

Impact of γ -irradiation and thermal processing on the antigenicity of almond, cashew nut and walnut proteins

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Abstract: Whole unprocessed almonds, cashew nuts and walnuts were each subjected to γ -irradiation (1, 5, 10 and 25 kGy) followed by heat processing including autoclaving (121°C, 15 psi for 15 and 30 min), dry roasting (138 and 160°C for 30 min each, 168 and 177°C for 12 min each), blanching (100°C for 5 and 10 min), oil roasting (191°C, 1 min) and microwave heating (500 W for 1 and 3 min). Rabbit polyclonal antibodies were raised against each major protein isolated from defatted, but not subjected to γ -irradiation and/or any thermal processing, almond, cashew nut and walnut flours. Immunoreactivity of almond, cashew nut and walnut proteins soluble in borate saline buffer, normalised to 1 mg protein ml⁻¹ for all samples, was determined by inhibition enzyme-linked immunosorbent assay (ELISA) and Western blotting. ELISAs and Western blotting experiments indicated that almond, cashew nut and walnut proteins exposed to γ -irradiation alone or followed by various thermal treatments remained antigenically stable.

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Keywords: almond; cashew nut; walnut; processing; food allergy; ELISA; γ -irradiation; antigenicity; protein

INTRODUCTION

Tree nuts are globally popular and are valued for their desirable sensory and nutritional/health attributes. Many tree nuts are an excellent source of proteins, good-quality lipids and certain vitamins and minerals. Almonds, cashew nuts and walnuts are some of the most popular tree nuts in the USA and are increasingly used in a variety of bakery, confectionery and snack foods. Despite their economic and health importance, tree nuts are often implicated in permanent, and sometimes fatal, food allergies in sensitive individuals.^{1,2} In a voluntary registry for peanut and tree nut allergies, walnut (34% of total), cashew nut (20%) and almond (15%) were the three most commonly implicated tree nuts.³

Currently there are no treatments available for food-induced allergies. Therefore the best way to avoid reactions is to eliminate the offending food

from one's diet. Avoidance of the offending food/agent is, however, frequently not possible for a variety of reasons. The two most important reasons in this regard are improper food labelling and cross-contamination by offending agent(s). Often, mislabelling and/or cross-contamination(s) lead to expensive food recalls. A recent FDA report indicated that, of the reported 659 recalls in 1999, 236 (36%) products were recalled because they contained one or more undeclared allergens. The three principal factors that contributed to the presence of undeclared allergens were ingredient statement omissions and errors (51% of recalled products), manufacturing equipment cross-contaminations (40%) and errors by ingredient suppliers or manufacturing firm employees (5%). Interestingly, consumers were most responsible (56%) for identifying the presence of an undeclared allergen.⁴ It is therefore

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important to explore ways to eliminate/modify allergenic food proteins.

Irradiation has been reported to be effective in reducing antigenicity of ovalbumin and bovine serum albumin⁵ and allergenicity/antigenicity of milk protein.⁶ The reduction in allergenicity/antigenicity has been mainly attributed to irradiation-induced changes in protein structure, notably the secondary and higher-order structures. These studies also suggested that the linear epitopes (stretches of amino acid sequences relevant to allergenicity/antigenicity) remained unchanged. With its increased global acceptance as a method to improve food safety, food irradiation is a processing method that needs to be evaluated for its effectiveness in reducing/eliminating tree nut allergenicity.

In our earlier studies, almond,^{7,8} cashew nut^{9,10} and walnut^{11–14} proteins that are relevant to human allergies were identified. Specifically, almond major protein (AMP), the major storage protein in almonds,¹⁵ cashew major protein (CMP), the major storage globulin in cashew nuts,⁹ and walnut glutelin (WG), the major storage glutelin fraction in walnuts,¹⁶ were shown to contain reactive polypeptides relevant to human allergies. In those studies it was also demonstrated that rabbit polyclonal antibodies raised against AMP, CMP and WG bind with most of the same polypeptides that were recognised by IgE from patients with pronounced and specific allergies to almonds, cashew nuts and walnuts respectively, although walnut has not yet been well studied. Simple heat processing treatments do not reduce antigenicity of almond proteins^{7,17} and cashew nut proteins.¹⁸ It is, however, not known whether exposing tree nuts to irradiation treatment alone or in combination with other thermal treatments can reduce/eliminate tree nut allergenicity/antigenicity. The present investigation therefore used rabbit polyclonal antibodies raised against AMP, CMP and WG to assess the influence of γ -irradiation alone and in combination with several common thermal treatments on antigenic stability of almond, cashew nut and walnut proteins.

