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Effects of heat and high-pressure treatments on the solubility and immunoreactivity of almond proteins

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ABSTRACT

The effects of dry and moist heat, autoclave sterilization and high-pressure treatment on the biochemical characteristics and immunological properties of almond proteins were investigated. Changes in the solubility and immunoreactivity of almond proteins extracted from treated almond flour were evaluated using a total protein assay, indirect competitive inhibition enzyme-linked immunosorbent assay (IC-ELISA), and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE). Almond proteins were stable during dry-heat treatment at temperatures below 250 °C. Dry heat at 400 °C, boiling, autoclave sterilization and high-pressure treatment in the presence of water at ⩾500 MPa greatly reduced the solubility and immunoreactivity of almond proteins. SDS–PAGE revealed that the protein profiles of almond flour samples treated under these conditions also changed significantly. The synergistic effects of heat, pressure and the presence of water contributed to significant changes in solubility and immunoreactivity of almond proteins.

1. Introduction

In the US, eight food groups (milk, egg, fish, shellfish, tree nuts, peanuts, wheat, and soybeans) must be included on labels when used as ingredients in packaged food. Almond is the seed of a drupe, despite being commonly referred to as a tree nut, and is on the FDA list of tree nut allergens (Sathe, Teuber, Gradziel, & Roux, 2001). US almond accounts for approximately half of world almond production (Holzhauser, Vieths, & Roder, 2010). Almond contains more than 188 proteins (Derbyshire, Wright, & Boulter, 1976) and the major seed storage protein, amandin, is officially recognized as an allergen (Pru du 6) by the International Union of Immunological Societies Allergen Nomenclature Sub-committee (Allergen Nomenclature, IUIS Allergen Nomenclature Sub-committee, http://www.allergen.org/search.php?allergensource=almond, Accessed 29.04.10.). Amandin is a hexamer and each monomer consists of two polypeptides: a 40–42 kDa acidic subunit and a 20–22 kDa basic subunit (Albillos, Menhart, & Fu, 2009; Albillos et al., 2008; Sathe et al., 2001).

Almond is used as an ingredient in a wide range of food products, which increases the potential for exposure to almond proteins by sensitized and/or allergic individuals. The presence of undeclared allergens introduced inadvertently to packaged food products, due to cross contact or production errors, can cause unintended exposure of allergic individuals (Acosta, Roux, Teuber, & Sathe, 1999). While strict avoidance of allergenic proteins remains the most effective means to prevent the occurrence of allergic reactions, developing new processing technologies and conditions to reduce food or protein allergenicity has attracted

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much interest in recent years (Cabanillas et al., 2012; Fu, Maks, & Banaszewski, 2010; Liu et al., 2013; Sathe & Sharma, 2009).

Foods, including almonds, are typically subjected to different processes before consumption. During processing, food proteins may undergo changes including unfolding, aggregation and chemical modifications (Fu & Maks, 2013; Mills, Sancho, Rigby, Jenkins, & Mackie, 2009). These changes can alter allergenicity of food proteins, increasing or decreasing the potential to be recognized by immunoglobulin E (IgE) (Cabanillas, Crespo, Burbano, & Rodríguez, 2010; Cuadrado et al., 2009). Even after γ-irradiation (1, 5, 10 and 25 kGy) and heat treatments (e.g., autoclaving, dry roasting, blanching or microwaving), almond proteins remained antigenically stable (Su, Venkatachalam, Teuber, Roux & Sathe, 2004). Similarly, amandin exhibits a high antigenic stability after roasting, blanching, and microwave heating (Venkatachalam, Teuber, Roux, & Sathe, 2002). After thermal (13–96 °C) and chemical (urea and dithiothreitol) treatments, amandin, in its native multimeric form, exhibits a higher structural stability than its acidic or basic subunits (Albillos et al., 2009). Chemical denaturation treatments, using guanidium hydrocholoride (GuHCl), urea, sodium dodecyl sulfate (SDS), reducing agents, and 2% v/v β-mercaptoethanol (β-ME) with heat, have been used to study the relationship between structural change and immunoreactivity of amandin. SDS and β-ME were found to significantly reduce the immunoreactivity of amandin, as determined by dot blot assays (Kshirsagar, Fajer, Sharma, Roux, & Sathe, 2011).

