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Rapidly detecting major peanut allergen-Ara h2 in edible oils using a new immunomagnetic nanoparticle-based lateral flow assay

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ABSTRACT

Ara h2 is a major peanut allergen that induces rashes, vomiting, diarrhea, and anaphylactic shock. Since peanut is a major source in producing edible oils globally, Ara h2 residues can be present in various edible oils. In this work, an immunomagnetic nanoparticle-based lateral flow assay for identifying Ara h2 in edible oils is developed. This assay exhibits high sensitivity with a visual detection limit of 0.1 mg/kg Ara h2 in oil, and favorable specificity in differentiating peanut from seeds and nuts. The calculated CV values of intra- and inter-assay were 6.73–10.21% and 4.75–8.57%, respectively, indicating high reproducibility. In an analysis of 26 oil products, Ara h2 was detected in two peanut oils as 0.122 ± 0.026 mg/kg and 0.247 ± 0.027 mg/kg. The entire method takes 5 h, including a 3.5-h sample preparation. Hence, this method has the potential to be an effective way to screen edible oils for Ara h2.

1. Introduction

Peanut allergy has become an important health concern owing to its growing prevalence, low outgrow rate, low inducing dose and life-threatening potential. In the United States, the prevalence of peanut allergy in children more than tripled in a decade from 0.4% in 1997 to 0.8% in 2002 and 1.4% in 2008 (Sicherer, Muñoz-Furlong, Godbold, & Sampson, 2010). Moreover, individuals allergic to peanut may retain their sensitization into adulthood. For instance, peanut-allergic patients with a peanut-specific IgE level of 5 kUA/L or less have an approximately 50% probability of outgrowing their allergy, and there possibility of re-sensitization exists, even after a negative skin prick test and negative food challenge test (Kerr & Pong, 2004). For some individuals who are hypersensitive to peanut, as little as 30–100 μg of peanut proteins can induce mild allergic reactions, such as swelling of the lips and tongue, rashes, vomiting, and diarrhea (Hourihane, Bedwani, Dean, & Warner, 1997). Food-induced anaphylaxis (FIA) is a serious allergic reaction that may cause the rapid death of sensitized patients. In the United States and the United Kingdom, peanut allergy is the most common cause of FIA in children (Pumphrey & Gowland, 2007; Rudders, Banerji, Clark, & Camargo, 2011). Accidental exposures to peanut allergens are typically caused by the contamination of raw food materials with peanuts or during food processing, or the presence of undeclared peanut residues in food products. Thus, accurately declaring the peanut content of food is crucial for peanut-allergic individuals.

Peanut allergy varies clinically and immunologically in different areas worldwide, possibly due to the difference in dietary tradition and pollen exposure (Cabanillas, Jappe, & Novak, 2018). In a study with 115 peanut allergic patients from 3 countries, American patients (n = 30) frequently reacted with rAra h1, 2, and 3 with sensitization rate as 56.7–90.0%; Swedish patients (n = 35) got the highest sensitization to rAra h8 (82.9%); as Spanish (n = 50) patients were more often sensitized to rAra h9 (60%) (Vereda et al., 2011). In a recent study with 68 patients from 11 European countries, it also found that rAra h 8 and rAra h 9 were major allergens for central/western and southern Europeans, respectively. rAra h2 was the only major allergen for the peanut-allergic population (Ballmer-Weber et al., 2015). Additionally, in a study with 32 peanut allergic patients from Netherlands, Ara h2 was recognized most frequently (26/32) in IgE immunoblotting and IgE-cross-link capacity tests, as compared with Ara h1 and Ara h3 (Koppelman, Wensing, Ertmann, Knulst, & Knol, 2004). In a study with serum from 12 highly sensitive, peanut allergic patients in the United States, Ara h 2 was more potent than Ara h 1 in the IgE-cross-link capacity and ImmunoCap assay with a median relative potency that was

