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Effect of high pressure processing on the immunoreactivity of almond milk

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ABSTRACT

Influence of high pressure processing (HPP) at 450 and 600 MPa, 30 °C for various holding times (0, 30, 60,180, 300 and 600 s) on almond milk amandin was investigated. The immunoreactivity of pressure treated almond milk was compared with raw and thermally processed (TP) almond milk (72, 85 and 99 °C for 0 to 300 s) using a sandwich enzyme-linked immunosorbent assay (ELISA), Western blot and dot blot. Monoclonal antibodies (mAbs) targeting linear (4F10) and conformational (4C10) epitopes on amandin were used to assess amandin immunoreactivity. To determine the aggregation of almond proteins, almond milk protein solubility was quantified after 300 s of HPP (up to 600 MPa, 30 °C) and TP (at 72, 85 and 99 °C, 0.1 MPa). After HPP (for all holding times), amandin can no longer be detected by the anti-conformational mAb in ELISA while signal generated from the anti-linear epitopes mAb was reduced by half ($P < 0.05$). On the other hand, most TP samples did not show significant reductions in immunoreactivity ($P > 0.05$) unless processed at 85 and 99 °C for 300 s. Western blot and dot blot also confirmed the loss of immunoreactivity by both antibodies for HPP almond milk. The reduced band intensity of the 61 and 63 kDa polypeptides and concomitant appearance of high molecular weight polypeptides in Western blot indicated that the observed decrease in immunoreactivity was partly due to the aggregation of amandin. The tested HPP and TP treatments respectively caused a maximum of ~70% and ~75% reduction in protein solubility. The study demonstrated that the loss of protein solubility, rather than the epitope destruction, may be responsible for the observed decrease in amandin immunoreactivity.

1. Introduction

Since its origination from the Mediterranean region, almond and the beverages made from it have been consumed for centuries. Almond milk is a colloidal dispersion obtained by disintegrating almonds with water. In recent years, almond milk has emerged as an alternative non-dairy beverage in the U.S., Europe and Australian market. This plant-based beverage is targeted to consumers suffering from lactose intolerance, and hypersensitive to cow milk, and those who seek for plant-based beverages as an alternative to dairy milk (Anonymous, 2013; Lacono, Lospalluti, Licastro, Scalici, & Pediatria, 2008; Salpietro, 2005). Almond milk is also rich in essential and non-essential nutrients like α-tocopherol, essential fatty acids, dietary fiber, and a wide range of other phytochemicals. The consumption of almond milk can also be linked to reduction of the risk of coronary heart disease by decreasing the plasma LDL cholesterol level (Chen, Milbury, Lapsley, & Blumberg, 2005). In spite of health benefits, they are one of the eight major allergenic foods affecting 0.5% of the adults in the U.S. (Sampson, 2004). The total population of allergenic individuals has been increasing in recent years (Jin et al., 2009). The Food Allergen Labeling and Consumer Protection Act (FALCPA) requires that the presence of any items causing allergy in any form should be mandatorily labeled (Tiwari et al., 2010). Almond seeds contain 188 different proteins as detected by two-dimensional electrophoresis (Li & He, 2004). Amongst these proteins, almond major protein (AMP) or amandin accounts for approximately 65% of total soluble proteins (Wolf, & Sathe,1998). Amandin belongs to the 11S globulin family and is reported to be stable after thermal treatments such as blanching, roasting, and autoclaving (Roux, Teuber, Robotham, & Sathe, 2001). It is therefore not surprising that the ultra-high temperature processed almond milk available in the market (Berger, Bravay, & Berger, 1997) retains considerable allergenicity.

High pressure processing (HPP) is an alternative food preservation technique that has the potential to preserve the organoleptic, textural,

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and nutritional qualities of food ingredients. HPP inactivates pathogenic microorganisms through elevated pressures (Balasubramaniam, Farkas, & Turek, 2008). It has been reported that HPP can modify the characteristics of some proteins depending upon the magnitude of pressure, temperature, and pressure holding time (Balny & Masson, 1993; Gross & Jaenicke, 1994; Heremans, 1982). Since HPP affects mostly the non-covalent bonds, it may cause irreversible changes in the quaternary, tertiary and secondary structures of proteins. Such alteration could potentially destroy the existing epitopes or, possibly, generate neo-allergen in foods (Sathe, Teuber, & Roux, 2005).

Due to the diversity of allergenic proteins, the effect of high-pressure treatment on immunoreactivity varies from study to study. In one study, HPP at 300–600 MPa for 5 min has been reported to decrease the allergenicity of several birch pollen allergy related foods including hazelnut, apple, celery, and peach (Meyer-Pittroff, Behrendt, & Ring, 2007). In addition, apple allergen Mal d 3 (Husband et al., 2011; Johnson et al., 2010), peanut allergen Ara h 2 (Hu et al., 2011), bovine gamma globulin (Yamamoto et al., 2010), soybean sprout (Peñas, Gomez, Frias, Baeza, & Vidal-Valverde, 2011), and soybean protein isolate (Li, Zhu, Zhou, & Peng, 2012) were also shown to be altered by pressure treatments. On the other hand, when subjected to HPP, the cod allergen Gad m1 (Somkuti, Bublin, Breiteneder, & Smeller, 2012), largemouth bass allergens (Liu, Tao, Liu, Chen, & Xue, 2012), and almond major protein allergens (Li et al., 2013) did not show a significant decrease in immunoreactivity. In one study, it was reported that the immunoreactivity of bovine β-lactoglobulin increased after HPP (Zhong, Liu, & Liu, 2011).

