.* (2007) showed degradation and proteolysis varies significantly depending upon the temperature and protein class.

According to data presented herein leaves are devoid of appreciable levels of gluten proteins as measured by specific antibody binding. The lack of antibody recognition can be explained as the induction of mRNA encoding gliadin and glutenin proteins are developmentally regulated with gluten specific transcripts present in developing seeds 8 days post anthesis (Altenbach *et al.*, 2002). Transcriptome analysis reveals differences in endosperm and leaf transcriptome profiles with no gluten proteins expressed in leaf tissue (Baudo *et al.*, 2006).

Dickey (2007) reports the high prevalence of ulcerative colitis and other colitis in people with CD. Other common symptoms are anaemia and osteoporosis, due to a malabsorption of nutrients (Fasano, 2011; Murray, 1999; Lewis *et al.*, 2011). Ben-Arye *et al.* (2002) and Ng *et al.* (2013) report wheatgrass as a treatment for this colitis and other symptoms common in CD. Moreover, Kulkarni *et al.* (2007, 2006) reported that wheatgrass is rich in minerals such as iron and calcium. Bauer (2014) at the Mayo Clinic confirms that wheatgrass includes iron, calcium, magnesium, amino acids, chlorophyll and vitamins A, C and E providing a concentrated amount of nutrients.

Flour gluten recovery

The recovery rate reflected the efficiency of extraction of gliadin upon measurement following the addition of a known amount of gliadin to the wheatgrass powders (Table 1). Wheatgrass was spiked with gliadin (5-100 mg/kg) to determine its rate of recovery. When 5 mg/kg gliadin was added, a lower recovery rate (90-103%) was obtained. Samples spiked with 10 mg/kg gliadin resulted in recovery rates that were close to 100% (103-113%).

Samples containing 100 mg/kg displayed the greatest variability. The most notable variability was in the R5 assay in the samples spiked with 100 mg/kg possibly due to extrapolation as the highest standard for this kit is 80 mg/kg.

Table 1. Mean recovery rate (% recovery) of spiked gliadin in commercial and home-grown wheatgrass (WG) using the R5 and G12 enzyme-linked immuno sorbent assay (ELISA) test kits.[1]

	R5 ELISA	G12 ELISA
Commercial WG		
5 mg/kg	91.53 (2.2)^{f}	93.45 (5.32)^{f}
10 mg/kg	103.75 (1.02)^{e}	105 (2.73)^{de}
100 mg/kg	128.36 (6.34)^{a}	107.87 (2.36)^{d}
Home-grown WG		
5 mg/kg	103.78 (1.76)^{e}	90.52 (2.04)^{f}
10 mg/kg	113.71 (1.32)^{bc}	109.62 (6.24)^{c}
100 mg/kg	132.96 (8.72)^{a}	116.19 (4.22)^{b}

[1] The reported results are means ± standard deviation. Means with same letter are not significantly different (P<0.05).

4. Conclusions

The gluten content of freshly grown and a commercial preparation of wheatgrass, the nutrient rich *Triticum aestivum* leaf tissue, was determined by ELISA. While gluten, the normal storage protein found in the grain of wheat, is safe for most people, it can be toxic to those with CD or gluten sensitivity and must be avoided. The R5 and G12 ELISA data confirmed that the leaves of the wheat plant used to make wheatgrass products do not contain gluten. Therefore, the gluten-free wheatgrass nutritional supplementation would be an appropriate consideration for people maintaining a gluten-free diet if the wheatgrass has been tested for gluten contamination and meets the Codex Alimentarius criteria.

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References

Abbott, M., Hayward, S., Ross, W., Godefroy, S.B., Ulberth, F., Van Hengel, A.J., Roberts, J., Akiyama, H., Popping, B., Yeung, J.M., Wehling, P., Taylor, S.L., Poms, R.E. and Delahaut, P., 2010. Validation procedures for quantitative food allergen ELISA methods: community guidance and best practices. Journal of AOAC International 93: 442-450.

