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An optimized extraction method for gluten analysis in cacao-containing products using an extraction buffer with polyvinylpyrrolidone

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A B S T R A C T

Enzyme-linked immunosorbent assay (ELISA) is commonly used to detect trace gluten in foods; however, ELISA for the determination of gluten is affected by matrices from various ingredients, such as plant proanthocyanidins (PACs). In the case of chocolate and other foods containing cacao, we found ELISA methods to be unsuitable for the determination of gluten contamination. The aim of this study was to improve methods for the determination of gluten in foods containing cacao by combining the extraction solution with polyvinylpyrrolidone (PVP), which binds PACs. We optimized the method for gluten determination in cacao-containing foods using PVP solution, and found that the best extraction method for the detection of gluten in cocoa powder was extraction with 1% PVP K15. This new extraction method can be applied to chocolate contaminated with wheat proteins during the manufacturing process, as well as cacao-containing products spiked with gliadin.

1. Introduction

Wheat is a major crop grown, processed, and consumed worldwide. Although it is a primary source of nutrition, it is also associated with both allergies and intolerance (Gujral, Freeman, & Thomson, 2012; Tatham & Shewry, 2008). An IgE-mediated immediate-type allergy to wheat is common in children, whereas wheat-dependent exercise-induced anaphylaxis (WDEIA) is common in adolescents and adults (Tatham & Shewry, 2008). Recently, WDEIA was also reported in Japan following transcutaneous sensitization using hydrolyzed wheat proteins containing soap (Yokooji et al., 2013). Wheat omega-5 gliadin and high molecular weight glutenin subunit are major allergens in WDEIA (Morita et al., 2009). The prevalence rate of wheat allergy is at least 0.21% among Japanese adults, which was determined using a combination of questionnaire-based examination, skin-prick test, and serum ω5-gliadin-specific IgE test (Morita et al., 2012).

Gluten proteins are the storage proteins of cereal grains, such as wheat, rye, and barley, and composed of two different protein families: alcohol-soluble monomeric prolamins and alcohol-insoluble polymeric glutenins. The prolamins can be further subdivided into α, β, γ, and ω-gliadins, whereas glutenins are classified into low-molecular weight (MW) and high-MW glutenins (Tatham & Shewry, 2008, 2012), with T cell stimulatory epitopes having been identified in all of these proteins (Tatham & Shewry, 2012). Gluten is characterized by high proline and glutamine content, and due to the occurrence of these amino acids in specific motifs, these proteins are stable against thermal processing and resistant to the digestive process (Mills, Jenkins, Alcocer, & Shewry, 2004). Celiac disease (CD) is one of the most frequent example of food intolerance worldwide caused by gluten ingestion and is found among ~1% of the population in Europe, North and South America, North Africa, and the Indian subcontinent (Cummins & Roberts-Thomson, 2009; Gujral et al., 2012).

Because the only therapy for a wheat allergy and CD is avoidance of the offending food, it is essential for food-allergy patients to eliminate gluten from their diet. A system of gluten labeling is necessary for people with allergies. According to the recently revised Codex Alimentarius, the regulatory threshold for a food to be able to claim gluten-free status is 20 mg gluten/kg. In Japan, the monitoring threshold for the labeling of wheat is 10 μg/g based on guidelines from the 2002 Food Sanitation Law (Akiyama, Imai, & Ebisawa, 2011; Mukoyama et al., 2007).

Effective testing methods are needed to accurately determine trace amounts of gluten in foods. Immunochemical methods are

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Abbreviations

PACs	Proanthocyanidins
PVP	Polyvinylpyrrolidone
WDEIA	Wheat-dependent exercise-induced anaphylaxis
CD	Celiac disease
ELISA	enzyme-linked immunosorbent assay
2-ME	2-mercaptoethanol
SDS	Sodium dodecyl sulfate
NVP	1-vinyl-2-pyrrolidone
MW	molecular weight
PVPP	Polyvinylpolypyrrolidone
PWG	Working Group on Prolamin Analysis and Toxicity

the most versatile for gluten analysis due to their specificity, sensitivity, and practical convenience (Haraszi, Chassaigne, Maquet, & Ulberth, 2011; Yin, Chu, Tsai, & Wen, 2016); however, the analysis of residual gluten levels in foods by enzyme-linked immunosorbent assay (ELISA) is difficult due to the physicochemical properties and low extraction efficiency of gluten in highly processed foods (Haraszi et al., 2011). Garcia et al. (2005) developed an extraction solution containing disaggregating guanidine hydrochloride and reducing 2-mercaptoethanol (2-ME) for heat-processed foods, and Watanabe et al. (2005) also developed an extraction buffer containing surfactant sodium dodecyl sulfate (SDS) and reducing 2-ME for extracting insoluble proteins caused by heat and pressure processing, as well as antibodies recognizing the denatured proteins. However, 2-ME was designated as a deleterious substance by the Poisonous and Deleterious Substances Control Act in 2008. Ito et al. (2016) developed a technique for extracting food components with an extract solution containing reducing sodium sulfite and surfactant SDS, with this method authorized as the revised official method for food-allergen analysis in Japan.