MATERIALS AND METHODS

Materials

Whole natural Nonpareil almonds (Blue Diamond Growers, Sacramento, CA, USA) and English walnuts (Diamond Walnut Growers, Stockton, CA, USA) were gifts. Raw natural unprocessed cashew nuts (kernels) were purchased from a local grocery store. Sources of electrophoresis chemicals and supplies have been reported earlier.¹⁹ Chemicals and supplies for rabbit antibody production, ELISA (enzyme-linked immuno sorbent assay) development and immunoblotting procedures have been reported earlier for almond,²⁰ cashew nut²¹ and walnut.²² All other chemicals were of analytical or better grade and were purchased from Fisher Chemical Co (Pittsburgh, PA, USA).

Tree nut processing

γ -Irradiation

Whole nuts were subjected to γ -irradiation (1, 5, 10 and 25 kGy) at Food Technology Service Inc (Mulberry, FL, USA). Each sample was irradiated in duplicate and then subjected to various thermal processing treatments as described below.

Thermal processing

Whole natural raw tree nuts (controls) and those subjected to γ -irradiation were processed as follows.

1. Pressure cooking in an autoclave at 121 °C and 15 psi for 15 and 30 min. Autoclaved samples were air dried at room temperature in a fume hood (until constant weight).
2. Blanching in boiling water (100 °C) for 5 and 10 min. The ratio of nut seeds to water was 1:10 w/v. Samples were patted dry on paper towels and further air dried at room temperature in a fume hood (until constant weight).
3. Frying in vegetable oil at 191 °C for 1 min. Excess oil was allowed to drain completely on paper towels prior to further handling.
4. Microwave heating in a Panasonic microwave oven (Panasonic Company, Secaucus, NJ, USA) at 50% power (500 W) for 1 and 3 min.
5. Dry roasting at 138 and 160 °C for 30 min each and at 168 and 177 °C for 12 min each. Samples were placed in crucibles and subjected to roasting in an oven previously set at the desired temperature (Thermolyne Corporation, subsidiary of Sybron Corporation, Dubuque, IA, USA).

Unprocessed nut samples (no γ -irradiation or any thermal treatment) were used as controls. All samples, processed and unprocessed, were stored at 4 °C in airtight plastic bottles until further use.

Preparation of nut flours, protein extracts and reference proteins

All samples were powdered manually using a mortar and pestle to about 40 mesh and the resultant flours were defatted for 6 h using a Soxhlet apparatus (Fisher Scientific Co, Orlando, FL, USA) with petroleum ether as solvent (flour/solvent ratio 1:10 w/v, boiling point range 38.2–54.3 °C). Defatted flours were air dried in a fume hood, powdered again using a mortar and pestle (to obtain a homogeneous sample) and stored in screw-capped plastic vials at 4 °C until further use.

Buffered saline borate (BSB; 0.1 M H₃BO₃, 0.025 M Na₂B₄O₇, 0.075 M NaCl, pH 8.45) was used to extract proteins from defatted flours. Typically, the defatted flours were extracted with BSB (flour/buffer ratio 1:50 w/v) at 25 °C for 1 h with vortexing at 10 min intervals. The samples were centrifuged in a tabletop microcentrifuge for 10 min (13 600 × *g*) and the collected supernatants were stored at –20 °C until further use.

Reference proteins AMP,²⁰ CMP²³ and WG²⁴ were prepared as described previously.

Rabbit polyclonal antibody production

Rabbit polyclonal antibodies to the reference proteins AMP, CMP and WG were produced by giving an initial injection of 0.5 mg of antigen in Freund's complete adjuvant. After boosting three times (at 0.5 mg per boost in incomplete Freund's adjuvant at 2–3 week intervals), the rabbits were bled and the resultant sera were stored at -20°C until further use.

