



## Comparison of gluten recovery in gluten-incurred buckwheat flour using different commercial test kits

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Recovery of gluten in buckwheat flour was evaluated as part of an effort to produce wheat-contaminated buckwheat flours that could be used as reference materials (RMs) for testing the presence of gluten in buckwheat. RMs of buckwheat containing 0, 20, 100 and 1000 ppm gluten were created and tested by ELISA. The Gluten-Check kit detected gluten accurately at all levels; RIDASCREEN and Biokits tests were accurate at 20 and 100 ppm levels, but at 1000 ppm both suffered from extraction saturation effect; Veratox kit read 60% higher for the 20 ppm RM (i.e., 31.9 ppm), but close to the target at 100 ppm RM; Veratox R5 kit showed low accuracy with around 30% recovery at 20 and 100 ppm and some 60% at 1000 ppm level. Overall, the results showed variations in recovery among different test kits which could have important implications in the accurate detection of gluten in buckwheat.

**Keywords:** celiac disease; gluten; buckwheat; reference material; wheat

### Introduction

Wheat-based foods and ingredients are common in the food supply. Consumption of wheat by sensitised individuals can elicit two mechanistically different reactions: an IgE-mediated allergic response (more rare) and the more prevalent gluten-sensitive enteropathy which often results in celiac disease (CD). Affecting nearly 1% of the general population, CD is triggered by gluten found in different species of wheat, barley, rye and cross-bred hybrids such as triticale (Rewers, 2005). Gluten elicits an autoimmune response that causes damage to the inner lining of the small bowel of celiacs, with the only available treatment being strict adherence to a gluten-free (GF) diet (Sandberg, Lundberg, Ferm, & Malmheden Yman, 2003; Weber, Cl  roux, & Godefroy, 2009). The versatility and functionality of the starch and protein found in wheat makes it extremely valuable in the development of a multitude of food products, but this also makes avoidance extremely difficult (Alvarez & Boye, 2012).

Foods that naturally do not contain wheat, barley, rye or their cross-bred varieties as well as foods that contain these ingredients but have been specially processed to remove gluten are considered GF foods. The Codex standard for a food product to be deemed GF is 20 ppm gluten or less, whereas food products with less than 100 ppm gluten are regarded as 'reduced in gluten' products (FAO/WHO, 2008).

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Although the historical production of buckwheat appears to vary markedly from year to year, in the period 2004–2009 Canada ranked as the fifth buckwheat exporter with an average production of 3.7 thousand tonnes (FAO). Buckwheat could be a good addition to the celiac diet, but purity of the supply can be a challenge; due to the use of shared farmland and equipment, it is not uncommon to find wheat, barley and/or rye in buckwheat (Gélinas, McKinnon, Mena, & Méndez, 2008; Størsrud, Malmheden Yman, & Lenner, 2003; Thompson, Lee, & Grace, 2010). We propose the development of a wheat-contaminated buckwheat material with defined amounts of gluten which can be used as RMs against which measurements of gluten contamination could be gauged. This would allow the presence of gluten in buckwheat to be accurately detected and quantified.

The specific objective of this research, therefore, was to produce well-characterised RMs consisting of Canada Western Red Spring wheat (CWRS)-contaminated buckwheat flours and to assess differences in recovery as measured by different commercial enzyme linked immune sorbent assay (ELISA) test kits. Buckwheat flours containing a mix of CWRS flours with final gluten concentrations of 0 and 20 ppm were thus prepared to simulate the threshold for GF products: 20 ppm for gluten-free foods, 100 ppm for 'low gluten' and 1000 ppm for heavily contaminated products. The RMs were characterised physicochemically, and the presence of gluten was detected by ELISA.

### Materials and methods

Wheat samples were provided by the Canadian Grain Commission (CGC) from the 2009 Harvest Sample programme, consisting of a homogenised composite of 1276 grade-1 CWRS samples from 1012 growers. Wheat originated in the Canadian provinces of Saskatchewan (42%), Alberta (40%) and Manitoba (18%) and consisted of at least 18 cultivars, four of which (Harvest, Kane, Lillian and Superb) made up to 50% of the tested kernels. CWRS wheat composites, calibrated at 12.5% (code 41465) and 13.5% protein (code 41463), were obtained and blended in a 1:1 ratio for the study.

