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Characterization of a gluten reference material: Wheat-contaminated oats

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ABSTRACT

Oat could be a good addition to the Gluten Free diet, but the purity of the oat supply is under scrutiny. As celiac disease becomes more prevalent, better detection tools for gluten in oats are necessary. We aimed to produce reference materials (RMs) of Canada Western Red Spring wheat (CWRS)-contaminated oat flour. Pure, uncontaminated oats flour (cultivars Navan and Gehl) was supplied by Cream Hill Estates. CWRS samples were provided by the Canadian Grain Commission from the 2009 Harvest Sample program. RMs containing 0, 20 and 100 ppm CWRS gluten-contaminated oats were created using a V-shell blender and tested by sandwich-type ELISA for gluten. Marked variations in ELISA results for the RMs were found among different test kits due to differences in capture antibodies and kit construction. The Veratox test was accurate at the 0, 20 and 100 ppm levels but detected only 30% (Veratox) and 50% (Veratox R5) of gluten at the 1000 ppm level; the Ridascreen test was accurate at all levels; the Biokits test detected roughly 10% of the gluten dosage; the Gluten-Check test detected some 30% of the gluten dosage. The RMs created could serve as standards for gluten detection in oat containing foods.

1. Introduction

In the period 2005–2009 with an average of 25.3 million tonnes, Canada was ranked the sixth top wheat producer. In 2008, Canada ranked third exporter by volume, after the United States and France (FAO, 2011). A cosmopolitan ingredient due to the versatility of its starch and protein, wheat can be found in a multitude of food products (CFIA, 2011). Wheat is one of the top eight allergenic foods which together account for 90% of food allergies (Poms and Anklam, 2004). Besides IgE-mediated wheat allergy, the more prevalent gluten sensitive enteropathy which often results in celiac disease affects nearly 1% of the general population and is triggered by gluten found in different species of wheat, barley, rye and crossbred hybrids such as triticale (Rewers, 2005). Gluten elicits an autoimmune response that causes damage to the inner lining of the small bowel of celiacs (Köppel et al., 1998; Sandberg et al., 2003). Currently, the only treatment is strict adherence to a gluten-free (GF) diet (Weber et al., 2009).

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

Oat could be a good addition to the celiac diet, but purity of the supply is under scrutiny (Koerner et al., 2011; Thompson, 2004). It is not uncommon to find wheat, barley and/or rye in oats, since these crops share farmland and equipment. Canada is the second producer of oats after Russia and the world leader in exports (2004–2008) of grain oats and rolled oats (FAO, 2011). Reference materials (RMs) of wheat-contaminated oats are, therefore, needed in order to offer to the scientific community well characterized standards to which measurements of gluten contamination could be gauged against.

The objective of this research, therefore, was to produce a standardized RM consisting of CWRS-contaminated oat flour for food-safety (gluten) testing and analytical research. Oat flours containing a mix of CWRS flours with final gluten concentrations of 0 ppm, and 20 ppm were prepared to simulate the threshold for GF products; and 100 ppm for “low gluten” products. RM containing 1000 ppm gluten was also prepared representing a highly contaminated sample (positive control). All raw materials and RMs were characterized physicochemically.

Abbreviations: 2-DE, two-dimensional electrophoresis; CWRS, Canada western red spring wheat; GF, gluten-free; HMW, high molecular weight; LMW, low molecular weight; MM, molar mass; RM, reference material; RP-HPLC, reversed-phase high-performance liquid chromatography; SOP, standard operation procedure; WGPAT, Working Group on Prolamin Analysis and Toxicity.

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2. Materials and methods

Wheat samples were provided by the Canadian Grain Commission from the 2009 Harvest Sample program, consisting of a homogenized composite of 1276 grade-1 CWRS samples from 1012 growers. Wheat originated in the 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 calibrated samples at 12.5% and 13.5% protein coded 41465 and 41463 respectively, were obtained. In the 2009 and 2010 harvests, hard red spring wheat accounted for 76% of the total Canadian wheat production, making them excellent candidates to develop gluten RMs. Additionally, the selected two (12.5% and 13.5%) top protein cut-offs constitute the most sought after in the wheat market extending the rationale for gluten source selection.

Wheat samples were cleaned and comminuted according to an internal SOP. Impurities were removed manually, wheat was washed 3 times with water of food ingredient quality, dried for 16–24 h at 37 °C, milled in a high speed processor Comitrol 3600 (Urschel, Valparaiso, IN) and stored at 4 °C. The progressive reduction of particle size involved 3 passes through the Comitrol 3600 using increasingly smaller mesh sizes in the cutting head, starting with mesh 3K-030050, followed by mesh 3K-030030 and finally 3K-015030.

Oats flour (cultivars Navan and Gehl) tested gluten-free by a third party commissioned by the vendor was supplied by Cream Hill Estates (LaSalle, QC, Canada). The oats supplier grows and processes only GF oats and has put in place a detailed workflow to minimize risks of contamination with gluten containing cereals. Oats flour lot 10026 in 25 kg bags was homogenized in-house with a double action mixer 100DA70 (Leland Southwest, Fort Worth, TX) and stored at 4 °C. Samples (~5 g) of 10026 oats flour from each box were taken after homogenization and tested for gluten using RIDAQUICK Gliadin lateral flow tests (R-Biopharm AG, Darmstadt, Germany). The inner surface of the 100DA70 mixer was also tested for gluten before mixing the oats flour using the RIDAQUICK test. Results were negative (<5 ppm gluten) in all cases (data not shown).