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52.5 and 2.3 folds more than Ara h1, respectively (Palmer et al., 2005). Two isoforms of Ara h2 have been identified to date. The large isoform (Ara h2.02; 19 kDa) has an insertion of 12 amino acids containing an additional repeat of a major IgE binding epitope, DPYSPS, relative to the small isoform (Ara h2.01; 17 kDa) (Chatel, Bernard, & Orson, 2003). Ara h2 is more resistant to protease digestion than Ara h1 or Ara h3, and the limited proteolysis of Ara h2 might be due to that its multiple intra-molecular disulfide bonds can effectively maintain the quaternary structure of Ara h2 (Mueller et al., 2011). The protease-resistant peptides of Ara h2 comprise a pool of relatively large peptides that can elicit allergic reactions (Koppelman, Hefle, Taylor, & de Jong, 2010). Moreover, the core of Ara h2 is highly stable against heating to 100 °C, resulting in persistence of its allergenic potency even after the implementation of various food processing methods (Lehman, Schweime, Reese, & Randow, 2006). All of these facts explain why Ara h2 is a potent target for detecting peanut allergenic residues in the food industry.

Many assays have been developed to detect peanut allergens; they can be classified as protein-based or DNA-based. Regarding protein-based assays, the target can be either a specific peanut allergen (Ara h1, Ara h2, and etc.) or total peanut proteins. Enzyme-linked immunosorbent assay (ELISA) and lateral flow assay (LFA) are the two most commonly used protein-based assays. Several ELISAs have been developed for detecting Ara h2. Montserrat et al. (2015) developed a sandwich ELISA and a competitive ELISA with sera against Ara h2. The competitive ELISA has a lower detection limit (0.06 mg/kg peanuts in biscuit) than the sandwich ELISA (0.13 mg/kg peanuts in biscuit). In 2015, a sandwich ELISA was established using a monoclonal antibody to detect Ara h2 with a sensitivity of 0.02 ng/mL (Peng, Song, Liu, Kuang, & Xu, 2015). LFA is a membrane-based immunochromatographic method whose results can be easily visualized by the naked eye after 10–20 min of reaction. Therefore, LFA is regarded as a useful method for on-site detection or point-of-care testing. Owing to its rapidity, simplicity, and low cost, LFA has been extensively used to detect various allergic foods, such as crustacean, peanut, milk, and wheat (Ji et al., 2011; Koizumi, Shirota, Akita, Oda, & Akiyama, 2014; Masiri et al., 2016; Yin, Chu, Tsai, & Wen, 2016). An LFA that uses gold nanoparticles as the detection reagent was developed to detect peanut allergens with a limit of detection of 1 ng/mL of Ara h2 (Peng et al., 2015). Unlike the traditional LFAs which only offer a yes/no answer, the barcode-style LFAs can rapidly provide a semi-quantitative result, based on the correlation between the number of test lines on a strip and the analyte concentration. For instance, a barcode-style LFA was developed for the rapid detection of gliadin, using two cut-off values (10 and 50 mg/kg gliadin) to provide a semi-quantification for identifying "gluten-free" and "very low gluten" foods (Yin et al., 2016).

Low sensitivity is a major disadvantage of LFA, especially in the analysis of foods is that it the food matrix effect interferes with the antibody-antigen interaction during analysis. Thus, numerous strategies for improving the sensitivity of LFA have been developed, including the use of magnetic beads (MBs) (Gas, Baus, Queré, Chapelle, & Dreanno, 2016), quantum dots (Le, Xie, Zhu, & Zhang, 2016), and silver treatment (Yang et al., 2011). MBs have been extensively used to isolate and concentrate targets from liquid samples in an applied external magnetic field. The three-dimensional structure of MBs significantly increases their surface-to-volume ratio, providing more binding sites for target molecules than are available in the wells of a microtiter plate with a two-dimensional structure; assay sensitivity is thus increased. The use of MBs also involves highly efficient washing and separation, significantly reducing interference by the food matrix (Chu & Wen, 2013). Hence, MB-based LFAs have been gradually applied for detecting contaminants in foods, such as allergens (Zheng, Wang, Lu, & Liu, 2012), foodborne pathogens (Fisher et al., 2009) and aflatoxin (Tang et al., 2009).