The wheat grains were cleaned and comminuted according to an internal standard operating procedure (SOP). Impurities were removed manually prior to milling; wheat was washed three times with water of food ingredient quality, dried for 16–24 h at 37 °C and then milled in a high-speed processor, Comitrol 3600 (Urschel, Valparaiso, IN), and stored at 4°C.

Canadian-grown buckwheat (*Fagopyrum esculentum*) flour, tested gluten-free by a third party and commissioned by the vendor, was supplied by Aliments Trigone Inc. (Saint-François-de-la-Rivière-du-Sud, QC, Canada). Buckwheat flour (T-20) lot 112633 in 20 kg bags was homogenised in-house with a double-action mixer 100DA70 (Leland Southwest, Fort Worth, TX) and stored at 4°C.

Bulk density was determined via an AccuPyc II 1340 gas pycnometer using helium at 23°C, 100 cm<sup>3</sup> sample chamber and ~30 g sample size (Micromeritics Instruments Corp., Norcross, GA). Moisture of flours was determined using AACC method 44-40.01 (AACC International, 1999). Crude fat was obtained by AOAC method 2003.06. Total ash was obtained by AACC method 08-03.01 (AACC International, 1999). Crude protein ( $N \times 5.53$ ) content of the buckwheat flour was achieved by the AACC method 46-30.01 (AACC International, 2010) using an

FP-428 nitrogen analyser (LECO Corporation, St. Joseph, MI), calibrated against EDTA. Protein and fat values were corrected to 13.5% moisture.

Tannin content was measured using the vanillin–HCl in methanol procedure (Price, van Scoyoc, & Butler, 1978), with some modifications (Khandelwal, Udipi, & Ghugre, 2010) and (+)-catechin as the reference standard. Briefly, flour samples (1 g) were extracted with 20 mL of 1% HCl in methanol for 20 min at 30°C in a water bath. The samples were centrifuged at  $931 \times g$  for 4 min. The supernatant (1.0 mL) was reacted with 5 mL vanillin solution (0.5% vanillin + 2% HCl in methanol) for 20 min at 30°C. Blanks were run with 4% HCl in methanol in place of vanillin reagent. Absorbance was read at 500 nm on a Cary 300 UV/VIS spectrophotometer (Agilent Technologies, Santa Clara, CA). A standard curve was prepared with catechin. Results were expressed in terms of catechin equivalents.

### ***Particle size***

Particle size of flours was determined by the AACC sieving method 66-20.01 (AACC International, 1999), using a Ro-Tap RX-29 (W.S. Tyler, Mentor, OH) and compared with AACC method 55-40.01 (AACC International, 2010), using a laser diffraction analyser Mastersizer 2000E (Malvern Instruments, Worcestershire, UK).

The SOP for laser diffraction was set as follows: refractive index of flour 1.469, refractive index of dispersant 1.39 (2-propanol), measurement/background time 5 sec in triplicates, 5000 snaps and pump at 1400 rpm. Equipment was triple rinsed with distilled water between samples and then dispersant was sonicated for 90 sec. Particle size distributions based on the sieving method were determined using the provided software.

### ***Preparation of RMs***

Samples (~5 g) of pure buckwheat flour from each box were taken upon receipt from Aliments Trigone and tested for gluten, using RIDAQUICK Gliadin lateral flow tests (R-Biopharm AG, Darmstadt, Germany). The inner surface of the V-shell blender and the double-action mixer 100DA70 were also tested before use for gluten, using the RIDAQUICK test. Results were negative (<5 ppm gluten) in all cases.

Gluten in buckwheat RMs was produced by a serial dilution of the wheat flour in the buckwheat flour, taking into consideration the protein and moisture content of the raw materials. The final concentration of gluten in the reference materials was 0, 20, 100 and 1000 ppm. In the V-shell mixer, 100 g flour per mixing event was found to be ideal to produce homogenous RMs. Mixing time was tested by adding dyed wheat flour into buckwheat flour to simulate 1% gluten-contaminated buckwheat; the sample was found to be well mixed after 5 min (results not shown). To ensure efficient mixing, mixing time was doubled to 10 min for each RM.

