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High hydrostatic pressure reducing allergenicity of soy protein isolate for infant formula evaluated by ELISA and proteomics via Chinese soy-allergic children's sera

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ABSTRACT

Proteomics was used to confirm allergen subunit differences between control and high hydrostatic pressure (HHP)-treated soy protein isolate (SPI), which could support further understanding of reduced allergenicity associated with HHP. SPI solutions (0.75% protein weight/solvent volume, pH 6.8) were subjected to HHP treatment at 200, 300, 400, 500 MPa for 5, 10, 15, 20 min, respectively. Enzyme-linked immunoabsorbent assay was used to assess the allergenicity, using soy-allergic human sera as the probe and non-soy-allergic human sera as the negative control. Proteomics was performed to analyze allergen differences in a mixed protein sample. HHP reduced allergenicity by 45.5% at 300 MPa for 15 min. Western blotting and mass spectrometry indicated that HHP treatment altered the allergenicity of < alpha > and < alpha > ′ subunits of 7S globulin and A1 and A1a subunits of 11S globulin. This suggests HHP could improve the safe use of SPI in infant formula.

1. Introduction

Soy-based infant formula (SBF) is being consumed more commonly, accounting for increased uptake in the US (up 25%) and Canadian (up 20%) infant formula markets (Bhatia, Greer, & the Committee on Nutrition, 2008), although it was initially developed for lactose intolerant and cows' milk allergic infants (Bhatia et al., 2008). However, some children are allergic to soy including a proportion of those who are cows' milk allergic (CMA, ca. 14%) (Klemola et al., 2002; Zeiger et al. 1999). In contrast to hydrolyzed milk formulae, SBF has dominated the market because of its low cost and better flavour (Klemola et al., 2002; Silva et al., 2015; Zeiger et al., 1999). Thus, reducing allergenicity of the soy protein isolate (SPI) in infant formulae is important for enhancing infant food safety.

High hydrostatic pressure (HHP), an emerging non-thermal technology, is widely utilized in food processing and facilitates sterilisation, modification of food proteins and carbohydrate functionalities, activation and inactivation of endogenous enzymes, preservation of micronutrients, and alteration of allergenicity (Bello, Martínez, Ceberio, Rodrigo, & López, 2014; Estrada-Girón, Swanson, & Barbosa-Cánovas, 2005). Potential mechanisms for reducing allergenicity of food proteins through HHP can be summarized in three theories. Firstly, HHP might incur the release of allergenic proteins, which could be caused by partial destruction of the endosperm, permeation of the surrounding solution, protein solubilization and/or solubilized protein diffusion (Kato, Katayama, Matsubara, Omi, & Matsuda, 2000). HHP could also breakdown proteins into subunits with low molecular weight, reducing the overall antigenicity (Zhou et al., 2016). Zhou et al. (2016) reported that ginkgo seeds proteins (GSP) disintegrated into small molecular weight subunits, ranging from 4 to 30 kD at 500, 600 and 700 MPa for 20 min, reducing antigenicity by more than 95%. Finally, HHP can induce aggregation and/or unfolding of food allergen, which might mask and/or inactivate conformational epitopes of allergenic proteins (Estrada-Girón et al., 2005; Somkuti & Smelle, 2013).

HHP-induced structural alteration of food proteins has been investigated extensively. These conformational changes have, most commonly, been monitored using spectroscopic techniques including infrared (IR) (Savadkoohi, Bannikova, Mantri, & Kasapis, 2016),

Abbreviations: ELISA, enzyme-linked immunoabsorbent assay; HHP, high hydrostatic pressure; SBF, soy-based infant formula; CMA, cow's milk-based formula; SPI, soy protein isolate; Ig, immunoglobulin; IR, infrared; NMR, nuclear magnetic resonance; CD, circular dichroism; UV, ultraviolet; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; 2DE, two-dimensional electrophoresis; MS, mass spectrometry

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fluorescence (Li, Zhu, Zhou, & Peng, 2012), nuclear magnetic resonance (NMR) (Hu, Zheng, Liu, Deng, & Zhao, 2016), ultraviolet (UV) absorption (Meng, Bai, Gao, Li, & Chen, 2017), circular dichroism (CD) spectroscopy (Li, et al.,2012; Vidacek, de las Heras, Solas, Mahillo, & Tejada, 2009).

