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Effects of grain species and cultivar, thermal processing, and enzymatic hydrolysis on gluten quantitation

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ABSTRACT

Gluten from wheat, rye, and barley can trigger IgE-mediated allergy or Celiac disease in sensitive individuals. Gluten-free labeled foods are available as a safe alternative. Immunoassays such as the enzyme-linked immunosorbent assay (ELISA) are commonly used to quantify gluten in foods. However, various non-assay related factors can affect gluten quantitation. The effect of gluten-containing grain cultivars, thermal processing, and enzymatic hydrolysis on gluten quantitation by various ELISA kits was evaluated. The ELISA kits exhibited variations in gluten quantitation depending on the gluten-containing grain and their cultivars. Acceptable gluten recoveries were obtained in 200 mg/kg wheat, rye, and barley-spiked corn flour thermally processed at various conditions. However, depending on the enzyme, gluten grain source, and ELISA kit used, measured gluten content was significantly reduced in corn flour spiked with 200 mg/kg hydrolyzed wheat, rye, and barley flour. Thus, the gluten grain source and processing conditions should be considered for accurate gluten analysis.

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1. Introduction

Gluten consists of proteins called prolamins and glutelins that are found in wheat, rye, and barley grains. The prolamins found in wheat, rye, and barley are called gliadin, secalin, and hordein, respectively. While most people are tolerant to gluten consumption, gluten sensitivity in others may cause IgE-mediated allergy or Celiac disease. Celiac disease is a digestive disorder that affects one percent of the population. Sensitivity to gluten triggers an immune response that damages the mucosal layer of the small intestine and prevents absorption of nutrients. In order to treat Celiac disease, patients must adhere to a gluten-free diet. To ensure the safety of gluten-sensitive consumers, Codex Alimentarius, European Commission Regulation, and the U.S. Food and Drug Administration have specified 20 mg/kg as the maximum threshold of gluten in foods labeled gluten-free.

Gluten content in foods is commonly quantified by immunoassays such as enzyme-linked immunosorbent assay (ELISA). Different types of ELISA kits target different epitopes of gluten, depending on the detection antibody. Monoclonal antibodies target one specific epitope, while polyclonal antibodies can identify multiple epitopes. Some monoclonal antibodies commonly utilized in immunoassays include Skerritt, R5, and G12. The Skerritt antibody is raised against wheat gliadin and recognizes ω-gliadins as well as high molecular weight glutenins (Skerritt & Hill, 1990). The R5 antibody is raised against secalin from rye and targets the epitopes QQPFP, QQQFP, LQPFP, and QLPFP (Kahlenberg et al., 2006). The G12 antibody is raised against a 33-mer peptide sequence of gliadin and selectively targets the epitopes QPQLPY, QPQLPF, QPQLPL, and QPQQPY (Morón et al., 2008). Some of the commercially available ELISA kits that use these antibodies include ELISA Systems Gliadin (Skerritt), R-Biopharm Gliadin (R5), and Romer Labs Gluten (G12) (Allred & Ritter, 2010; Bruins Slot, van der Fels-Klerx, Bremer, & Hamer, 2015; Bugyi et al., 2013; Halbmayr-Jech et al., 2012; Méndez, Vela, Immer, & Janssen, 2005). The Morinaga Wheat Protein ELISA kit uses a polyclonal antibody, which displayed immunoreactivity to prolamin and glutelin fractions from wheat, rye, and barley (Rallabhandi, Sharma, Pereira, & Williams, 2015).

Gluten detection can vary among ELISA kits and be affected by various processing conditions. The detection antibodies, target epitopes, sample extraction buffers, and calibration standards differ among kits and may result in differences in gluten quantitation (Diaz-Amigo & Popping, 2013). Extraction buffers such as aqueous ethanol extract prolamin but may not be suitable for gluten extraction in thermally processed foods. For such foods, extraction buffers containing dissociating and reducing agents, such as the

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Mendez cocktail, may be helpful for obtaining better extraction efficiency. The source of gluten can also affect gluten detection due to the variations in the type and proportion of gluten among wheat, rye, and barley. Within each type of grain are various cultivars that can also differ in the type of gluten and consequently its reactivity to the antibody. Different processes that can also affect gluten detection include thermal treatment (Bugyi et al., 2013; Gomaa & Boye, 2013; Török et al., 2014), enzymatic hydrolysis (Buddrick, Cornell, & Small, 2015; Walter, Wieser, & Koehler, 2015), fermentation (Panda, Zoerb, Cho, Jackson, & Garber, 2015), microwave heating (Susanna & Prabhasankar, 2011), and irradiation (Leszczynska, Lacka, Szemraj, Lukamowicz, & Zegota, 2003). Studies have shown that, depending on the ELISA kit and processing conditions, thermal processing can reduce the recovery of wheat gluten by altering the protein structure, thus inhibiting antibody binding (Bugyi et al., 2013; Gomaa & Boye, 2013; Török et al., 2014). Similarly, gluten hydrolysis by various enzymes has resulted in significantly reduced gluten recovery potentially due to cleavage of the target epitopes, preventing detection by the antibody (Buddrick et al., 2015; Walter et al., 2015).

