



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.

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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 hydrochloride (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 11,000g for 10 min and the supernatant passed through a 0.45 μ m pore size cellulose filter. Protein concentrations in filtrates were determined using a QuantiPro™ BCA assay kit. The protein

filtrate was diluted appropriately and tested by indirect competitive ELISA and SDS–PAGE.

2.6. Indirect competitive inhibition enzyme-linked immunosorbent assay (IC-ELISA) for almond protein extracts

2.6.1. ELISA buffers and solutions

The buffers and solutions used were either purchased from Sigma or were prepared in our lab as follows:

PBS: 38.4 mmol L⁻¹ Na₂HPO₄·H₂O, 11.5 mmol L⁻¹ NaH₂PO₄·H₂O, 154 mmol L⁻¹ NaCl, pH 7.4;

Washing buffer: PBS with 0.05% Tween 20;

Coating buffer: 50 mmol L⁻¹ sodium carbonate buffer, pH 9.6;

Blocking buffer: 1% BSA in PBS;

Substrate solution: TMB (3.3 mg) in DMSO (250 µL) was added to phosphate-citrate buffer (25 mL, 0.1 mol L⁻¹ citric acid, 0.2 mol L⁻¹ Na₂HPO₄, pH 4.3) containing 3.25 µL of 30% H₂O₂ solution.

Stop solution: 1.25 mol L⁻¹ H₂SO₄.

2.6.2. ELISA procedure

A 96-well polyvinyl microtiter plate was coated with amandin (purified according to Albillos et al., 2008) at a concentration of 1 µg per well in 100 µL coating buffer and incubated at 4 °C overnight. The plate was washed three times with washing buffer, and unbound sites were blocked with 200 µL of blocking buffer per well for 1 h at 37 °C. After washing, the plate was blotted dry. PBS was added to control (50 µL) and blank wells (100 µL), and diluted almond extract (50 µL) was applied to specified wells. 50 µL of anti-amandin antibody diluted 1:80,000 in PBS was added immediately to all wells except for the blanks, and the plate was incubated for 1 h at room temperature. The plate was washed four times and 100 µL of HRP-labeled goat anti-rabbit IgG diluted 1:30,000 in PBS was applied to all wells and the plate was incubated at 37 °C for 0.5 h. After the plate was washed five times, 100 µL of the substrate solution was added to each well and the plate incubated for 20 min. The reaction was stopped by adding 50 µL of stop solution, and the absorbance was recorded in dual-wavelength mode (450 and 650 nm as the test and reference wavelength, respectively) using the microplate reader.

Inhibition curves were constructed by plotting percentage inhibition against the concentrations of almond protein. The percentage inhibition for a given concentration of inhibitor (i.e., the test proteins) was calculated using the following equation:

$$\% \text{inhibition} = \frac{A_0 - A_2}{A_0 - A_1} \times 100$$

where A_0 is the absorbance of sample that contained the primary antibody only (maximum absorbance), A_2 is the absorbance at any given antigen concentration, and A_1 is the average absorbance of control wells that contained no primary antibody. The half maximal inhibitory concentration (IC₅₀) was determined from the inhibition curve as the almond protein concentration that gives 50% inhibition.

2.7. Electrophoresis

SDS polyacrylamide gel electrophoresis (SDS–PAGE) was carried out according to Sathe et al. (2001) and Albillos et al. (2008) with minor modifications. Protein extracts from high-pressure treated almond powder were boiled for 10 min in sample buffer (50 mM Tris/HCl, pH 6.8, 4% SDS, 0.05% bromophenol blue, 20% glycerol, 2% β-ME). Samples (10 µL) were loaded on the polyacrylamide gel (separating and stacking gels were 12 and 4% total acrylamide, respectively) and electrophoresed at a constant voltage (80 V) in an electrophoresis buffer (0.025 M Tris/HCl buffer containing 0.1% SDS and 0.192 M glycine). The gels were stained with 0.25% Coomassie Brilliant Blue R-250 in 40% ethanol/10% acetic acid, and destained with 30% methanol/7% acetic acid.