### ***ELISA***

Five different sandwich ELISA test kits were employed to test the gluten-contaminated buckwheat flour: RIDASCREEN Gliadin (R-Biopharm AG, Darmstadt, Germany); Veratox R5 for Gliadin; Biokits Gluten Assay Kit; Veratox for Gliadin (Neogen Corporation, Lansing, MI, USA) and imutest Gluten-Check

Table 1. Summary of the extraction protocols used for the ELISA test kits.

Test kit	RIDASCREEN	Veratox R5	Gluten-Check	Biokits	Veratox
Sample size raw (g)	0.25	1.0	1.0	2.0	1.0
Additive	Yes <sup>a</sup>	Yes <sup>b</sup>	No	No	Yes <sup>c</sup>
Extraction (min)	100	Raw: 10	15	1.5 <sup>d</sup>	Raw: 15

<sup>a</sup>Extraction cocktail for all samples; 0.25 g of skimmed milk for buckwheat containing samples.

<sup>b</sup>Additive for raw samples.

<sup>c</sup>Additive for raw samples, and if they contain buckwheat special additive.

<sup>d</sup>With the use of a high-speed homogeniser, a Polytron in our case.

(Diagnostic Innovations Limited (DIL), Denbighshire, UK) (Tables 1 and 2). ELISA tests were implemented as per manufacturer's instructions, using two extractions and two test wells per extraction for a total of four observations per sample. ELISA tests were performed with the recommended protocol of each kit. The 100 and 1000 ppm RMs were diluted post-extraction as recommended, except in the case of the 100 ppm RM with Gluten-Check since the calibration curve accommodated for this high value.

## Results and discussion

### Material characterisation

The buckwheat flour employed to create the RMs was extensively characterised (Table 3). Bulk density of the flour was  $1.455 \pm 0.0032 \text{ g/cm}^3$  and moisture, fat, ash and protein were, respectively,  $9.26 \pm 0.18\%$ ,  $2.24 \pm 0.08\%$ ,  $2.08 \pm 0.04\%$  and  $10.58 \pm 0.09\%$ . As tannins can affect ELISA measurement, tannin content of the buckwheat flour was analysed and was determined to be  $1104.7 \pm 133.7 \text{ mg/100 g}$  expressed as catechin in 100 g of flour ( $n = 10$ , standard deviation).

Particle size is another important descriptor that could affect ELISA results mainly by altering the extractability of allergens from food matrices. Particle size distribution of buckwheat flour was compared, using two methods and the results are presented in Figure 1. Both methods produced comparable particle size

Table 2. General information about the ELISA test kits used to determine gluten recoveries.

Test kit	RIDASCREEN	Veratox R5	Gluten-Check	Biokits	Veratox
Company	R-Biopharm	Neogen	DIL	Neogen (formerly Tepnel)	Neogen
Target	Gliadins	Gliadins	HMW- glutenins and $\omega$ -gliadins	HMW- glutenins and $\omega$ - gliadins	Gliadins
Antibody	R5 (Mendez)	R5 (Mendez)	401.21 (Skerritt)	401.21 (Skerritt)	Undisclosed set of two monoclonal
Gluten LOQ (ppm)	5	5	5	3	10

Table 3. Characteristics of buckwheat flour; data dispersion is expressed as standard deviation ( $n = 10$ ).

Properties	Buckwheat flour
Bulk density ( $\text{g}/\text{cm}^3$ )	$1.455 \pm 0.003$
Moisture (%)	$9.26 \pm 0.18$
Crude fat (%)	$2.24 \pm 0.08$
Total ash (%)	$2.08 \pm 0.04$
Crude protein (%)	$10.58 \pm 0.09$
Tannins ( $\text{mg}/100\text{g}$ )	$1104.7 \pm 133.7$

distributions of the flour with more than half of the particles in the buckwheat flour being smaller than  $150 \mu\text{m}$ . The rest of the flour had particle sizes distributed among the other five collected fractions ranging from  $150 \mu\text{m}$  to  $> 600 \mu\text{m}$ .