Altenbach, S.B., Kothari, K.M. and Dao Lieu, D., 2002. Environmental conditions during wheat grain development alter temporal regulation of major gluten protein genes. Cereal Chemistry 79: 279-285.

Bar-Sela, G., Tsalic, M., Fried, G. and Goldberg, H., 2007. Wheat grass juice may improve hematological toxicity related to chemotherapy in breast cancer patients: a pilot study. Nutrition and Cancer 58: 43-48.

Bar-Sela, G., Cohen, M., Ben-Arye, E. and Epelbaum, R., 2015. The medical use of wheatgrass: review of the gap between basic and clinical applications. Mini Reviews in Medicinal Chemistry 15: 1002-1010.

Baudo, M.M., Lyons, R., Powers, M.J.S., Pastori, G.M., Edwards, K.J., Holdsworth, M.J. and Shewry, P.R., 2006. Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. Plant Biotechnology Journal 4: 369-380.

Bauer, B.A., 2014. Healthy lifestyles: Q&A: what is wheatgrass, and should I add it to my smoothies for better health? Mayo Clinic, 22 January 2014. Available at: http://tinyurl.com/jqgrhus.

Ben-Arye, E., Goldin, E., Wengrower, D., Stamper, A., Kohn, R. and Berry, E., 2002. Wheat grass juice in the treatment of active distal ulcerative colitis: a randomized double-blind placebo-controlled trial. Scandinavian Journal of Gastroenterology 37: 444-449.

Benincasa, P., Galieni, A., Manetta, A.C., Pace, R., Guiducci, M., Pisante, M. and Stagnari, F., 2015. Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species. Journal of the Science of Food and Agriculture 95: 1795-1803.

Bugyi, Z., Török, K., Hajas, L., Adonyi, Z., Popping, B. and Tömösközi, S., 2013. Comparative study of commercially available gluten ELISA kits using an incurred reference material. Quality Assurance and Safety of Crops & Foods 5: 79-87.

Catassi, C., Bai, J.C., Bonaz, B., Bouma, G., Calabrò, A., Carroccio, A., Castillejo, G., Ciacci, C., Cristofori, F., Dolinsek, J., Francavilla, R., Elli, L., Green, P., Holtmeier, W., Koehler, P., Koletzko, S., Meinhold, C., Sanders, D., Schumann, M., Schuppan, D., Ullrich, R., Vécsei, A., Volta, U., Zevallos, V., Sapone, A. and Fasano, A., 2013. Non-celiac gluten sensitivity: the new frontier of gluten related disorders. Nutrients 5: 3839-3853.

Chu, P.T. and Wen, H.W., 2013. Sensitive detection and quantification of gliadin contamination in gluten-free food with immunomagnetic beads based liposomal fluorescence immunoassay. Analytica Chimica Acta 787: 246-253.

Codex Alimentarius Commission, 1979 (revised 2008). Codex standard for foods for special dietary use for persons intolerant to gluten. Codex Stan 118, 3 pp.

Codex Alimentarius Commission, 2014. Codex Standard 234-1999. Recommended methods of analysis and sampling. Gluten-free foods: enzyme-linked immunoassay R5 Mendez (ELISA) method. FAO/WHO, Rome, Italy.

Commission of the European Communities, 2009. Commission regulation (EC) no. 41/2009 of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten. Official Journal of the European Union 52: 3-5.

Cosnes, J., Cellier, C., Viola, S., Colombel, J.-F., Michaud, L., Sarles, J., Hugot, J.-P., Ginies, J.-L., Dabadie, A., Mouterde, O., Allez, M. and Nion-Larmurier, I., 2008. Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet. Clinical Gastroenterology and Hepatology 6: 753-758.

Department of Health and Human Services – Food and Drug Administration, 2013. Food labeling; gluten-free labeling of foods; final rule. Federal Register 78: 47154-47179.