Proteins extracts from the heat-treated and autoclaved samples were electrophoresed according to Albillos et al. (2008) using Novex® 10–20% gradient acrylamide gel in a XCell Sure Lock unit (Invitrogen).

2.8. Statistical analysis and experimental design

The thermal and high-pressure treatments were repeated three times. For each trial, duplicate almond flour samples were treated under each set of conditions. Protein extracts from the control and treated samples obtained from each trial were assayed with separate BCA or IC-ELISA analyses and each with triplicate wells. Differences in the normalized concentrations between samples treated at different conditions were analyzed by analysis of variance using IBM SPSS for Windows (version rel. 19.0, 2010, SPSS Inc., Chicago, IL, USA). The Duncan test was used for comparison of the means. In all cases, the level of significance was set at $p < 0.05$.

Table 1
Effect of food processing on almond protein solubility ($n = 3$).

Food processing		Relative protein recovery (mean ± SD)	CV (%)	Food processing		Relative protein recovery (mean ± SD)	CV (%)
Unprocessed Dry heat (°C)	100	1.00 ^a 0.98 ± 0.020 ^a	2.72	Unprocessed	400 (dry flour)	1.00 ^a 0.84 ± 0.059 ^b	7.11
	200	0.95 ± 0.021 ^a	2.19		400 (with PBS)	0.54 ± 0.056 ^c	10.39
	250	0.86 ± 0.016 ^b	1.87		500 (dry flour)	0.91 ± 0.015 ^{ab}	1.68
	400	0.06 ± 0.001 ^d	1.64		500 (with PBS)	0.43 ± 0.027 ^d	6.16
Moist heat (°C)	60	0.96 ± 0.035 ^a	3.62	High pressure (MPa)	580 (dry flour)	0.84 ± 0.060 ^b	7.15
	100	0.78 ± 0.030 ^c	3.85		580 (with PBS)	0.38 ± 0.027 ^d	7.16
Autoclave sterilization	Dry flour	0.90 ± 0.055 ^b	6.12				
	With PBS	0.75 ± 0.089 ^c	11.49				

Relative protein recovery was statistically analyzed by one way ANOVA (Duncan). Values with the same letters in the same column are not significantly different ($p > 0.05$).

3. Results and discussion

This study investigated the effects of thermal and high-pressure processing on the solubility and immunoreactivity of almond proteins. Defatted almond flours were subjected to various heat and high-pressure treatments under conditions commonly used for home cooking or industrial food manufacturing. Treatment with dry heat at 400 °C was used to determine if almond proteins could be completely eliminated at high temperatures. Heating in water at 60 °C or 100 °C was also investigated. Hydrostatic high pressure processing is a non-thermal food processing technique that has been used increasingly for food sterilization, enzyme deactivation, and improving the functional properties of food products (Pellerin & Balny, 2002; Tian et al., 2014). How the presence of water might affect the heat- or pressure-induced changes in the solubility and immunoreactivity of almond proteins was investigated by subjecting almond flour samples to autoclaving and high-pressure treatments with and without water in the form of PBS.

Protein recovery, as determined by the BCA total protein assay, was selected to assess quantitatively the changes in almond proteins solubility as a result of thermal and high-pressure treatments. This approach differs from other studies (e.g., Su et al., 2004) where changes in the solubility of almond proteins following heat treatments were only analyzed qualitatively by SDS–PAGE (Su et al., 2004).

IC-ELISA, based on rabbit polyclonal antibodies against amandin, was used to evaluate the changes in the immunoreactivity of almond proteins after different heat and pressure treatments. The IC_{50} value provided an estimate of the immunoreactivity between the antibody and target antigen where a high value indicates low immunoreactivity. To avoid data fluctuation that can occur between different trials, all protein recovery and IC_{50} values obtained from the processed samples were normalized and calculated as the ratio of values for processed samples to those of unprocessed samples. SDS–PAGE was used to investigate changes in the protein/peptide profiles of almond protein extracts after thermal and high-pressure treatments.