Peanut is one of the materials from which edible oils are produced, and the annual production of peanut oil is about 4% of the total vegetable oil worldwide (Gunstone, 2011). Accordingly, residues of peanut allergens may be present in other types of edible oils, owing to the cross-contamination of raw materials or in production lines. Isolating proteins from oil samples is typically difficult, due to the low protein contents in final oil products and the hydrophobicity of oil (Martin-Hernandez, Benet, & Obert, 2008). In this study, for the sensitive and specific detection of Ara h2 residues in edible oil, an immunomagnetic nanoparticle (IMNP)-based LFA was developed and was used herein to analyze 26 commercial edible oils to validate its feasibility in detecting Ara h2 residues.

2. Materials and methods

2.1. Materials

Raw peanuts (Tainan #14) were provided by the Agricultural Improvement Station (Taichung, Taiwan). Seeds, nuts, edible oils and nonfat dry milk (NFDM) powder were purchased from local supermarkets (Taichung, Taiwan). Carboxylic acid magnetic beads were bought from Ocean NanoTech, LLC (San Diego, CA, USA). Polyvinylpyrrolidone (PVP10), 2-(N-morpholino)ethanesulfonic acid (MES), N-hydroxysuccinimide (NHS), Tween-20, and bovine serum albumin (BSA) were purchased from Merck (Darmstadt, Germany). Ammonium sulfate, dithiothreitol (DTT), phenylmethanesulfonyl fluoride (PMSF) and casein were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Tris hydrochloride (Tris-HCl) and protein A agarose were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Sephadex G50 and HiTrap Q media were bought from GE Healthcare (Chicago, IL, USA). With respect to nitrocellulose (NC) membranes, HF075, HF120, and HF135 were purchased from Merck Millipore; AE99 was purchased from GE Healthcare, and CN140 was purchased from Sartorius Corp. (Göttingen, Germany).

2.2. Production and purification of antibodies against Ara h2

Antibodies against peptide 1 and peptide 2 (α-P1 Ab and α-P2 Ab) were obtained from GenScript USA, Inc. (Piscataway, NJ, USA). The amino acid sequence of peptide 1 is RDPYSPSQDPYSPSC, and that of peptide 2 is RQQWELQGDRRC. These two peptides were chosen based on the expected antigenicity, surface possibility, and secondary structure of Ara h2.02. Peptides were synthesized, conjugated onto KLH (keyhole limpet hemocyanin) and then immunized into New Zealand White rabbits. Antibody against the whole Ara h2 molecule (α-Ara h2 Ab) was generated from Ara h2 purified from raw peanuts. Ara h2 from peanuts was purified as described in an earlier study (Koppelman et al., 2005), and the purification protocol was provide in Supplementary information. The antibodies from three batches of sera were purified through a protein A column. The purified antibodies were conjugated on magnetic nanoparticles (Chu et al., 2013) to be IMNPs. Detailed conjugation protocol is described in Supplementary information.

2.3. Assembly of strips of laterals flow assay

On strips, 7.5 μg/cm anti-Ara h2 antibody was coated on the test line and 1.0 μg/cm anti-rabbit antibody was coated on the control line using a Linomat V automatic sample spotter (CAMAG, Muttenz, Switzerland). The distance between each line was 5 mm. The membrane was dried at 37 °C for 1 h, blocked with a mixed solution (1% (w/v) PVP 10, 1% (w/v) glycine, 0.3% (v/v) Tween 20 in PB, pH 7.5) for 30 min, and then dried at 37 °C for 1 h. For assembly, the NC membrane was firstly attached to a backing card and the absorbent pad was adhered to the upper end of the test strip with a 2 mm overlap with the NC membrane. The complete set of components was cut into 4 mm-wide, 55 mm-long strips using a guillotine cutter (KinBio, Shanghai, China).

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