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Gluten-containing grains skew gluten assessment in oats due to sample grind non-homogeneity

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ABSTRACT

Oats are easily contaminated with gluten-rich kernels of wheat, rye and barley. These contaminants are like gluten 'pills', shown here to skew gluten analysis results. Using R-Biopharm R5 ELISA, we quantified gluten in gluten-free oatmeal servings from an in-market survey. For samples with a 5–20 ppm reading on a first test, replicate analyses provided results ranging <5 ppm to >160 ppm. This suggests sample grinding may inadequately disperse gluten to allow a single accurate gluten assessment. To ascertain this, and characterize the distribution of 0.25-g gluten test results for kernel contaminated oats, twelve 50 g samples of pure oats, each spiked with a wheat kernel, showed that 0.25 g test results followed log-normal-like distributions. With this, we estimate probabilities of mis-assessment for a 'single measure/sample' relative to the <20 ppm regulatory threshold, and derive an equation relating the probability of mis-assessment to sample average gluten content.

1. Introduction

Celiac disease (CD) is a genetic autoimmune disease that affects approximately 0.2–1.0% of the population worldwide (Catassi & Fasano, 2008; Ludvigsson et al., 2013; Mooney et al., 2016; Mustalahti et al., 2010; Sanders et al., 2003). Its prevalence has continued to increase (Ludvigsson et al., 2013; Rubio-Tapia & Murray, 2010). CD patients cannot tolerate the gluten proteins in wheat, barley and rye, which trigger autoimmune damage of the small intestinal mucosa (Janatuinen et al., 1995). Consequently, CD patients have to strictly observe a gluten-free (GF) diet in order to avoid adverse consequences. In addition to CD patients, GF diets are attracting increased numbers of consumers, being viewed as part of a healthy life style (Sharma, Pereira, & Williams, 2015). Consequently, food products with GF claims are becoming more popular in the marketplace (Sapone et al., 2012). To be valid for a GF claim, the gluten content of a food product has to be below a threshold level. One widely accepted GF threshold is 20 ppm, which is recognized by food regulatory agencies, such as Codex Alimentarius, the European Union, and the US Food and Drug Administration (Sharma et al., 2015).

Oats provide dietary fiber, B-complex vitamins (thiamin, niacin and riboflavin), iron and proteins (Comino, Moreno, & Sousa, 2015; Rebello, O'Neil, & Greenway, 2016). They have a long history of human consumption and are considered one of the most important whole grains in one's diet (Jacobs & Gallaher, 2004; Slavin, Martini, Jacobs, & Marquart, 1999). There is abundant evidence to support that the consumption of oats or oat products provides health benefits (Cerio, Dohil, Magina, Mahé, & Stratigos, 2010; Kale, Hamaker, & Bordenave, 2014; Rebello, O'Neil, & Greenway, 2016). Regarding oat's suitability for CD patient consumption, there has been debate. For instance, although avenins, the storage proteins in oats, lack the well-recognized epitopes found in the corresponding gluten proteins of wheat, rye and barley (that can trigger autoimmune conditions (Londono et al., 2013)), there has been discussion whether certain amino acid sequences harbored in oat avenins pose potential risks to CD patients (as they show some degrees of similarity to the gluten epitopes (Comino et al., 2011, 2015; Londono et al., 2013)). Increasing amounts of clinical data however show that most CD patients can tolerate oats in their diets (Lundin et al., 2003; Tapsas, Fälth-Magnusson, Högberg, Hammersjö, & Hollén, 2014; Thompson, 2003). This has been demonstrated in multiple studies where moderate inclusion of oats in gluten-free diets (for both adult and child CD patients) has caused no adverse effects (Janatuinen et al., 1995; Tapsas et al., 2014). In fact, a recent clinical study using a daily oat consumption of 100 g indicates that the amount of pure oats commonly consumed does not trigger clinical relapse in celiac disease patients (Hardy et al., 2015). This supports results found in previous long-term feeding studies that

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oats are safe for most CD patients (Janatuinen et al., 1995; Lundin et al., 2003; Tapsas et al., 2014; Thompson, 2003). As a consequence, inclusion of oats in a GF diet may expand the dietary options and improve the nutritional status of GF consumers (Comino et al., 2015).

So, although pure oats, which are free of any non-oat cereal contaminants, are safe for most CD patients (Janatuinen et al., 1995; Lundin et al., 2003; Tapsas et al., 2014; Thompson, 2003), oats can be easily contaminated with gluten-containing kernels of wheat, rye and barley. This can occur in the field, during transportation, in storage and during processing (Hernando, Mujico, Mena, Lombardia, & Mendez, 2008; Koerner et al., 2011; Thompson, 2004; Thompson, Lee, & Grace, 2010). Removal of these contaminant kernels seems a conceptually straightforward way to produce gluten-free oats, but our in-market survey suggests this is not a simple task to accomplish or assess. We have paid attention to gluten analysis of oats at serving-size level (a pouch or ~50 g), because this sample size is what GF consumers including CD patients may consume on a regular basis. As shown herein, non-compliant servings are getting onto store shelves and assessment issues related to kernel-based gluten contamination are a prime suspect for that. This is because contaminant kernels are hardened, 'pill like' pockets of concentrated gluten, not evenly distributed throughout oats and as we have found not easily distributed within a ground sample.