Bulk density was determined via an AccuPyc II 1340 gas pycnometer using helium at 23 °C, 100 cm^3 sample chamber, and ~30 g sample size (Micromeritics Instruments Corp., Norcross, GA). Moisture of flours was determined using AACC method 44-40.01. Crude fat was obtained by AOAC method 2003.06. Total ash was obtained by AACC method 08-03.01. Crude protein of oat (N × 5.83) and wheat (N × 5.70) flours was achieved by AACC method 46-30.01 using a nitrogen analyzer FP-428 (LECO Corporation, St. Joseph, MI), calibrated against EDTA. Protein and fat values were corrected to 13.5% moisture.

2.1. Particle size

Particle size of flours was determined by the AACC sieving method 66-20.01 using a Ro-Tap RX-29 (W.S. Tyler, Mentor, OH), and compared with AACC method 55-40.01 using a laser diffraction analyzer 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 s in triplicate, 5000 snaps, and pump at 1400 rpm. Equipment was triple rinsed with distilled water between samples, and then dispersant was sonicated for 90 s. Particle size distributions based on the sieving method were simulated using the provided software.

2.2. Protein composition

Protein was extracted from 5 mg of flour for two-dimensional electrophoresis (2-DE) using OFFGEL reagents and loaded onto IPG low-resolution strips pH 3–10. Isoelectric focusing was performed using an OFFGEL 3100 fractionator (Agilent Technologies, Santa Clara, CA). Collected fractions were then separated based on molar mass (MM) in denaturant conditions on a 4–12% Bis-Tris Criterion XT gel in a Criterion cell (Bio-Rad Laboratories, Hercules, CA) with a low molecular weight (LMW) protein standard (GE Healthcare, Baie d'Urfe, QC, Canada). Gels were stained with Coomassie blue R-250 and recorded on an Image Scanner III with Labscan and analyzed using ImageQuant TL V2005 (GE Healthcare, Baie d'Urfe, QC, Canada).

Protein extractions for RP-HPLC and turbidimetry measurements were done as described in the literature (Mamone et al., 2000; Wieser, 2000a; Wieser, 2000b; Wieser et al., 1998) with slight modifications, using 1 mg/mL BSA in water as standard. Wheat and oat flours (100 mg) were extracted stepwise three times with 1 mL of 0.4 M NaCl to recover albumins; stepwise three times with 0.5 mL of 60% (v/v) ethanol to recover the gliadins fraction; and stepwise twice with 1 mL of 50% (v/v) 1-propanol, 2 M urea, 0.05 M Tris–HCl (pH 7.5) and 1% (w/v) DTT under nitrogen to recover glutenins. Between steps samples were vortexed 2 min, mixed by magnetic stirring for 10 min and centrifuged for 20 min at 5000 g and 20 °C. Samples and standard were filtered through a 0.45 μm membrane.

The reverse-phase high-performance liquid chromatography (RP-HPLC) analysis was performed using a 1200 series ChemStation instrument (Agilent Technologies, Santa Clara, CA) with a Jupiter 5 μm C18 300 Å 250 × 4.6 mm column (Phenomenex, Torrance, CA), column temperature 45 °C, flow rate 1 mL/min, and sample injection 25 μL. The elution system was: water + TFA (0.1% v/v) (A) and ACN + TFA (0.1% v/v) (B); with a linear gradient of 0 min 20% B, 30 min 60% B, 32 min 20% B, 50 min 20% B. Absorbance was measured at 210 nm.

For turbidimetry, 0.10 mL of the gliadin extract was mixed with 0.40 mL of the gliadin extraction solution and 1 mL of 2-propanol. A volume of 0.15 mL glutenin extract was mixed with 0.35 mL of the glutenin extraction solution and 1 mL of 2-propanol. Samples were incubated at 20 °C for 40 min and A_{450} was read in a Cary 300 Bio UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA).

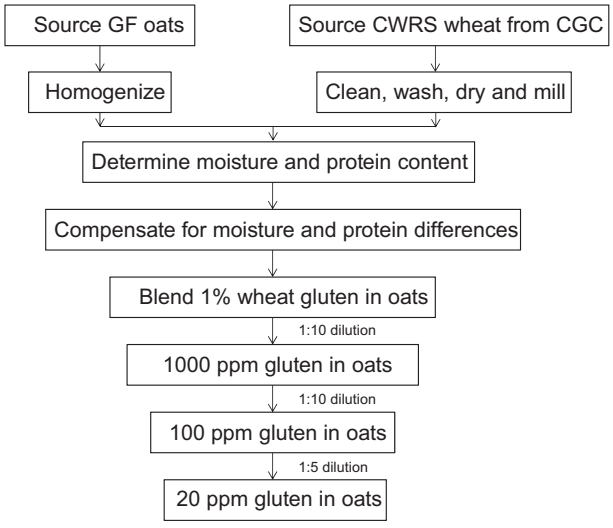

Fig. 1. Diagram for preparation of wheat-contaminated oats reference materials.