### Gluten recovery by ELISA

Five commercial ELISA test kits were used to test the RMs: RIDASCREEN and Veratox R5, which use monoclonal R5 antibody calibrated to the Working Group on Prolamin Analysis and Toxicity (WGPAT) standard; Biokits and Gluten-Check, which use monoclonal antibody 401.21 against glutenin and gliadin (Devery, La Brooy, Krillis, Davidson, & Skerritt, 1989; Skerritt, Jenkins, & Hill, 1989); and Veratox, which uses a set of two different capture monoclonal antibodies (Geng, Westphal, & Yeung, 2008).

Results from the ELISAs test kits are presented in Table 4 as actual gluten recovered in parts per million with standard deviation values. To help visualise the

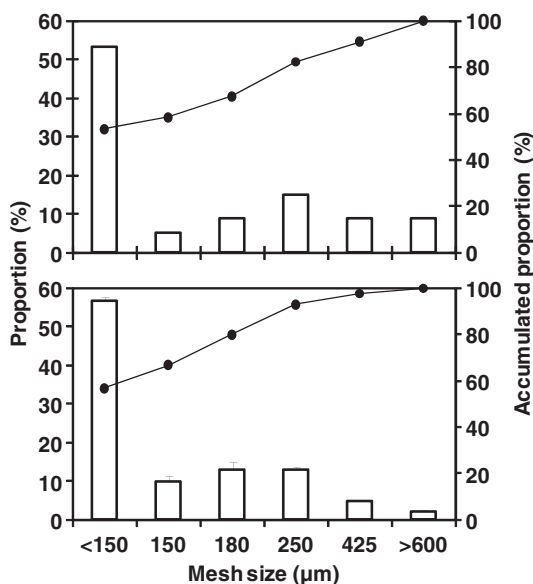


Figure 1. Particle size distribution of buckwheat flour is studied by laser diffraction (top) and sieving (bottom). Error bars for the sieved fractions are expressed as standard deviation ( $n = 10$ ).

Table 4. Average ELISA results of buckwheat contaminated with wheat gluten at 0, 20, 100 and 1000 ppm levels (results presented in ppm of detected gluten). Standard deviations represent a population of  $n=4$ .

RMs (ppm)	RIDASCREEN Gluten (ppm)	Veratox R5 Gluten (ppm)	Gluten-Check Gluten (ppm)	Biokits Gluten (ppm)	Veratox Gluten (ppm)
0	1.1±2.0 <sup>a</sup>	2.0±0.5 <sup>a</sup>	0.9±0.1 <sup>a</sup>	2.1±0.2 <sup>a</sup>	3.7±2.7 <sup>a</sup>
20	18.9±3.3	6.4±0.6	17.5±2.4	14.3±5.9	31.9±5.4
100	96.1±7.1	32.1±3.7	117.5±23.6	92.2±18.7	86.5±27.4
1000	613.4±47.7	630.1±43.5	918.3±49.7	459.5±61.2	702.7±163.9

<sup>a</sup>Below the limit of quantitation.  
Dispersion is expressed as standard deviation.

results, values were converted to recoveries over the expected values expressed as percentages and dispersion as coefficients of variation (Figure 2). As anticipated, the 0 ppm RM gluten recovery was below the quantitation limit of all five test kits which confirmed the absence of false positives.

The best gluten recoveries in terms of accuracy and dispersion across all RM levels were achieved using the Gluten-Check kit. These results are in good agreement with a previous report (Geng et al., 2008), which detected the presence of gluten in different food matrices. The RIDASCREEN and Biokits test kits offered good accuracy for the 20 and 100 ppm RMs, but some saturation was observed for the 1000 ppm samples. The results for RIDASCREEN contrasts with the previously reported kit's precision (Geng et al., 2008); however, the matrices utilised on those studies differed from the one in this work.

The Veratox kit read 1.6 times higher for the 20 ppm RM; conversely, the average reading for the 100 ppm RM was close to the target but with a low precision, and in the case of the 1000 ppm RM, the kit detected a lower amount of the incurred gluten. The Veratox R5 kit, on the other hand, gave low gluten recoveries for all the RMs, and at 20 and 100 ppm, only around 30% of the gluten was detected and some 60% at 1000 ppm level.