3.1. Effect of food processing on the solubility of almond proteins

Heat can lower protein solubility and can cause denaturation and damage to lysine, arginine, and cysteine residues. It can also change protein digestibility to some degree (Gerrard et al., 2012). The effect of heat on the almond protein solubility is shown in Table 1. When almond flour was subjected to dry-heat treatment at 100 or 200 °C for 10 min, the relative protein recovery remained largely constant ($p > 0.05$). Protein recovery began to decrease following treatment at 250 °C (10 min). Dry-heat treatment at 400 °C for 10 min resulted in almost no protein recovery. Although no loss of protein was observed in samples heated in PBS at 60 °C for 10 min, moist heating in general resulted in a greater decrease in

protein recovery than dry heat. For example, samples moist-heated at 100 °C had a lower relative protein recovery than those subjected to dry heat at 100 °C. We conclude that treatment at high temperature reduces the solubility of almond proteins, and the presence of water enhances the thermally induced changes in protein solubility. Similar results were observed for egg and peanut proteins when spray-dried whole egg and peanut flours were subjected to both moist- and dry-heat (Cabanillas et al., 2012; Fu & Maks, 2013; Fu et al., 2010).

A similar pattern was observed for the autoclaved samples. While there was little change in protein solubility when dry almond powder samples underwent autoclave sterilization (121 °C and 0.15 MPa for 10 min), there was a clear decrease in protein recovery for samples autoclaved in PBS. The decrease in solubility of samples autoclaved with PBS was greater than those subjected to moist heat at 100 °C. This suggests the pressure and higher temperature associated with autoclaving accelerated the changes. The effect of water was also apparent in samples subjected to high-pressure treatment. Table 1 shows that high-pressure treatment in the absence of water had little effect on protein solubility. However, when samples were subjected to high pressure, in the presence of water, the solubility of almond protein

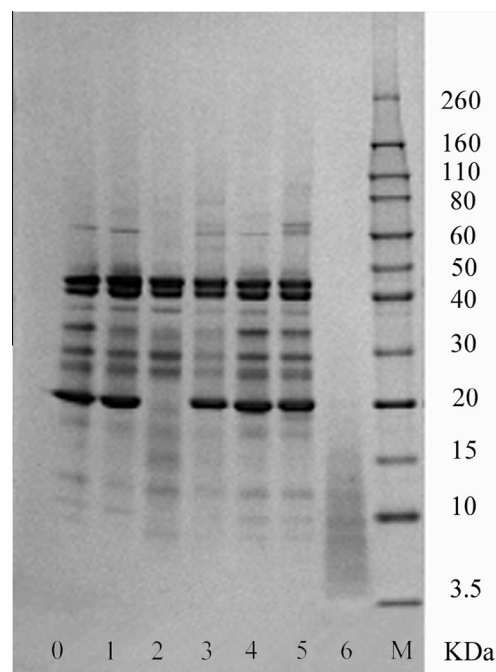


Fig. 1. SDS–PAGE results of almond proteins extracted from flours that were subjected to different heat treatments: M, marker (with MW 3.5–260 kDa); 0, unprocessed; 1, moist heat 60 °C; 2, moist heat 100 °C; 3, dry heat 100 °C; 4, dry heat 200 °C; 5, dry heat 250 °C; 6, dry heat 400 °C.

Table 2

Effect of food processing on almond protein immunoreactivity ($n = 3$).

Food processing	Relative IC_{50} (mean $\pm$ SD)	CV (%)	Food processing	Relative IC_{50} (mean $\pm$ SD)	CV (%)
Unprocessed	1.00 ^d		Unprocessed	1.00 ^d	
Dry heat (°C)			High pressure (MPa)		
100	1.20 $\pm$ 0.21 ^d	17.7	400 (dry flour)	1.28 $\pm$ 0.12 ^d	9.06
200	0.81 $\pm$ 0.010 ^d	12.1	400 (with PBS)	5.68 $\pm$ 0.36 ^c	6.41
250	4.36 $\pm$ 0.25 ^c	5.67	500 (dry flour)	1.14 $\pm$ 0.10 ^d	9.06
400	–	–	500 (with PBS)	41.5 $\pm$ 0.34 ^b	0.82
Moist heat (°C)					
60	0.75 $\pm$ 0.14 ^d	18.5			
100	26.8 $\pm$ 0.41 ^b	1.53	580 (dry flour)	1.19 $\pm$ 0.14 ^d	11.8
Autoclave sterilization			580 (with PBS)	74.4 $\pm$ 0.43 ^a	0.58
Dry flour	0.85 $\pm$ 0.15 ^d	17.6			
With PBS	70.3 $\pm$ 0.12 ^a	0.167			

– : cannot detect.