Gluten can be quantitatively analyzed via enzyme-linked immunosorbent assay (ELISA) (Moron et al., 2008; Valdes, Garcia, Llorente, & Mendez, 2003), mass spectrometry (Fiedler, McGrath, Callahan, & Ross, 2014; Simonato, Mainente, Tolin, & Pasini, 2011) and polymerized chain reaction (Dahinden, von Büren, & Lüthy, 2001; Zeltner, Glomb, & Maede, 2008). ELISA is the most widely utilized analytical method both in industry and amongst regulatory agencies to determine GF compliance (Sharma et al., 2015). Because of this, we report here the effects of gluten kernel contaminants on gluten ELISA analysis using the well accepted ELISA method (Koerner et al., 2011; Sharma et al., 2015; Thompson, 2004; Thompson et al., 2010), R-Biopharm R5 sandwich ELISA R7001.

There were two parts to this research, first was an 'in-market survey' where repeated measures of gluten positive yet compliant (i.e., <20 ppm) servings were conducted. Secondly we attempted to characterize the distribution of gluten in 0.25 g sub-samples (coming from a larger ground sample) given a gluten containing kernel existed in the ground sample.

2. Materials and methods

2.1. Materials

For the 'in-market survey', gluten-free oatmeal was acquired from the market by a third party sample acquisition company and then tested by a third party laboratory using the R-Biopharm R5 ELISA RIDASCREEN Gliadin (R7001) kit, purchased from R-Biopharm, Inc. (Washington, MO, USA).

For the 'within ground sample gluten distribution characterization of kernel contaminated oats' part of this research, clean, pure oat groats spiked with Hard Red Winter wheat kernels (Western Canada Origin, 2014 crop year) were prepared by hand-picking and provided by PepsiCo, Inc. These samples were also analyzed with the R-Biopharm R5 ELISA RIDASCREEN Gliadin (R7001) kit purchased from R-Biopharm, Inc. (Washington, MO, USA).

2.2. Gluten analysis specifics

To prepare a solid sample for gluten analysis with the R-Biopharm R5 ELISA RIDASCREEN Gliadin kit (R7001), the manufacturer's instruction recommends at least 5 g of sample be ground and 0.25 g of the ground sample be analyzed to assess gluten content. Since FDA has not provided an advisory procedure relating to sample grinding in terms of grinder type, sample size, and grinding time, analytical labs usually come up with their own procedures on the basis of the test kit manufacturer's instruction. In our study, sample grinding was performed by commercial labs X and Y according to their best practice, where, as mentioned a serving size of oatmeal (a pouch) or oat groats (50 g) were ground with household coffee grinder or food processor for two minutes. R-Biopharm R5 ELISA RIDASCREEN Gliadin kit (R7001) has a quantification range of 5–80 ppm. In case the gluten content was beyond the upper quantification range (i.e., >80 ppm) of the R-Biopharm kit, the sample extraction was appropriately diluted with 60% ethanol and was subjected to another round of ELISA assay to obtain a numerical gluten reading.

2.3. 'In-market survey' repeated measures

Six hundred thirty-six servings (e.g., a serving pouch) of gluten-free oatmeal which were produced by two large gluten-free oatmeal producers were acquired from store shelves by a third party sample acquisition company (14 date codes all 8/16/15 or later). The identifiers of the brand names and producers on the packages were covered by non-transparent tapes. The samples were shipped directly from the sample acquisition company to a well-recognized third party analytical lab, denoted as Lab Y. Lab Y ground each serving for two minutes using a Kitchen Aid coffee grinder. A clean grinding head and sample cup was used to grind each sample. Gluten content of each sample was analyzed by Lab Y and reported back to the authors at PepsiCo, Inc. The remainders of these 636 ground samples were retained at Lab Y where a portion was eventually selected for repeated analyses by Lab Y.

2.4. Gluten distribution in ground wheat-spiked oat groats

Twelve samples of pure oat groats (50 g each) were spiked with a wheat kernel (of approximately 0.027 g). Six of these spiked samples were sent to each of two recognized laboratories, denoted Labs X and Y. Lab X used an Osterizer food processor and Lab Y used a Kitchen Aid coffee grinder to do the grinding. In both labs, a sample was ground for two minutes with a clean grinding head and sample cup. After grinding, the gluten content of each sample was analyzed in triplicate (0.25 g per analysis). The remainders of the 12 ground samples were sent back to the authors, and were then aliquot into 0.25 g portions. Each aliquot was subjected to gluten analysis performed by the PepsiCo analytical team with nearly 2300 total analyses conducted.

2.5. Probability distribution

Data analysis and data fitting were performed in Excel, Microsoft Office 2013. The log-normal distribution of the test results of the spiking experiments was determined by chi-square goodness of fit tests (Snedecor & Cochran, 1989). Estimation of the confidence intervals for the mean of data following a lognormal distribution was performed with the Modified Cox Method (Olsson, 2005).

3. Results and discussion

3.1. 'In-market survey' repeated measures

In our 'in-market survey', 636 servings (e.g., a serving pouch) of gluten-free oatmeal were ground and tested for gluten content by