Electrophoresis and Western blotting

Electrophoresis and Western blotting experiments were done as described earlier.⁸ Proteins were subjected to Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE; 8–25% linear monomer acrylamide gradient) and transferred onto 0.2 μm nitrocellulose (NC) paper according to the method of Towbin *et al.*²⁵ Unbound sites on the NC paper were blocked using 5% non-fat dry milk (NFDM) in Tris-buffered saline (TBS-T; 10 mM Tris, 0.9% (w/v) NaCl, 0.05% (v/v) Tween 20) for 2 h. The blots were washed twice, 5 min each, in TBS-T and then incubated with rabbit antiserum ($1:10^4$ v/v dilution) in TBS-T for 1 h. NC sheets were rinsed twice with TBS-T and then washed once for 15 min followed by three 5 min washes with TBS-T. The blots were then incubated for 1 h with horseradish peroxidase (HRP)-labelled goat anti-rabbit antibody diluted ($1:40\,000$ v/v) in TBS-T. The blots were washed as before and developed using a luminol/*p*-coumaric acid system and Kodak X-O-MAT photo film.

Competitive inhibition ELISA

Competitive ELISAs were performed as described previously,⁸ with some modifications. A 96-well polyvinyl microtitre plate was coated with reference antigen (AMP, CMP or WG) by adding 50 μl per well of antigen solution ($10\,\mu\text{g ml}^{-1}$ in citrate/phosphate coating buffer (pH 5, 48.5% 0.1 M citric acid, 51.5% 0.2 M Na_2HPO_4)) and incubated at 37°C for 1 h. Wells were washed and blocked with 100 μl of 0.5% bovine serum albumin (BSA) in 0.05% Tween 20 and 1 mM ethylene diamine tetraacetic acid (EDTA) in phosphate-buffered saline (PBS; 10 mM, pH 7.2) for 1 h at 37°C . Primary antibody (rabbit polyclonal antibodies) was diluted ($1:10^5$ v/v) in 0.1% BSA in BSB and 54 μl of diluted primary antibody solution was added to each well of a second uncoated plate. Proteins extracted from unprocessed as well as processed samples were used as soluble inhibitor. Soluble inhibitor (6 μl per well at $1\,\text{mg ml}^{-1}$) was serially diluted (10-fold) into the antiserum and incubated for 1 h at 37°C . A 45 μl aliquot of the mixture from these wells was transferred to the coated plate and incubated for an additional 1 h at 37°C . The plates were then washed with BSB buffer three times (5 min each) and developed using alkaline phosphatase (AP)-labelled goat anti-rabbit IgG for 1 h at 37°C and phosphatase

substrate (50 μl of 1 mg *p*-nitrophenyl phosphate (PNPP) ml^{-1} alkaline phosphatase substrate buffer). The alkaline phosphatase buffer comprised 48 ml of diethanolamine and 24.5 mg of MgCl_2 in a final volume of 500 ml adjusted with distilled water after adjusting the pH of the solution to 9.8 with 5 M HCl. Reactions were stopped by adding 50 μl of 3 M NaOH. Plates were read at 405 nm using an ELISA reader (Power Wave 200, Bio-Tek Instruments Inc, Riverton, NJ, USA), and 50% inhibitory concentration (IC_{50}) values were calculated for all samples. The appropriate reference protein (AMP, CMP or WG) was used in a corresponding plate as a control. All experiments were performed in duplicate and each sample was analysed two or three times. Data are reported as mean $\pm$ standard error of mean (SEM).

Protein determination

Soluble proteins were determined by the method of Lowry *et al.*²⁶ BSA was used as the standard protein. BSA standard curves were prepared (for each assay) in appropriate buffer, and suitable blanks were used in all assays.

Statistical analyses

Appropriate data were analysed for statistical significance using ANOVA (SPSS Inc, Chicago, IL, USA) and Fisher's least significant difference (LSD, $p = 0.05$) test.

RESULTS AND DISCUSSION

Food processing treatments often cause some protein denaturation that can lead to some loss of protein solubility. The extent of loss of protein solubility depends on the severity and duration of processing. To compensate for the decrease in protein solubility, all protein extracts were normalised to $1\,\text{mg ml}^{-1}$ in BSB prior to inhibition ELISAs. However, normalising protein concentration does not necessarily relate to the qualitative profile of protein polypeptides. Western blotting experiments were therefore included to visualise the stability of protein polypeptide profiles for processed samples when compared with the corresponding controls.