Dickey, W., 2007. A case of sequential development of celiac disease and ulcerative colitis. Nature Clinical Practice Gastroenterology and Hepatology 4: 463-467.

El-Salhy, M., Hatlebakk, J.G., Gilja, O.H. and Hausken, T., 2015. The relation between celiac disease, nonceliac gluten sensitivity and irritable bowel syndrome. Nutrition Journal 14: 92.

Fasano, A., 2011. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiological Reviews 91: 151-175.

Food and Drug Administration (FDA), 2013. Gluten-free labeling of foods; final rule. Federal Register, Vol. 78, No. 150. Rules and regulations. FDA, College Park, MD, USA, pp. 47154-47179.

Halbmayr-Jech, E., Hammer, E., Fielder, R., Coutts, J., Rogers, A. and Cornish, M., 2012. Characterization of G12 sandwich ELISA, a next-generation immunoassay for gluten toxicity. Journal of AOAC International 95: 372-376.

Halbmayr-Jech, E., Rogers, A., Don, C. and Prinster, M., 2015. Gluten in rice flour and baked rice products by G12 sandwich ELISA: First Action 2014.03. Journal of AOAC International 98: 103-111.

Koehler, P., Hartmann, G., Wieser, H. and Rychlik, M., 2007. Changes of folates, dietary fiber, and proteins in wheat as affected by germination. Journal of Agricultural and Food Chemistry 55: 4678-4683.

Kulkarni, S.D., Acharya, R., Nair, A.G.C., Rajurkar, N.S. and Reddy, A.V.R., 2006. Determination of elemental concentration profiles in tender wheatgrass (*Triticum aestivum* L.) using instrumental neutron activation analysis. Food Chemistry 95: 699-707.

Kulkarni, S.D., Acharya, R., Rajurkar, N.S. and Reddy, A.V.R., 2007. Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum* L.) by *in vitro* digestion method. Food Chemistry 103: 681-688.

Lewis, N.R., Hubbard, R.B., Sanders, D.S., Logan, R.F., Holmes, G.K. and West, J., 2011. Treating occult coeliac disease with a gluten-free diet is associated with a significant improvement in quality of life. Gut 60: A1-A2.

Michalcova, E., Potocka, E., Chmelova, D. and Ondrejovic, M., 2012. Study of wheat protein degradation during germination. Journal of Microbiology, Biotechnology and Food Sciences 1: 1439-1447.

Murray, J.A., 1999. The widening spectrum of celiac disease. American Journal of Clinical Nutrition 69: 354-365.

Ng, S.C., Lam, Y.T., Tsoi, K.K.F., Chan, F.K.L., Sung, J.J.Y. and Wu, J.C.Y., 2013. Systematic review: the efficacy of herbal therapy in inflammatory bowel disease. Alimentary Pharmacology and Therapeutics 38: 854-863.

Osman, A.A., Uhlig, H.H., Valdes, I., Amin, M., Mendez, E. and Mothes, T., 2001. A monoclonal antibody that recognizes a potential coeliac-toxic repetitive pentapeptide epitope in gliadins. European Journal of Gastroenterology and Hepatology 13: 1189-1193.

Riddle, M.S., Murray, J.A. and Porter, C.K., 2012. The incidence and risk of celiac disease in a healthy US adult population. American Journal of Gastroenterology 107: 1248-1255.

Sapone, A., Bai, J.C., Ciacci, C., Dolinsek, J., Green, P.H., Hadjivassiliou, M., Kaukinen, K., Rostami, K., Sanders, D.S., Schumann, M., Ullrich, R., Villalta, D., Volta, U., Catassi, C. and Fasano, A., 2012. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Medicine 10: 13.

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

Valdes, I., García, E., Llorente, M. and Mendez, E., 2003. Innovative approach to low-level gluten determination in foods using a novel sandwich ELISA protocol. European Journal of Gastroenterology 15: 465-474.