Generally, extraction solutions from food samples are not purified by ELISA, given that ELISA for the determination of gluten in food might also be affected by matrices derived from various ingredients. The complexity of the various food matrices might cause variations in immunoassays by changing protein conformations and antibody binding. Plant polyphenols, such as proanthocyanidins (PACs), bind to and precipitate proteins, especially proline-rich proteins (e.g., gluten proteins and gelatin) (Siebert, 1999; Wu & Siebert, 2002); therefore, PACs might interfere with the detection of gluten proteins by ELISA.

To avoid this problem, we developed a protocol to measure gluten proteins in foods containing PACs by adding polyvinylpyrrolidone (PVP) to the extraction solution. PVP is a water-soluble polymer produced by the polymerization of vinylpyrrolidone. Polymers with degrees of polymerization ranging from 10 to 100,000 are obtained by radical polymerization, with polymers with MWs of between 2,500 and 10,000,000 mainly produced in industry (Haaf, Sanner, & Straub, 1985). PVP has been used as a blocking agent in immunochemical studies (Haycock, 1993) and is also used in DNA-isolation methods for foods containing high levels of PACs (Porebski, Bailey, & Baum, 1997).

Recently, PVP was used to improve the extraction of gluten from food samples containing PACs (Mena, Lombardia, Hernando, Mendez, & Albar, 2012); however, this method has not yet been fully evaluated. In this study, we optimized the methods for detection of gluten in cacao products by combining the extraction solution with PVP.

2. Materials and methods

2.1. Chemicals

1-Vinyl-2-pyrrolidone (NVP), PVP K15 (MW: 10,000), K60 (MW: 160,000), and K90 (MW: 360,000) were obtained from Tokyo Chemical Industry (Tokyo, Japan). PVP K25 (MW: 25,000) and K30 (MW: 40,000) were purchased from Wako Pure Chemicals (Osaka, Japan). Polyvinylpolypyrrolidone (PVPP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade, and all aqueous solutions were prepared with distilled water purified by a water-distilling apparatus (Yamato WAG-203; Yamato Scientific, Tokyo, Japan).

2.2. Samples

Food samples were purchased from Osaka, Japan. Chocolates contaminated with wheat were kindly gifted from EZAKI Glico Co., Ltd., Institute for Safety Research (Osaka, Japan). Each item was thoroughly ground to a fine, homogenized sample using a food processer (Iwatani IFM-800DG; Iwatani Corporation, Tokyo, Japan).

2.3. Reference material

The reference gliadin standard was purchased from the Working Group on Prolamin Analysis and Toxicity (PWG). This gliadin standard consisted of a mixture of 28 wheat cultivars representative of European wheat-producing countries (van Eckert et al., 2006). The calculations in this study were based on a gliadin protein content of 91.4% on a dry matter basis (van Eckert et al., 2006).

2.4. Extraction procedures

Procedures for extraction from food samples were based on reducing sodium sulfite and surfactant SDS reagents in Tris-HCl-buffered saline, as previously described (Ito et al., 2016; Oyama et al., 2010). For extraction using conventional methods, a 1-g sample of ground food was weighed in a 50-mL polypropylene tube, and a 19-mL aliquot of the extraction solution was then added to the tube containing the sample. The tubes were closed tightly, mixed thoroughly by vortexing, and then placed in a rack for shaking horizontally overnight (over 12 h) at 25 °C on a reciprocal shaker (Taitec BR-23UM; Taitec Corporation, Koshigaya, Japan) at between 90 rpm and 110 rpm. The tubes were then centrifuged (Kokusan H-9R; Kokusan Denki, Saitama, Japan) for 20 min at 7,000 *g* and 25 °C. The supernatant was filtered through filter paper (ADVANTEC 5A; Advantec, Tokyo, Japan) to obtain the extract, followed by a 20-fold dilution and ELISA.

For extraction with the PAC-binding agent, 1 g of cocoa powder was weighed and placed in a 50-mL polypropylene tube, followed by addition of 25 μL of PWG-gliadin stock solution before addition of a 19-mL aliquot of the extraction solution containing 1% (w/v) PAC-binding agent. After vortexing, the extraction method proceeded as described.

2.5. Determination of minimum PVP concentration

To determine the minimum PVP concentration required, a 1-g sample cocoa powder was weighed and placed in a 50-mL polypropylene tube, and 25 μL of PWG-gliadin stock solution was used to spike the tube, followed by addition of a 19-mL aliquot of extraction solution containing 0.5%, 1%, 2%, or 4% (w/v) PVP K15. After vortexing, the extraction method proceeded as described.