The inconsistent findings in the literature are probably due to a) the distinct characteristics of the various allergenic proteins in the investigated foods, b) type of epitope(s) investigated, c) methods used for investigating the immunoreactivity of the targeted protein(s), d) food matrix effects on the targeted immunoreactive protein(s), e) effects of food processing on the protein solubility, f) or a combination thereof. Since there are very limited studies documenting the influence of pressure treatment on almond allergens, we chose to evaluate the impact of HPP on the immunoreactivity of almond milk using antibodies targeting both conformational and linear epitopes of the major almond allergen — amandin.

2. Material and methods

2.1. Reagents and chemicals

Microtiter plates (12 × 8-well) were from Costar I (Cambridge, MA). Nitrocellulose membrane and blotting papers were from Schleicher and Schuell, Inc. (Keene, NH). Sathe, Teuber, Gradziel, and Roux (2001) described sources of chemicals for electrophoresis. All other chemicals and supplies were of reagent or better grade and were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich Corporation (St. Louis, MO).

2.2. Almond processing and milk preparation

Raw almond seeds were purchased from a local supermarket and stored at room temperature for few weeks. Almonds (200 g) were soaked overnight (15–18 h) in 600 mL water at 4 °C. The soaked almonds were drained and rinsed with cold water and re-weighed to determine the amount of water absorbed by the seeds. The skins of soaked almonds were manually removed (unless otherwise specified). One part, by weight, of deskinned almonds was mixed with nine parts, by weight, of water and disintegrated in a Waring Laboratory blender (Torrington, CT) for 3 min in low speed (~15,000 rpm). The resulting mixture was then put through two layers of muslin cloth and the filtered milky almond suspension was passed through a 180-micron (μ) sieve. This final filtered aqueous suspension of almond milk was then loaded into 500 mL pouches, heat sealed and stored at 4 °C for not more than 24 h before processing.

All analyses were performed at least in triplicate for independent samples.

2.3. High pressure processing

The schematic of experimental procedures and analysis conducted during the study is presented in Fig. 1. Almond milk was processed in a high-pressure kinetic tester (PT-1, Avure Technologies Inc., Kent, WA). The procedure described by Thai Nguyen, Rastogi, and Balasubramaniam (2007) was used. Briefly, aliquots of 2.5 mL almond milk were placed in a high barrier pouch made from a sterile filter bag (# 01-002-57, Fisher Scientific), air bubbles were removed, and the pouch was heat-sealed. Two pouches were placed inside a 10 mL polypropylene syringe (model 309604, Becton Dickinson and Co., Franklin Lakes, NJ), within a sample carrier, and the remaining space inside the syringe was filled with cold water (2–5 °C). The syringes loaded with samples were kept in ice water to precool before loading into the pressure chamber. The pressurization was started when the samples reached the predetermined temperature, taking into account the heat of compression of the test samples (Patazca, Koutchma, & Balasubramaniam, 2007). Representative pressure–time–temperature profile obtained during high pressure processing of almond milk is presented in Fig. 2. The almond milk samples were processed at 30 °C (±2 °C) at 450 MPa and 600 MPa, each with pressure holding time of 0, 30, 60, 180, 300 and 600 s. Then the immunoreactivity of the samples was determined (see Sandwich mAb-based Enzyme Linked Immunosorbent Assay (ELISA) for amandin detection and immunoreactivity and Western blot and dot blot analyses sections). Additional experiments were also carried out at 30 °C (±2 °C) over 150–600 MPa for 300 s to estimate pressure effects on protein solubility (Fig. 1). The equipment had a pressurization rate of about 20 MPa/s and rapid depressurization time (~1 s).

2.4. Thermal processing

Aliquots of two 2.5 mL almond milk at room temperature were placed into sterile plastic pouches as described in the High pressure processing section. The pouch were then loosely wrapped with the muslin cloth and dipped in a steam kettle at different temperatures (72, 85, and 99 °C) for four different time intervals, 0, 30, 180 and 300 s at each temperature. During a preliminary experimentation, sample temperature was monitored by using a k-type thermocouple temperature data logger (DCC Corporation, Pennsauken, NJ, model#3211) and come up time (105 to 115 s) was determined. The reported thermal process holding times do not include come-up time. The thermally treated samples at specified process temperature and holding times were then immediately removed and cooled in ice water (~0 °C).

The HPP and TP samples were immediately stored at −30 °C until further use. The samples were then shipped under frozen condition (−30 °C) to Florida State University (Tallahassee, FL) for immunoreactivity analyses.

2.5. Determination of pH, °Bx and protein content

The pH and °Bx of almond milk were measured using a pH meter (Accumet XL 15, Fisher Scientific, Mississauga, ON, Canada) and brix/RI refractometer (Reichert Analytical Instruments, model #13940000, Japan) respectively. pH and brix values were measured before and after processing.

Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin (BSA, 0–200 μg/mL) in borate saline buffer (BSB, 0.1 M boric acid, 0.025 M sodium borate, 0.075 M sodium chloride, pH 8.45) as the standard protein to normalize the samples in SDS-PAGE, Western blots, dot blots, and ELISA.