Food Control 42 (2014) 290–295

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Effects of high pressure processing on immunoreactivity and microbiological safety of crushed peanuts

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form
10 February 2014
Accepted 14 February 2014
Available online 24 February 2014

Keywords:
High pressure
Peanut
Immunoreactivity
Aspergillus flavus
Aflatoxin

ABSTRACT

Peanuts are a common economical food source consumed worldwide but exist health concern of food allergy and are particularly susceptible to infection by the mold fungus *Aspergillus flavus* during storage, accumulating highly toxic substance aflatoxin. In this study, the effect of high pressure treatments on peanut immunoreactivity, peanut amino acid composition, *A. flavus* growth and aflatoxin contents on crushed peanuts was evaluated. Results showed that immunoreactivity of peanuts treated with 600 MPa and 800 MPa for 10 min was significantly lower ($P < 0.05$) than those of the control group by 69.2 ± 5.3% and 73.3 ± 1.9%, respectively. High pressure treatment at 800 MPa decreased total essential amino acid content as well as two nutritional indexes, the chemical score and the essential amino acid index, by 32.4 ± 2.1% and 31.1 ± 3.2%, respectively. The growth of aflatoxigenic fungi was inhibited in peanuts with aflatoxin accumulation that were subjected to different levels of pressure treatments during 30 days of storage. Peanuts treated with 600 MPa and 800 MPa had considerably lower aflatoxin levels, 0.26 μg/g and 0.22 μg/g in wet basis, respectively, than the control peanut aflatoxin level (9.08 μg/g) on day 30. Results were demonstrated that high pressure treatment had a significant inhibitory effect on *A. flavus* growth in peanuts and this contributes to reduction of aflatoxin production and accumulation instead of directly destroy aflatoxin. Taken together, the findings of this study indicated that high pressure treatment could preserve peanut quality by reducing food immunoreactivity and by eliminating *A. flavus* in peanuts.

1. Introduction

High pressure processing (HPP) application is an emerging non-thermal technology that can improve food safety by inactivating food spoilage microorganisms and increasing the shelf life of agricultural and other types of food products without affecting their organoleptic properties. Different microorganisms have different degrees of resistance to HPP treatment, and a broad HPP sensitivity range varies among microbiological species and even across strains (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008). HPP can damage microbial membranes and thereby interfere with nutrient uptake and cell waste disposal. Additional damaging events include extensive solute loss during pressurization, protein denaturation, and key enzyme inactivation (Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, & Torres, 2011). Inactivation of food spoilage microorganisms by HPP application has been extensively used to improve food safety and to increase the shelf life of various food products. Kaur, Kaushik, Rao, and Chauhan (2013) reported that HPP treatment (435 MPa) induced a hardening effect in fresh shrimp that exhibited an extended shelf life to 15 d, which was longer than the 5-d shelf life of untreated shrimp. The treated shrimp maintained lower viable microbe counts throughout the storage period and had superior microbial quality than that of the untreated samples. Evert-Arriagada, Hernández-Herrero, Juan, Guamis, and Trujillo (2012) showed that cheese treated with 300 and 400 MPa pressure and stored at 4 °C had a shelf-life of 14 and 21 d, respectively. In contrast, untreated control cheese had a shelf life of 7 d. Garriga, Grèbola, Aymerich, Monfort, and Hugas (2004) reported that HPP at 600 MPa for 6 min was an efficient method for inhibiting the growth of yeasts and Enterobacteriaceae for curbing off-flavors production, and for delaying the growth of spoilage microorganisms such as lactic acid bacteria in sliced marinated beef loin. Calligaris, Foschia, Bartolomeoli, Maifreni, and Manzocco (2012) also reported that HPP is a reliable technological alternative to conventional heat treatments for the

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http://dx.doi.org/10.1016/j.foodcont.2014.02.030
0956-7135/

production of fresh-like banana juice. Banana juice pressure treatment at 200 MPa allows a reduction of mesophilic bacteria by 4 log cycle and also facilitates pectate lyase inactivation. Tribst, Franchi, Cristianini, and Demassaguer (2009) have reported that mango nectar treated with high pressure homogenization at 300 MPa resulted in more than 6 log reduction in *Aspergillus niger*. Voldřich, Dobiáš, Tichá, Čeřovský, and Krátká (2004) reported that the number of *Talaromyces avellaneus* in apple juice was reduced by 6 log CFU/mL following thermally assisted high pressure treatment at 600 MPa for 60 min at 60 °C.