Savadkoohi, Bannikova, Mantri, and Kasapis (2016) investigated the impact of HHP on conformational changes in soy glycinin though IR spectra and argued that HHP reduced numbers of < alpha > -helix and < beta > -sheets and increased random coils in 10, 30 and 60% (w/w) soy glycinin solutions. Li et al. (2012) showed that HHP could reduce allergenicity SPI in infant formula, using a commercial ELISA kit. Extrinsic emission fluorescence spectroscopy indicated an approximate 11.5-fold increase in fluorescence intensity and a shift in the maximum emission wavelength from 516 to 466 nm. CD spectra also suggested there was a significant increase in helix 1 and turn contents but fewer strand 1 and unordered contents. Li et al. (2012) speculated that SPI allergens epitopes could be closely related to the secondary structures of < alpha > -helix and < beta > -sheets. Hu, Zheng, Liu, Deng, and Zhao (2016) argued that HHP caused the unfolding of < alpha > -casein, thus slightly increasing < alpha > -helix content and decreasing < beta > -sheet and < beta > -turn contents. The NMR peaks of the main allergenic characteristics distributed in the regions of 15–17, 23–26, 40, 53, 59 and 85–88. The 200 MPa HHP evidently decreased the relative ratio of 23–26 and 40 and increased the relative ratio of 59 and 85–88. Application of targeted proteomics to food allergens has increased rapidly in the last five years (Ahsan, Rao, Gruppuso, Ramratnam, & Salomon, 2016). Among the allergen proteomic research conducted so far, over half has concentrated on plant allergen identification and/or quantification (Ahsan et al., 2016; Gagnon, Poysa, Cober, & Gleddie, 2010; Rouquié, Capt, Eby, Sekar, & Hérouet-Guicheney, 2010). Gagnon et al. (2010) reported a total of 19 potentially allergenic proteins in soybeans including five novel allergens identified using proteomics in their study of North American patients. Rouquié et al. (2010) adopted 2DE to compare known endogenous food allergens in seeds from five different soybean lines to determine if there were any unintended impacts of genetic modification.

Alteration of allergenicity in some food products or food extracts induced by HHP has been detected using SDS-PAGE combined with immunoblotting, which can evaluate changes in allergenicity of food protein subunits (Kato et al., 2000; Zhou et al., 2016). In these examples, most adopted animal sera as the probe for Western blotting (Kato et al., 2000; Vidacek et al., 2009; Zhou et al., 2016) but some have used sera from allergic patient (Hajós, Polgár, & Farkas, 2004; Kobayashi et al., 2016; Liang, Xu, Pan, Ge, & Zong, 2015). Verhoeckx et al. (2015) predicted that understanding of soybeans allergenicity is limited owing to insufficient numbers of high-quality studies conducted with soy-allergic human sera. To our knowledge, little research assessing the influences of HHP on SPI allergenicity in infant formula has been performed by 2DE combined with immunoblotting using allergic children sera. Thus, the primary objectives of this study were to use 2DE and Western blotting to separate and identify SPI allergens capable of binding immunoglobulin E (IgE) in infant formulae using sera from Chinese soybean-allergic children, and apply mass spectrometry to confirm differences in allergenic subunits between control and HHP-treated SPI, which might further aid understanding of reduced allergenicity induced by HHP.