Almond

γ -Irradiation treatment alone or in combination with autoclaving, blanching, frying, microwaving or roasting treatments did not qualitatively alter the protein profile (Fig 1) or significantly change the IC_{50} value when compared with the control (Table 1), indicating an antigenic stability of the almond proteins. These data are consistent with our earlier results on antigenic stability of almond proteins.^{7,17} The data, taken together with our previously published results,^{7,15} also indicate that AMP is not only an excellent marker protein for detection of trace amounts of raw and unprocessed almonds (regardless of the genotype/hybrid) but is also a reliable marker for

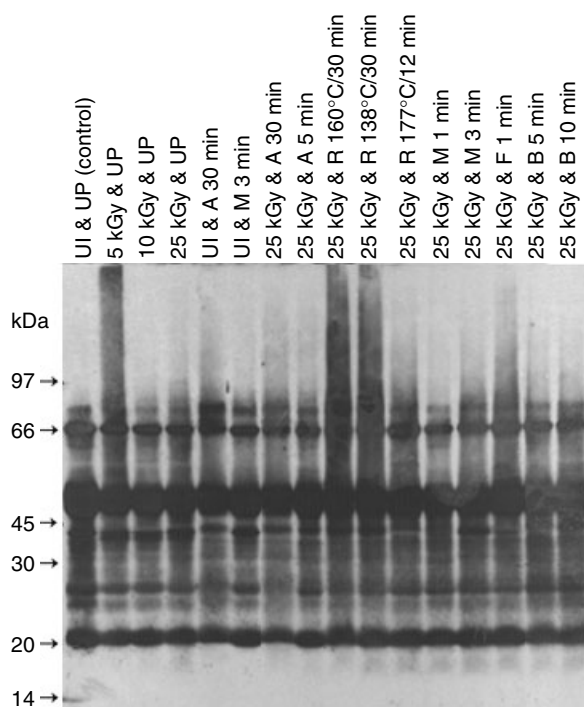


Figure 1. Western blot of almond samples probed with rabbit anti-AMP polyclonal antibodies. Protein load in each sample lane was 30 µg. Legends: UI, unirradiated; UP, unprocessed (thermal); A, autoclaving; B, blanching; F, frying in oil; M, microwave heating; R, roasting.

detection of trace amounts of processed almonds as well. The Western blotting profile and SDS-PAGE profile of the extracted almond proteins were similar (data not shown). The similarity of the SDS-PAGE and Western blotting profiles is consistent with our earlier observations^{7,8,17,20} and was expected, since AMP is the major storage protein in almonds and accounts for almost 65% of total seed proteins.

Similar findings were also noted for cashew nut and walnut protein SDS-PAGE and corresponding Western blotting profiles, as CMP (~50% of total seed proteins) and WG (~70% of total seed proteins) account for the major portion of cashew nut and walnut storage proteins respectively.

Cashew nut

Inhibition ELISAs of cashew nut proteins also indicated stability of cashew nut proteins towards the aforementioned processing methods (Table 2). The only exceptions to this were the samples exposed to 25 kGy dose followed by 30 min autoclave treatment (Fig 2, lane 7) or roasting at 160 °C for 30 min (Fig 2, lane 9), where a significant qualitative difference in the extractable polypeptide profile (compared with the control) was seen. The difference in polypeptide profile did not, however, diminish our qualitative ability to detect the presence of cashew proteins using inhibition ELISA. The polypeptide band (~50 kDa, indicated by the solid arrow in the right-hand margin) is the Ana o 1 allergen (a vicilin group protein) in cashew nut¹⁰ that is not only recognised by the rabbit polyclonal antibodies but is also recognised by human serum IgE from patients known to be allergic to cashew nuts. An additional band (~33 kDa, indicated by the open arrow in the right-hand margin) that remains stable to all the processing methods investigated belongs to the legumin-type (11S) allergen, termed Ana o 2, that we have recently shown to be a major allergen in cashew nut.²⁷ The linear epitopes recognised by human allergic sera have been mapped for both allergens (Ana o 1 and Ana o 2). The antigenic stability of CMP polypeptides towards commonly encountered food processing methods suggests that CMP can also serve as an excellent marker protein for the purpose of

Table 1. Effect of γ -irradiation and thermal processing on antigenicity of almond proteins^a