High-pressure treatment is used increasingly in food processing and many studies have examined the effect of high-pressure treatment on proteins (Knorr, Heinz, & Buckow, 2006; Pellerin & Balny, 2002; Silva, Foguel, & Royer, 2001; Tian, Li, Zhao, Xu, & Jin, 2014). An unfolding transition midpoint of the apple allergen Mal d 1 was observed at 150–250 MPa, and the unfolding was irreversible (Somkuti, Houska, & Smeller, 2011). However, the allergenicity of Mal d 1 from apple juice and apple homogenates was not affected significantly by high pressure (450–550 MPa) (Houska et al., 2009). A gradual unfolding of soy protein was observed during pressure treatment, ranging from 200 to 600 MPa; however, the unfolded proteins regained their secondary and tertiary structures when the pressure was returned to atmospheric pressure (Tang & Ma, 2009). High-pressure treatment (300 MPa) over an extended period of time (5–15 min) induced modifications in the α-helix and β-sheet structures of soy proteins, and significantly altered the allergenicity of these proteins (Li, Zhu, Zhou, & Peng, 2012). While high-pressure treatment has been used to reduce the antigenicity of various allergens (Cabanillas et al., 2012; Houska et al., 2009; Somkuti et al., 2011; Tang & Ma, 2009; Li et al., 2012), it has not yet been applied to almond proteins.

In this study, we investigated changes in the solubility and immunological properties of almond proteins following various heat and pressure treatments, including dry heat (100, 200, 250 and 400 °C), moist heat (60 and 100 °C), autoclave sterilization and high-pressure treatment (400, 500 and 580 MPa). The synergistic effects of moisture, temperature and pressure on almond proteins were evaluated.

2. Materials and methods

2.1. Materials

Raw whole Nonpareil Supreme almonds were purchased from VineTreeOrchards (Burbank, USA). The rabbit polyclonal antibody against amandin was produced in the US Food and Drug Administration Module One Laboratory (Laurel, USA). Horseradish peroxidase (HRP) labeled goat anti-rabbit IgG was obtained from Promega (Madison, USA). Unstained protein molecular weight marker and 96-well polystyrene microtiter plates were purchased from Thermo Scientific (Rochester, USA). QuantiPro™ BCA assay kit, 3,3′,5,5′-tetramethylbenzidine (TMB), dimethyl sulfoxide (DMSO) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, USA). Tris (hydroxymethyl) aminomethane (Tris), glycine and SDS were obtained from Bio-Rad Laboratories (Richmond, USA). Gradient acrylamide separating gel (Novex® 10–20% tricine gel 1.0 mm, 15 well) was obtained from Invitrogen (Carlsbad, USA). Coomassie Brilliant Blue R-250 was obtained from Fisher Scientific (Pittsburgh, USA). Tween-20, salts and other chemical reagents were purchased from Tianjin Fengchuan Chemical Reagent Science and Technology Co., Ltd. (Tianjin, China).

2.2. Instrumentation

Ultrahigh pressure processing equipment (HPP.L3-600/0.6, 0–580 MPa) was purchased from Huatai (Tianjin, China). A microtiter plate reader was obtained from Thermo Scientific. The grinding mill and shaker (IKA MTS 2/4 digital) were obtained from Linda (Zhejiang, China) and IKA (Staufen, Germany), respectively.

2.3. Preparation of defatted almond flour

The protocol as described in Albillos et al. (2008) was followed. Whole almonds were ground in a mill and defatted with hexane in 50 mL centrifugal tubes (flour/solvent ratio was 1:10 w/v). Tubes were shaken at 250 rpm at room temperature (c.a. 22 °C) for 3 h. The solvent was decanted and the extraction process repeated twice. Residual hexane in the slurry was evaporated in a fume hood overnight. The defatted almond powder was homogenized using a mortar with a pestle, and stored at −20 °C in airtight plastic bottles.

2.4. Sample treatment

2.4.1. Dry heat treatment

Defatted almond flour (500 mg) samples in crucibles were heated in a muffle furnace (Barnstead International, Dubuque, USA) at 100, 200, 250 or 400 °C for 10 min.

2.4.2. Moist heat treatment

Defatted almond flour (500 mg) was mixed with 12 mL of PBS in a 15-mL centrifuge tube. The mixtures were subjected to heating in a water bath at 60 or 100 °C for 10 min.

2.4.3. Autoclave sterilization

Defatted almond flour (250 mg) in a 15-mL centrifuge tube was subjected to autoclave treatment (121 °C, 0.15 MPa) for 10 min. For autoclave sterilization in the presence of water, the almond flour was first dissolved in 6 mL of PBS prior to autoclaving.

2.4.4. High-pressure treatment

Plastic pouches each containing 500 mg of defatted almond flour, with or without addition of 6 mL of PBS, were sealed and subjected to high-pressure treatment at 400, 500 or 580 MPa at ~20 °C for 3 min.

2.5. Protein extraction

Defatted almond flour samples were extracted with PBS (pH 7.4) by shaking at 200 rpm for 1 h. The mixture was centrifuged at 11,000*g* for 10 min and the supernatant passed through a 0.45 μm pore size cellulose filter. Protein concentrations in filtrates were determined using a QuantiPro™ BCA assay kit. The protein