Relative IC_{50} values were statistically analyzed by one way ANOVA (Duncan). Values with the same letters in the same column are not significantly different ($p > 0.05$).

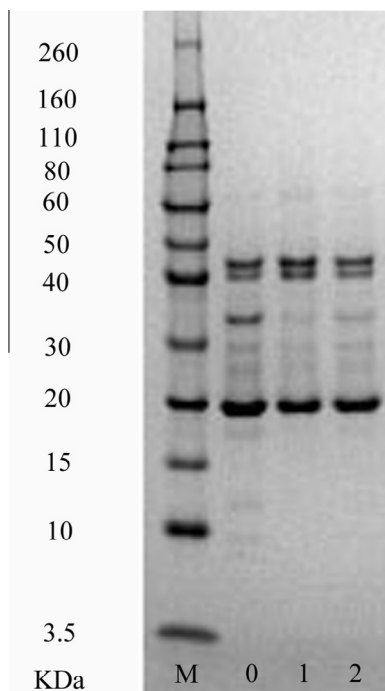


Fig. 2. SDS–PAGE results of almond proteins extracted from control and autoclaved samples: M, marker (with MW 3.5–260 kDa); 0, unprocessed; 1, sterilization without PBS; 2, sterilization with PBS.

was greatly reduced, with lower solubility observed in samples treated with higher pressures.

3.2. Effect of food processing on immunoreactivity of almond proteins

The antibody used in the IC-ELISA assays was raised against native amandin and, therefore, changes in IC_{50} values, as a result of heat and/or high-pressure treatments, might indicate changes in the immunoreactivity of amandin. Table 2 shows the relative IC_{50} values for the almond protein extract were not affected significantly by dry heat for 10 min at 100 or 200 °C ($p > 0.05$), indicating that almond proteins are stable under these dry heat conditions. This is consistent with the results reported previously where

amandin was readily detected in almonds that were subjected to dry heat at temperatures below 200 °C (Sathe & Sharma, 2009). After dry heat for 10 min at 400 °C, the sample was unable to bind with the antibody, consistent with the lower protein recovery (Table 1). Therefore, dry heat at 400 °C might provide a mean to reduce allergen residues on surfaces and minimize allergen cross contact during food production.

Moist heating at 100 °C (i.e., boiling) for 10 min lowered the immunoreactivity of almond extract approximately 27-times (Table 2), and this change was much greater than the corresponding change in protein recovery. While the solubilities presented in Table 1 reflect almond protein as a whole, the immunoreactivity measured only represent that of amandin, as the antibody was raised specifically against amandin. Boiling might affect the conformational epitopes of amandin, affecting the binding of the protein with the antibody. Albillos et al. (2009) found that amandin formed aggregates after heating in PBS at temperatures >77 °C. These aggregates might bind to the antibody differently compared with the native amandin and, therefore, generated higher IC_{50} values and decreased immunoreactivity. Such a decrease in binding between amandin-specific antibody and thermally treated almond proteins agrees with the observation that an ELISA test kit (ELISA Systems Almond Residues™), which specifically targets amandin, under-estimated the amount of almond allergens in boiled or autoclaved samples (Chang & Fu, 2012).

IC_{50} values for samples autoclaved with PBS were much greater than those of boiled samples (i.e., moist heated at 100 °C), suggesting the higher temperature and pressure applied during autoclaving resulted in a greater loss of the immunoreactivity. A similar trend was observed for samples subjected to high-pressure treatment at ~20 °C. Table 2 shows that IC_{50} values were similar for samples subjected to high-pressure treatment in the absence of PBS ($p > 0.05$). Treatment in the presence of PBS, especially at 500 and 580 MPa, decreased the IC_{50} values by 42- and 74-times, respectively. As with the autoclaved samples, the immunoreactivity of the almond proteins showed greater sensitivity to high-pressure treatment than protein solubility.