Thermal processing	γ -Irradiation dose				
	Unirradiated	1 kGy	5 kGy	10 kGy	25 kGy
Unprocessed	1.00 ± 0.08	1.45 ± 0.17	1.80 ± 0.22	1.82 ± 0.13	1.80 ± 0.13
Autoclaving (121 °C)					
15 min	0.91 ± 0.59	0.65 ± 0.31	0.63 ± 0.35	0.64 ± 0.42	0.83 ± 0.43
30 min	1.38 ± 0.42	1.41 ± 0.11	1.16 ± 0.33	1.51 ± 0.10	1.11 ± 0.14
Blanching (100 °C)					
5 min	0.36 ± 0.12	0.36 ± 0.12	0.34 ± 0.06	0.30 ± 0.08	0.90 ± 0.35
10 min	0.92 ± 0.40	0.85 ± 0.32	1.12 ± 0.66	0.89 ± 0.31	0.53 ± 0.02
Frying (191 °C)					
1 min	0.73 ± 0.11	0.73 ± 0.28	0.79 ± 0.27	0.48 ± 0.00	0.54 ± 0.03
Microwave (500 W)					
1 min	0.51 ± 0.17	0.58 ± 0.27	0.49 ± 0.18	0.68 ± 0.33	0.67 ± 0.23
3 min	0.61 ± 0.25	0.55 ± 0.24	0.61 ± 0.41	0.86 ± 0.54	0.98 ± 0.67
Roasting					
138 °C/30 min	0.86 ± 0.23	0.87 ± 0.29	0.74 ± 0.16	0.77 ± 0.21	0.48 ± 0.10
160 °C/30 min	0.83 ± 0.11	1.90 ± 1.23	1.01 ± 0.44	0.91 ± 0.35	1.52 ± 0.93
168 °C/12 min	1.48 ± 0.74	1.28 ± 0.10	1.07 ± 0.19	1.08 ± 0.06	1.24 ± 0.44
177 °C/12 min	0.90 ± 0.34	0.88 ± 0.23	0.75 ± 0.27	0.57 ± 0.07	0.72 ± 0.28

^a Data are expressed as ratio of IC₅₀ of processed/IC₅₀ of unprocessed samples (mean ± SEM). Least significant difference (LSD) = 1.02 ($p = 0.05$, $n = 4$). Differences between two means exceeding LSD value are significant.

Table 2. Effect of γ -irradiation and thermal processing on antigenicity of cashew nut proteins^a

Thermal processing	γ -Irradiation dose				
	Unirradiated	1 kGy	5 kGy	10 kGy	25 kGy
Unprocessed	1.00 $\pm$ 0.11	1.05 $\pm$ 0.11	1.13 $\pm$ 0.04	1.02 $\pm$ 0.09	1.06 $\pm$ 0.03
Autoclaving (121 °C)					
15 min	2.32 $\pm$ 0.34	2.41 $\pm$ 0.03	2.24 $\pm$ 0.05	2.33 $\pm$ 0.90	3.22 $\pm$ 1.02
30 min	7.95 $\pm$ 4.12	3.71 $\pm$ 0.30	4.52 $\pm$ 1.55	10.93 $\pm$ 0.73	10.94 $\pm$ 4.35
Blanching (100 °C)					
5 min	1.69 $\pm$ 0.19	1.69 $\pm$ 0.19	1.80 $\pm$ 0.34	2.03 $\pm$ 0.59	2.32 $\pm$ 0.24
10 min	1.76 $\pm$ 0.45	1.48 $\pm$ 0.00	1.70 $\pm$ 0.23	2.45 $\pm$ 0.17	3.33 $\pm$ 0.61
Frying (191 °C)					
1 min	10.34 $\pm$ 1.76	10.34 $\pm$ 6.91	10.36 $\pm$ 4.95	6.98 $\pm$ 0.00	8.07 $\pm$ 3.94
Microwave (500 Watts)					
1 min	1.05 $\pm$ 0.08	1.03 $\pm$ 0.21	0.90 $\pm$ 0.17	0.98 $\pm$ 0.05	1.64 $\pm$ 0.04
3 min	0.72 $\pm$ 0.27	1.10 $\pm$ 0.18	1.01 $\pm$ 0.33	1.84 $\pm$ 0.95	1.05 $\pm$ 0.05
Roasting					
138 °C/30 min	4.10 $\pm$ 2.43	2.15 $\pm$ 0.05	2.92 $\pm$ 0.60	3.35 $\pm$ 0.41	3.00 $\pm$ 0.66
160 °C/30 min	136.75 $\pm$ 1.31	170.69 $\pm$ 33.71	96.78 $\pm$ 86.46	156.84 $\pm$ 151.47	1315.28 $\pm$ 437.72
168 °C/12 min	3.49 $\pm$ 0.82	2.93 $\pm$ 1.43	2.93 $\pm$ 2.00	3.13 $\pm$ 1.21	2.11 $\pm$ 0.66
177 °C/12 min	2.74 $\pm$ 0.21	2.07 $\pm$ 0.34	3.24 $\pm$ 1.63	3.44 $\pm$ 2.05	11.16 $\pm$ 3.50

^a Data are expressed as ratio of IC₅₀ of processed/IC₅₀ of unprocessed samples (mean $\pm$ SEM). Least significant difference (LSD) = 172.56 (p = 0.05, n = 4). Differences between two means exceeding LSD value are significant.