Peanuts (*Arachis hypogaea*) are globally used as an economical food source as they contain high quality proteins, unsaturated fatty acid, and are rich in minerals. Peanuts are consumed whole roasted and boiled, and as ingredients in a variety forms of products include peanut brittle, peanut butter, and peanut oil, and so on (Chang, Sreedharan, & Schneider, 2013). However, peanut is one of the eight most common causes of IgE-mediated food allergies worldwide, and the incidence of peanut allergy is expected to escalate due to the increasing consumption of peanut products. Peanut is often considered the most potent allergen. Eleven peanut allergens (Ara h 1–11) have been officially recognized by the Allergen Nomenclature Sub-committee of the International Union of Immunological Societies (http://www.allergen.org/Allergen.aspx). Among these allergens, Ara h 1 and Ara h 2 are considered major allergens as they are immunologically recognized by more than 90% individuals with peanut allergies (Hu et al., 2011). The increased prevalence of peanut food allergy in recent years has resulted in a demand for methods that control allergens in peanut products (Kumar, Verma, Das, & Dwivedi, 2012). Previously, effects of various food processing methods, including thermal, nonthermal, genetic modification, and chemical methods were studied for evaluating their impact on the structural and allergenic properties (Shriver & Yang, 2011). Beyer et al. (2001) reported that frying and boiling peanuts resulted in a decreased IgE binding capacity to Ara h 2 and Ara h 3. High pressure microfluidization treatment decreased the antigenicity, changed the secondary structure, and increased the UV absorption intensity and surface hydrophobicity of peanut allergen Ara h 2 (Hu et al., 2011). Autoclaving at 2.56 atm/30 min induced a significant decrease of IgE-binding capacity of peanut allergens (Ara h 1, Ara h 2 and Ara h 3) (Cabanillas et al., 2012). Chu et al. (2008) reported that transgenic silencing of Ara h 2 and Ara h 6 is a feasible approach to produce hypoallergenic peanuts. Chung, Yang, and Krishnamurthy (2008) also demonstrated that UV-light treatment resulted in reduced solubility of peanut allergens in the peanut extracts and liquid peanut butter. Nevertheless, it has been recognized that roasted peanuts are more allergenic than raw peanuts (Chung & Champagne, 2001). Besides, peanut contamination with toxigenic strains of *Aspergillus* spp. is a major problem for farmers as it tends to grow in the stored crop after harvest and then causes crop spoilage by synthesizing highly poisonous aflatoxins, which are toxic, carcinogenic, mutagenic, immunosuppressive, and teratogenic agents. Heavily contaminated peanuts cannot be used in edible products and cause huge economic losses in the peanut industry (Chang et al., 2013). Globally, aflatoxin contamination is major concern for food and feed safety. Thus, there is an increased research interest for discovering effective treatment methods that preserve food quality and decontaminate food by inactivating *Aspergillus* spp.

The objective of this study was to investigate the effects of high pressure treatment on the allergenic properties and amino acid composition of crushed peanuts. High pressure treatments for inactivating *Aspergillus flavus* toxigenic strains, inhibiting their growth, and reducing aflatoxin content of crushed peanuts were also evaluated.

2. Material and methods

2.1. Plant material and high pressure treatments

Shelled raw peanuts (*A. hypogaea* L. TN11) purchased from local markets of Chiayi, Taiwan, were used in this study. The peanuts were visually inspected for defects, and defective peanuts were removed from the batch. The sound peanuts were then milled in masher. Grinded peanut (10 g) were vacuum packed in polyethylene bags and were treated in a 300-mL pressure-vessel (Foodlab Plunger Press model S-FL-850-9 W; Stansted Fluid Power Ltd, Stansted, UK). The pressure come-up rate was approximately 300 MPa/min, the deviation at targeted pressure was ±10 MPa, and the pressure release time was less than 10 s. Water was used as the pressure-transmitting fluid in this study and the compression and decompression time were not included in the pressurization time. The packets were placed in a cylindrical loading container at room temperature and pressurized at 200, 400, 600 or 800 MPa for 5 and 10 min and compared with the untreated samples (controls). After processing, pressurized samples were stored at 4 °C and processed for allergen assay and amino acid composition analysis. All analyses were performed in triplicate.

2.2. Allergen assay

The allergen contents were determined using sandwich enzyme-linked immunosorbent assay (ELISA) kits for the quantitative analysis of high pressure-treated peanuts (RIDASCREEN® FAST Peanut, R-Biopharm). Briefly, 5 g of the samples were ground well and thoroughly mixed, and then weighed 1 g of sample mix intensively with 20 mL extraction buffer which were already heated to approx. 60 °C for 10 min at 60 °C by shaking casually. The extract was centrifuged at 2500 × *g* for 10 min at 4 °C and then filtered the supernatant. The filtrate was used in the assay. Afterwards, the analysis was proceeding according to manufacturer's protocols. The kit has been approved by the AOAC Research Institute according to the Performance Tested Method Program with the license No. 030404. The polyclonal antibody specifically detects peanut proteins, including the peanut allergens Ara h 1 and Ara h 2. All experiments were performed in triplicate. Accuracy and precision of the peanut allergen enzyme-linked immunosorbent assay kit was approved by Whitaker, Williams, Trucksess, and Slate (2005) and Nogueira, McDonald, Westphal, Maleki, and Yeung (2004) have demonstrated its utilization in major peanut allergen detection.

2.3. Determination of amino acid composition

The protein amino acids were determined using the freeze-dried samples for acid hydrolysis, derivatization, and HPLC quantification with slight modifications to the method described by Martinez-Villaluenga, Gulewicz, Frias, Gulewicz, and Vidal-Valverde (2007). Briefly, 200 μL of DL-norleucine (0.2 mmol/mL, Sigma) were added to 100 mg of sample as internal standard. The protein hydrolysis was performed by incubating the samples with 3 M hydrochloric acid for 21 h at 110 °C in vacuum, and then rinsing the samples twice with ultrapure water. Phenyl-isothiocyanate (PITC, 99%, Sigma) was used for amino acid derivatization. Tryptophan was measured according to the method described in the Official Methods of Analysis of the Association of Analytical Chemists (AOAC, 1990). The results are shown as mg/g of peanut. Essential amino acid index (EAAI) and chemical score (CS) were calculated to the method described by Oser (1959) by using the