The RIDASCREEN and Veratox R5 kits are calibrated to gliadin from a European wheat composite (WGPAT gliadin), and the reactivity with Canadian wheat varieties has not been systematically studied. The R5 antibody reacts strongly

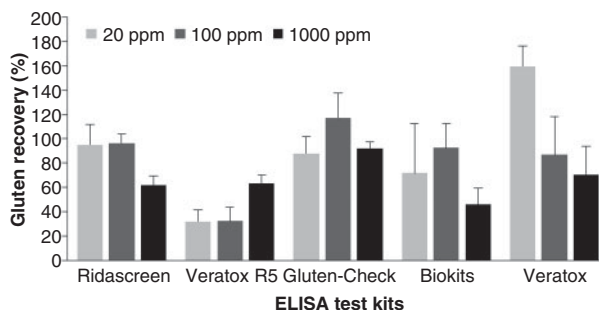


Figure 2. Average gluten recovery (expressed as percentage of target) for buckwheat-based RM, using five ELISA test kits ( $n=4$ ). Data dispersion is expressed as coefficient of variation.

with  $\omega$ -,  $\gamma$ - and  $\alpha$ -gliadin, and the reactivity is dependant on the amount of these gliadin subunits in each variety of wheat present (van Eckert et al., 2010). These two test kits provided very different results suggesting that influences from other factors, such as extraction procedure and reagents, buffers, secondary antibodies, etc., could play a very important role in the performance of the test kit.

Surprisingly, two of the ELISA test kits that performed the best with the buckwheat RMs, Gluten-Check and Biokits, did not include any additive in the extraction procedure to handle tannins (Table 1). Also, despite the higher sample size (doubled) from Gluten-Check to Biokits, which are constructed around the same antibody, the latter kit provided less accuracy and decreased precision; this could relate to the shorter extraction time even with the use of a high-speed homogeniser.

The Codex Standard for Foods for Special Dietary use for Persons Intolerant to Gluten requires food products to be tested using an ELISA with mono- or polyclonal antibodies against gliadin (FAO/WHO, 2008). Many ELISA tests have been developed around diverse capture antibodies: 401.21, validated by the AOAC (Skerritt & Hill, 1990); R5, adopted as Type 1 method by Codex (Sorell et al., 1998); PN3, raised against a synthetic  $\alpha$ -gliadin peptide (Ellis, Rosen-Bronson, O'Reilly, & Ciclitira, 1998); and G12, against a 33-mer peptide from  $\alpha 2$ -gliadin (Morón et al., 2008). A summary of relevant information for the ELISA test kits used to study gluten recoveries within this work is provided in Table 2.

Variations in results between different ELISA kits can arise due to differences in antibody specificity, extraction method and kit construction (van Eckert et al., 2010). Additionally, ELISA testing may not be quantitative for mixtures of wheat, barley and rye, as the antibodies have varying affinities for prolamins in different cereals (Sandberg et al., 2003). A summary of the extraction protocols for the ELISA test kits used to study gluten recoveries within this work is presented in Table 1. It is clear from the results obtained that there is a marked variation in gluten recovery, depending on the test kit used, highlighting the need for standardised reference materials.

The purified gliadin standard developed by the WGPAT (van Eckert et al., 2006) has been used by European laboratories for research on gluten (Dahinden, von Büren, & Lüthy, 2001; Gabrovská et al., 2006; Köppel, Stadler, Lüthy, & HuÈbner, 1998; van Eckert et al., 2010). The WGPAT standard consists of a gliadin extract from 28 European wheat cultivars of the 1999 harvest. The WGPAT-gliadin was submitted to the Institute for Reference Materials and Measurements of the European Commission, but dropped as a candidate due to stability concerns. There, therefore, continues to be a need for reference materials with incurred quantities of gluten that could be used for accurate gluten detection as demonstrated through the RMs prepared for this study.