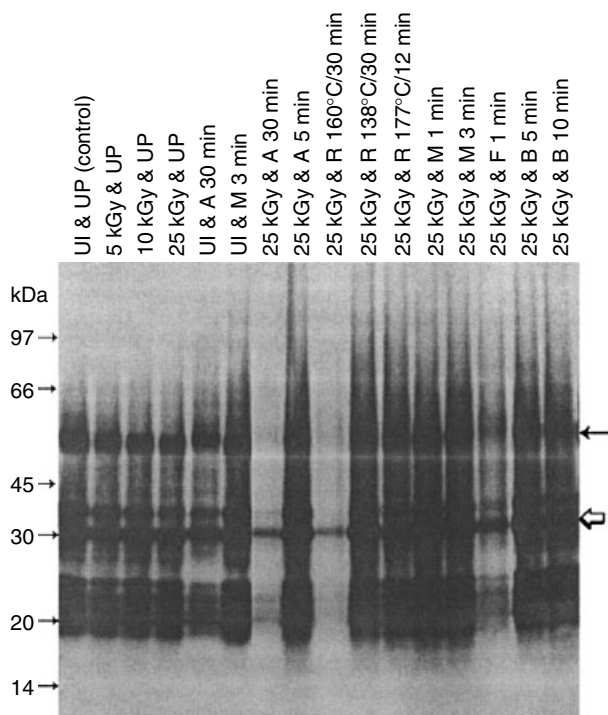


Figure 2. Western blot of cashew nut samples probed with rabbit anti-CMP polyclonal antibodies. Protein load in each sample lane was 30 μ g. Legends: UI, unirradiated; UP, unprocessed (thermal); A, autoclaving; B, blanching; F, frying in oil; M, microwave heating; R, roasting.

detection of trace amounts of cashew nuts in foods and food products. We have already developed a sandwich ELISA using rabbit anti-CMP and goat anti-whole extract polyclonal antibodies for this purpose.²⁸

Walnut

Similar to almond and cashew nut proteins, walnut proteins were also antigenically stable when subjected

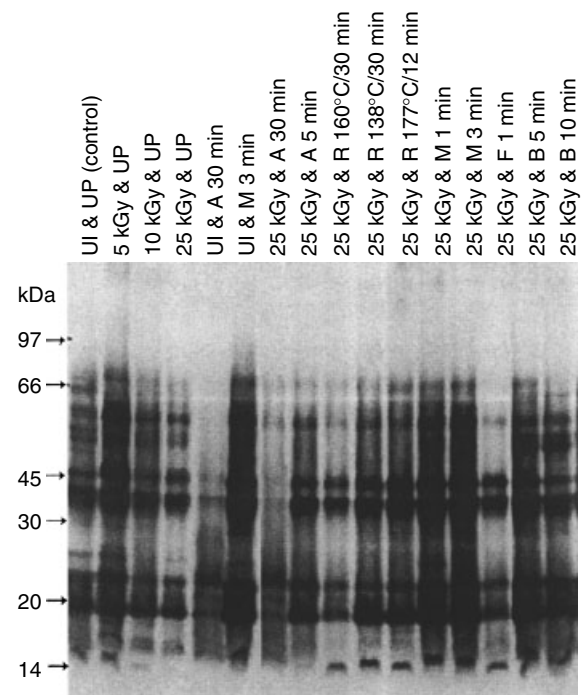


Figure 3. Western blot of walnut samples probed with rabbit anti-WG polyclonal antibodies. Protein load in each sample lane was 30 μ g. Legends: UI, unirradiated; UP, unprocessed (thermal); A, autoclaving; B, blanching; F, frying in oil; M, microwave heating; R, roasting.

to the various processing treatments investigated, as indicated by the stability of polypeptide profiles (Fig 3). With the exception of 25 kGy + 30 min autoclave treatment (Table 3), none of the IC₅₀ values were significantly different from those of the control. Western blotting analysis (Fig 3, lane 7) indicated a decrease in the intensity of major polypeptide bands of ~42–45 kDa; however, such a decrease in intensity for polypeptides of 20–25 kDa was not observed.