These various processing conditions and gluten sources can affect the accurate detection of gluten in foods by ELISA, consequently leading to poor labeling compliance and increased risk for gluten-sensitive individuals. While studies have been done on the effects of processing on the detection of wheat gluten, there is very little information known about its effects on the detection of gluten from rye and barley. Also, differences in gluten content among the various grain cultivars have not been extensively studied. The purpose of this study was to evaluate the effect of different grain cultivars, thermal processing, and enzymatic hydrolysis on gluten quantitation by ELISA.

2. Materials and methods

2.1. Materials

Ten different wheat cultivars were obtained from the Grain Inspection, Packers and Stockyards Administration (GIPSA) of the U.S. Department of Agriculture, whereas seven winter rye and three barley cultivars were kindly provided by the Carrington Research Extension Center (North Dakota State University). The three sandwich ELISA kits used for determining gluten concentration were wheat protein ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan), RIDASCREEN Gliadin (R-Biopharm AG, Darmstadt, Germany), and AgraQuant gluten assay (Romer Labs UK Ltd, North Wales, UK). The competitive ELISA kit used was RIDASCREEN Gliadin competitive (R-Biopharm AG, Darmstadt, Germany). Papain, bromelain, alcalase, flavourzyme, pepsin, and trypsin were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Preparation of flour

The grains were ground using a blender (LB10, Waring Laboratory) and sifted through a sieve. The flour obtained was stored in a plastic container at 4 °C until further use. The total protein content in the flour was determined by the micro-Kjeldahl method (Micro Analysis Inc., Wilmington, DE) using 5.83 as the nitrogen to protein conversion factor.

2.3. Thermal processing of gluten-spiked corn flour

Wheat (Billings), rye (Hancock), and barley (Tradition) flours were spiked at a level of 200 mg/kg in corn flour (120 mg of each flour in 600 g corn flour) and mixed for 48 h using a rotator (TC-7, New Brunswick Scientific). An ELISA was run using the R-Biopharm kit to test for the homogeneity of the spiked corn flour. Four sub-samples from each of the three spiked corn flours run in duplicates exhibited a coefficient of variation below 20%, indicating good homogeneity.

The spiked corn flour was thermally processed in dry and moist conditions. The 200 mg/kg spiked corn flour was used directly for the dry condition, whereas for the moist condition, water was added to the spiked corn flour in a 1:1 ratio, resulting in a final spiked level of 100 mg/kg. Both dry (18–20 g each) and moist (35–40 g each) samples were heated in duplicate using an incubator (Thelco, Precision Scientific) under the following conditions: untreated, 60 °C for 20 min, 120 °C for 20 min, 170 °C for 20 min, and 170 °C for 40 min. These treatments were selected to assess the effect of increasing temperatures on gluten quantitation. The treatment of 170 °C for 20 min is close to baking conditions, whereas 170 °C for 40 min serves to determine the effect of excess thermal treatment on gluten quantitation. Samples were weighed before and after each treatment to calculate the moisture loss. The samples were ground and stored at -20 °C until further use. Two sub-samples were drawn from each duplicate sample, and two aliquots from each sub-sample were used for the ELISA analysis (n = 8).

2.4. Preparation of enzymatic hydrolysate spiked corn flour

Stock solutions of various enzymes (1 mg/ml for powdered enzymes; 1:100 v/v for liquid enzymes) were prepared in distilled water and adjusted to the pH for optimum activity. The pH used was 6.5 for papain and bromelain and 7.5 for alcalase, flavourzyme, and trypsin. Pepsin was prepared in 0.2 N HCl solution. In separate tubes, 100 mg each of wheat (Billings), rye (Hancock) and barley (Tradition) flour was incubated with 100 µl of enzyme stock solution and 900 µl of distilled water at optimum pH for 4 h at the following temperatures: 65 °C for papain, 60 °C for alcalase, 50 °C for flavourzyme, and 37 °C for bromelain, pepsin, and trypsin. Thereafter, the hydrolyzed samples were frozen at −80 °C and lyophilized. Appropriate flour and enzyme controls were also prepared under similar conditions.

The hydrolyzed samples were finely ground and 20 mg each were spiked into 100 g of corn flour (200 mg/kg). The spiked corn flour was then mixed for 24 h using a rotator. Three sub-samples from each control and enzyme-treated spiked corn flour were run in duplicates for the ELISA analysis (n = 6).

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot

The flour samples were extracted with 50 mM Tris-HCl, pH 7.5, containing 1% sodium dodecyl sulfate (SDS) and 2% β-mercaptoethanol (β-ME) (1:10 w/v) for one hour at room temperature, followed by centrifugation at 10,000 × *g* for 15 min. The protein content of the supernatant was estimated by a 660 nm protein assay using bovine serum albumin as a standard. The assay uses a dye that is deprotonated by basic amino acid residues of proteins, causing a color change from reddish-brown to green (Pierce, Rockford, IL). An appropriate stock solution was made in SDS-PAGE sample buffer (50 mM Tris-HCl, pH 6.8, 1% SDS, 30% glycerol, 0.01% bromophenol blue, and 2% β-ME) and incubated in a boiling water bath for 10 min. Five micrograms of protein was loaded onto a 4–12% Novex Bis-Tris gel (Invitrogen, Grand Island, NY) and run at a constant voltage of 100 V until the dye reached the bottom of the gel. The gel was stained overnight with 0.25% Coomassie Brilliant Blue R in 10% acetic acid and 50% methanol, followed by the removal of unbound dye with destaining solution (50% methanol, 10% acetic acid in deionized water). For Western blotting, the polypeptides were transferred from the