## Conclusion

CWRS-incurred buckwheat flour RMs containing 0, 20, 100 and 1000 ppm gluten were created in this study and used to compare gluten recoveries detected by different commercial ELISA kits. Marked variations in recoveries were observed, suggesting differences in the accuracy and precision of the commercial ELISA kits used. Although no false positives or false negatives were detected, appropriate labelling of 'gluten-free' or 'low-gluten' foods requires accurate quantification of gluten in grains



and flours used for product development. There, therefore, continues to be a need for appropriate reference materials that could be used to quantify gluten in allergen-controlled foods. The wheat-incurred buckwheat materials used in this study could serve as a guide for the production of standardised reference materials for kit manufacturers, the food industry and the scientific community.

## References

- AACC International. (1999). *Approved methods of analysis* (11th ed.). Method 08-03.01. Ash-rapid (2 hr, 600°C) Method. Approved 1999. St. Paul, MN: AACC International. Retrieved from <http://dx.doi.org/10.1094/AACCIntMethod-08-03.01>
- AACC International. (1999). *Approved methods of analysis* (11th ed.). Method 44-40.01. Moisture-Modified Vacuum-Oven Method. Approved 1999. St. Paul, MN: AACC International. Retrieved from <http://dx.doi.org/10.1094/AACCIntMethod-44-40.01>
- AACC International. (1999). *Approved methods of analysis* (11th ed.). Method 66-20.01. Determination of Granularity of Semolina and Farina: Sieving Method. Approved 1999. St. Paul, MN: AACC International. Retrieved from <http://dx.doi.org/10.1094/AACCIntMethod-66-20.01>
- AACC International. (2010). *Approved methods of analysis* (11th ed.). Method 46-30.01. Crude Protein-Combustion Method. Approved 2010. St. Paul, MN: AACC International. Retrieved from <http://dx.doi.org/10.1094/AACCIntMethod-46-30.01>
- AACC International. (2010). *Approved methods of analysis* (11th ed.). Method 55-40.01. Particle Size of Wheat Flour by Laser Instrument. Approved 2010. St. Paul, MN: AACC International. Retrieved from <http://dx.doi.org/10.1094/AACCIntMethod-55-40.01>
- AOAC. (2006). AOAC Official Method 2003.06. Crude fat in feeds, cereal grains, and forages. randall/soxtec/hexanes extraction-submersion method. Retrieved from [http://www.aoac.org/omarev1/2003\\_06.pdf](http://www.aoac.org/omarev1/2003_06.pdf)
- Alvarez, P. A., & Boye, J. I. (2012). Food production and processing considerations of allergenic food ingredients: A review. *Journal of Allergy*, 2012, 14. doi:10.1155/2012/746125
- Dahinden, I., von Büren, M., & Lüthy, J. (2001). A quantitative competitive PCR system to detect contamination of wheat, barley or rye in gluten-free food for coeliac patients. *European Food Research and Technology*, 212(2), 228–233. doi:10.1007/s002170000252
- Devery, J., La Brooy, J., Krillis, S., Davidson, G., & Skerritt, J. (1989). Anti-gliadin antibody specificity for gluten-derived peptides toxic to coeliac patients. *Clinical & experimental immunology*, 76(3), 384. Retrieved from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1541896/>
- Ellis, H., Rosen-Bronson, S., O'Reilly, N., & Ciclitira, P. (1998). Measurement of gluten using a monoclonal antibody to a coeliac toxic peptide of A gliadin. *Gut*, 43(2), 190. doi:10.1136/gut.43.2.190
- FAO. (2012). FAOSTAT. Retrieved from <http://faostat.fao.org>
- FAO/WHO. (2008). Codex standard for foods for special dietary use for persons intolerant to gluten. Retrieved from [www.codexalimentarius.net/download/standards/291/cxs\\_118e.pdf](http://www.codexalimentarius.net/download/standards/291/cxs_118e.pdf), 118–1979.
- Gabrovská, D., Rysová, J., Filová, V., Plicka, J., Cuhra, P., Kubík, M., & Baršová, S. (2006). Gluten determination by gliadin enzyme-linked immunosorbent assay kit: Interlaboratory study. *Journal of AOAC International*, 89(1), 154–160. Retrieved from <http://www.ingentaconnect.com/content/aoac/jaoac/2006/00000089/00000001/art00021>
- Gélinas, P., McKinnon, C. M., Mena, M. C., & Méndez, E. (2008). Gluten contamination of cereal foods in Canada. *International Journal of Food Science & Technology*, 43(7), 1245–1252. doi:10.1111/j.1365-2621.2007.01599.x
- Geng, T., Westphal, C. D., & Yeung, J. M. (2008). Detection of gluten by commercial test kits: Effects of food matrices and extraction procedures. In D. P. Siantar, M. W. Trucksess, P. M. Scott, & E. M. Herman (Eds.), *Food contaminants* (vol. 1001, pp. 462–475). ACS Symposium Series. doi:10.1021/bk-2008-1001.ch030