Table 3. Effect of γ -irradiation and thermal processing on antigenicity of walnut proteins^a

Thermal processing	γ -Irradiation dose				
	Unirradiated	1 kGy	5 kGy	10 kGy	25 kGy
Unprocessed	1.00 $\pm$ 0.12	1.33 $\pm$ 0.39	2.18 $\pm$ 0.93	2.22 $\pm$ 0.92	2.07 $\pm$ 0.75
Autoclaving (121 °C)					
15 min	4.00 $\pm$ 1.43	6.16 $\pm$ 0.97	8.30 $\pm$ 5.51	1.03 $\pm$ 0.07	4.77 $\pm$ 3.50
30 min	10.68 $\pm$ 3.40	23.30 $\pm$ 1.52	33.61 $\pm$ 5.26	11.10 $\pm$ 4.98	46.03 $\pm$ 27.56
Blanching (100 °C)					
5 min	1.42 $\pm$ 1.00	1.42 $\pm$ 1.00	1.43 $\pm$ 0.76	1.55 $\pm$ 0.65	0.48 $\pm$ 0.33
10 min	1.71 $\pm$ 0.00	0.84 $\pm$ 0.12	0.98 $\pm$ 0.11	0.94 $\pm$ 0.26	0.96 $\pm$ 0.02
Frying (191 °C)					
1 min	0.92 $\pm$ 0.15	0.92 $\pm$ 0.13	0.71 $\pm$ 0.24	1.04 $\pm$ 0.00	1.24 $\pm$ 0.50
Microwave (500 watts)					
1 min	1.05 $\pm$ 0.12	1.34 $\pm$ 0.04	1.02 $\pm$ 0.46	0.77 $\pm$ 0.36	1.01 $\pm$ 0.18
3 min	1.19 $\pm$ 0.49	0.99 $\pm$ 0.50	1.37 $\pm$ 0.84	1.01 $\pm$ 0.42	0.68 $\pm$ 0.14
Roasting					
138 °C/30 min	1.46 $\pm$ 0.75	0.62 $\pm$ 0.10	0.88 $\pm$ 0.30	0.63 $\pm$ 0.17	0.86 $\pm$ 0.05
160 °C/30 min	0.47 $\pm$ 0.21	0.61 $\pm$ 0.23	0.50 $\pm$ 0.07	0.60 $\pm$ 0.02	0.70 $\pm$ 0.51
168 °C/12 min	1.07 $\pm$ 0.17	0.71 $\pm$ 0.12	0.75 $\pm$ 0.20	0.66 $\pm$ 0.26	0.48 $\pm$ 0.04
177 °C/12 min	0.68 $\pm$ 0.02	0.67 $\pm$ 0.13	0.61 $\pm$ 0.05	0.41 $\pm$ 0.13	0.52 $\pm$ 0.16

^aData are expressed as ratio of IC₅₀ of processed/IC₅₀ of unprocessed samples (mean $\pm$ SEM). Least significant difference (LSD) = 10.83 (p = 0.05, n = 4). Differences between two means exceeding LSD value are significant.

We have reported earlier that walnut glutelin mainly comprises these same polypeptides²⁴ and that this fraction contains reactive polypeptides relevant to human allergies to walnuts.¹⁶

CONCLUSIONS

Western blotting experiments using rabbit polyclonal antibodies raised against AMP, CMP and WG recognised human allergy-relevant proteins in almonds, cashew nuts and walnuts respectively. The results of this study suggest that the antigenicity of almond, cashew nut and walnut proteins remains stable towards not only γ -irradiation (1–25 kGy) but also the combination of γ -irradiation and common thermal processing methods that these tree nuts are likely to encounter in commercial processing. The data also suggest that AMP, CMP and WG are excellent marker proteins for detection of almonds, cashew nuts and walnuts respectively in processed foods. The assays that can detect the AMP, CMP and WG in a sensitive, reproducible and accurate manner should therefore be useful in monitoring the presence of trace amounts of almonds, cashew nuts and walnuts in foods and food products.

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