3.3. SDS–PAGE analysis

Fig. 1 shows the protein/peptide profiles for dry- and moist-heat treated samples. Most bands remained unchanged in samples

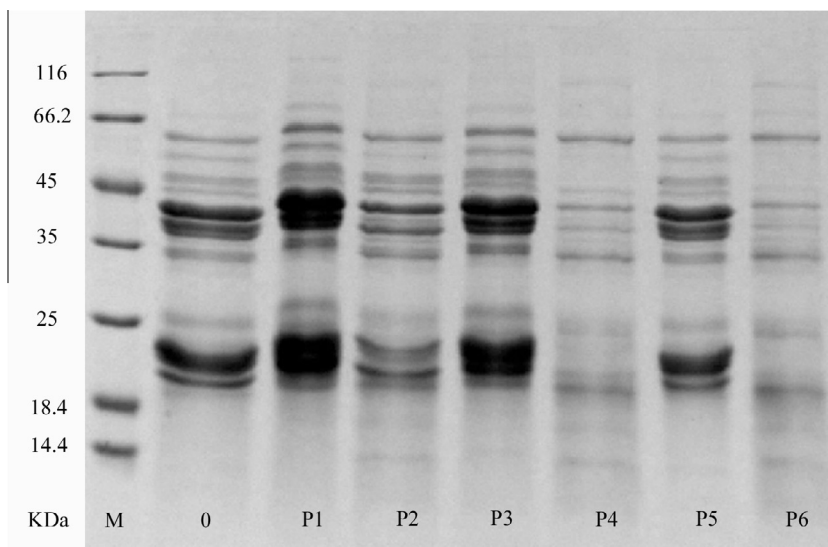


Fig. 3. SDS–PAGE results of almond proteins extracted from control and high-pressure treated samples: M, marker (with MW 14.4–116 kDa); 0, unprocessed; P1, 400 MPa; P2, 400 MPa with PBS; P3, 500 MPa; P4, 500 MPa with PBS; P5, 580 MPa; P6, 580 MPa with PBS.

subjected to dry heat at 100, 200 and 250 °C compared with those of the unprocessed sample. Characteristic amandin bands (20–22 and 40–42 kDa) (Albillos et al., 2009) were absent from the dry heated samples at 400 °C. There remained only a few light bands of MW <15 kDa, which may not be recognized by the antibody. Boiling caused the breakdown of the 20–22 kDa bands while the 40–42 kDa subunits did not appear to be affected. It is possible that the breakdown of the 20–22 kDa basic subunit affected significantly binding with the anti-amandin antibody and caused or is otherwise related to the decrease in the immunoreactivity of boiled samples (Table 2).

Fig. 2 shows SDS–PAGE analyses of almond proteins after autoclave sterilization. The protein profiles of the samples autoclaved without PBS were similar to those of unprocessed samples. As with the boiled sample, the 20–22 kDa bands were absent from the samples autoclaved with PBS, again indicating that this subunit is more susceptible to thermal denaturation in the presence of water.

SDS–PAGE profiles of almond proteins in samples subjected to high pressure at 400, 500, or 580 MPa without PBS remained unchanged (Fig. 3). Protein bands in samples subjected to high pressure in the presence of water were less intense as pressure increased. Both the 20–22 and 40–42 kDa subunits exhibited changes after high-pressure treatment leading to the changes observed in immunoreactivity (Table 2).

In conclusion, the synergistic effects of heat, pressure and water significantly changed the solubility and immunoreactivity of almond proteins. Almond flour samples exhibiting significant changes in protein recovery after thermal or high-pressure treatments also exhibited changes in immunoreactivity, indicating that thermal and high-pressure induced structural changes contributing significantly to the changes in the immunoreactivity of almond proteins; autoclave sterilization and high-pressure treatment, which are typically performed with liquid samples or samples with high water contents, might provide a means to reduce the antigenicity of almond proteins. Additional studies are needed to evaluate the impact of these processing methods in reducing almond allergenicity using sera from patients allergic to almond proteins, and determine whether similar results occur in other food matrices that contain almond proteins.

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