- Khandelwal, S., Udipi, S. A., & Ghugre, P. (2010). Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. *Food Research International*, 43(2), 526–530. doi:10.1016/j.foodres.2009.09.036
- Köppel, E., Stadler, M., Lüthy, J., & HuEbner, P. (1998). Detection of wheat contamination in oats by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, 206(6), 399–403. doi:10.1007/s002170050281
- Morón, B., Bethune, M. T., Comino, I., Manyani, H., Ferragud, M., López, M. C., . . . Sousa, C. (2008). Toward the assessment of food toxicity for celiac patients: Characterization of monoclonal antibodies to a main immunogenic gluten peptide. *PLoS ONE*, 3(5), 2294. doi:10.1371/journal.pone.0002294
- Price, M. L., van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, 26(5), 1214–1218. doi:10.1021/jf60219a031
- Rewers, M. (2005). Epidemiology of celiac disease: What are the prevalence, incidence, and progression of celiac disease? *Gastroenterology*, 128(4, Suppl. 1), S47–S51. doi:10.1053/j.gastro.2005.02.030
- Sandberg, M., Lundberg, L., Ferm, M., & Malmheden Yman, I. (2003). Real time PCR for the detection and discrimination of cereal contamination in gluten free foods. *European Food Research and Technology*, 217(4), 344–349. doi:10.1007/s00217-003-0758-4
- Skerritt, J. H., & Hill, A. S. (1990). Monoclonal antibody sandwich enzyme immunoassays for determination of gluten in foods. *Journal of Agricultural and Food Chemistry*, 38(8), 1771–1778. doi:10.1021/jf00098a029
- Skerritt, J. H., Jenkins, K. L., & Hill, A. S. (1989). Monoclonal antibody based two-site enzyme immunoassays for wheat gluten proteins. 2. Specificity analysis. *Food and Agricultural Immunology*, 1(3), 161–171. doi:10.1080/09540108909354687
- Sorell, L., López, J. A., Valdés, I., Alfonso, P., Camafeita, E., Acevedo, B., . . . Méndez, E. (1998). An innovative sandwich ELISA system based on an antibody cocktail for gluten analysis. *FEBS Letters*, 439(1–2), 46–50. doi:10.1016/S0014-5793(98)01336-2
- Storsrud, S., Malmheden Yman, I., & Lenner, R. A. (2003). Gluten contamination in oat products and products naturally free from gluten. *European Food Research and Technology*, 217(6), 481–485. doi:10.1007/s00217-003-0786-0
- Thompson, T., Lee, A. R., & Grace, T. (2010). Gluten contamination of grains, seeds, and flours in the United States: A pilot study. *Journal of the American Dietetic Association*, 110(6), 937–940. doi:10.1016/j.jada.2010.03.014
- van Eckert, R., Berghofer, E., Ciclitira, P., Chirido, F., Denery-Papini, S., Ellis, H., . . . Mamone, G. (2006). Towards a new gliadin reference material-isolation and characterisation. *Journal of Cereal Science*, 43(3), 331–341. doi:10.1016/j.jcs.2005.12.009
- van Eckert, R., Bond, J., Rawson, P., Klein, C. L., Stern, M., & Jordan, T. W. (2010). Reactivity of gluten detecting monoclonal antibodies to a gliadin reference material. *Journal of Cereal Science*, 51(2), 198–204. doi:10.1016/j.jcs.2009.11.012
- Weber, D., Clérout, C., & Godefroy, S. B. (2009). Emerging analytical methods to determine gluten markers in processed foods—method development in support of standard setting. *Analytical and Bioanalytical Chemistry*, 395(1), 111–117. doi:10.1007/s00216-009-2943-1