2. Materials and methods

2.1. Materials

Soy protein isolate (model: specially prepared for infant formula) was purchased from Henan Province Anyang Mantianxue Food Co., Ltd. (Anyang, China). The defatted soybean meal was mixed with 85% ethanol solution at a ratio of 1:5 (w/v) and stirred for 1 h at 20 °C. Then the mixture was suction filtered at a vacuum pressure of 0.09 MPa. The filter cake was mixed with 95% ethanol solution at a ratio of 1:5 (w/v). The mixture was stirred for 1 h at 20 °C and was then suction filtered. The filter cake was naturally dried at 20 °C. The soybean meal washed by the ethanol was mixed with deionized water in a ratio of 1:10 (w/v) and adjusted to pH 7.0. The mixture was stirred for 1 h at 30 °C at a speed of 60 r/min. Then, the mixture was centrifuged at 8000*g* for 30 min. The supernatant was adjusted to pH 4.5 and was centrifuged at 4000*g* for 20 min. After centrifugation, the solid content of the curd was adjusted to 10%, and its pH was changed to 7.5; then, the curd was dried using the spray drying equipment (model LPG; Wuxi Shangde Drying Equipment Co. Ltd., Wuxi, China). The spray drying conditions were as follows: outlet pressure of 30 MPa, inlet temperature of 125 °C, an inner tower temperature of 75 °C and an outlet temperature of 80 °C. The SPI was mainly made from non-genetically modified soybean cultivars Zhonghuang 35 and Beidou 10 with a protein content on a dry basis of 91.61% and NSI of 82.8%. The urease activity of the SPI was negative.

Urea, iodoacetamide, Tween 20, glycine, thiourea, bromophenol blue, 3-[3-(cholamidopropyl) dimethylamino] propyl sulfonate (CHAPS), bovine serum albumin (BSA), sequencing-grade trypsin, < alpha > -cyano-4-hydroxy-cinnamic acid, tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). The Bradford assay kit, standard protein, pH 4–7 IPG strips, carrier ampholytes, mineral oil, dithiothreitol (DTT), Bio-Safe Coomassie and 2-mercaptoethanol were purchased from Beijing Bio-Rad Life Science Development Company (Beijing, China). Spain Biowest low melting point agarose was purchased from Gene Co., Ltd. (Shanghai, China). Santa Cruz horseradish peroxidase labelled goat anti-human IgE was purchased from Shanghai Univ Biological Technology Company (Shanghai, China). The ECL kit # 34079 was purchased from Thermo Fisher Scientific China Co., Ltd. (Shanghai, China). Polyvinylidene fluoride (PVDF) membranes were purchased from Amersham Biosciences Company (Staffanstorp, Sweden). Film was purchased from Kodak China Investment Co., Ltd. BJ Branch (Beijing, China). The remaining reagents were analytical grade products. All solutions were prepared with MilliQ water (Milli-Q Integral 10, Millipore Corporation, Billerica, USA).

2.2. High hydrostatic pressure (HHP) treatment

HHP treatment was performed according to the method of Li et al. (2012). High-pressure equipment (model UHPF-750 MPa-3L; maximum pressure 750 MPa; Baotou KEFA Hitech Food Machine Co., Ltd., Baotou, Inner Mongolia, China) was utilized, which has a hydraulic type chamber with an inner capacity of 3 L (1000 mm in diameter and 2000 mm in height) and whose temperature is controlled by a water jacket to avoid overheating. Distilled water was used as the solvent. Prior to pressure processing, SPI was dissolved in distilled water at a ratio of 1% (w/v), and then, the mixture was magnetically stirred for 20 min at 20 °C at a speed of 60 r/min. The prepared SPI solution was vacuum-conditioned in a double-layer polyethylene bag. The pressure transmitting medium in the HHP machine was water, and its temperature was kept at 20 °C throughout the HHP processing. SPI solutions (0.75% protein weight/solvent volume, pH 6.8) were respectively subjected to HHP at 200, 300, 400 or 500 MPa for 15 min. In addition, SPI solutions were respectively subjected to HHP treatment at 300 MPa for 5, 10, 15 or 20 min. The pressure was increased at a rate of approximately 250 MPa/min and decreased at a rate of approximately 300 MPa/min. The SPI solutions treated by HHP were freeze-dried. The unpressurized product was adopted as the experimental control. Each sample was processed in duplicate.