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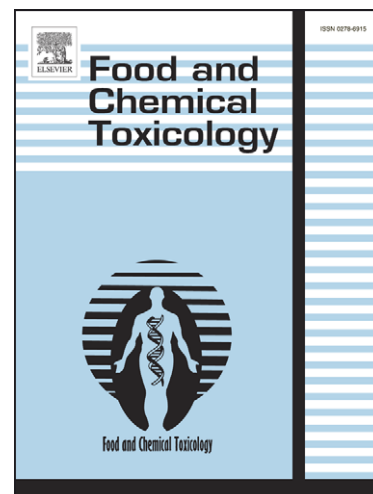
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**Low-dose gamma irradiation of food protein increases its
allergenicity in a chronic oral challenge**

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1 INTRODUCTION

Food allergy refers to a group of disorders with an IgE-mediated immune response to specific food proteins that affects between 5% of children and 2% of adults (Kagan, 2003). Food allergy has been an important and recurring health problem potentially due to the food consumed becoming increasingly processed (Taylor and Hefle, 2006). Most proteinaceous food allergens are thermostable and resistant to proteases belonging to different groups of plant proteins (Breiteneder and Ebner, 2000). Lectins, also known as plant agglutinins, are a class of proteins that bind to specific moieties of sugar on cells and glycoproteins (Lis and Sharon, 1998). Lectins are found in seeds, especially those of legumes. Some lectins are generally recognized as an important anti-nutrient of food (Shibasaki et al., 1998; Hamid and Masood, 2009). Concanavalin A (Con-A) is a lectin extracted from jack bean (*Canavalia ensiformis*) that has been extensively characterized and widely applied in biotechnology (Edelman et al., 1972; Saleemuddin and Husain, 1991).

Current approaches for the management of allergic disease are based on the removal of specific allergens avoidance diets, drug treatments and preventive measures (Sicherer, 2002). Unfortunately, treatment with medications such as antihistamines and mast cell stabilizers usually play an insignificant role in the treatment of gastrointestinal manifestations (Teitelbaum et al., 2002; Ngo and Furuta, 2005). Genetic engineering offers the opportunity to reduce or even eliminate the compounds in foods that cause allergies (Metcalf et al., 1996). Nevertheless, associated concerns about so-called cross-contact allergens cannot be eliminated by the incorporation of genetically modified foods. In addition, preventive measures and elimination of allergens are interdependent and has been a target of regulatory agencies around the world (FAO/WHO, 2001).

In this scenario, the development of effective methods to eliminate the antigenic properties of food allergens has been evaluated (Sathe et al., 2005). Among them food irradiation, which is applied to increase the safety and shelf life of foods, has been used (Su et al., 2004). Food irradiation can offer a wide range of benefits to the food industry and the consumer. In recent research, we have seen that radiation damages allergens directly by rupturing covalent bonds as a result of transfer of photon energy, or indirectly, by producing reactive oxygen species which can reduce allergenicity by compromising structure-function relationships (Vaz et al., 2012).

Animal models, despite their individual limitations, are useful tools for the study of allergic reactions in vivo (Helm, 2002). They have been instrumental in advancing our understanding of the pathophysiological mechanisms for assessing the risk of allergenicity of food products (Knippels and Penninks, 2002). Historically, classic food allergy models have been used to investigate allergenicity of food proteins (Helm, 2002). However, we still need to clarify with the use of other models, if a low dose of radiation attenuates or exacerbates the antigenicity of food allergens when compared to a high dose.

The aim of this study was to establish a chronic allergic mouse model with histological changes in mice and to use the model to examine whether a low dose of ionizing radiation is as effective as a high dose to eliminate the antigenicity of food protein. We monitored lymphocyte migration in the jejunum and representative cytokines of Con-A-induced allergy as well as the structural and molecular profile of irradiated food protein.

2 MATERIAL AND METHODS

2.1 Materials

The broad-range molecular weight protein standard, external fluorescence probe 4,4'-bis-1-Anilinonaphthalene 8-sulphonate (bis-ANS) and Concanavalin A from *Canavalia ensiformis* (Jack bean) Type IV were purchased from Sigma Chemical Co., USA. All solvents and other chemicals used were of analytical grade from Merck, Germany. All solutions were made with water purified by the Milli-Q system.

2.2 Irradiation of food protein

Con-A in phosphate buffer (pH 7.2) was lyophilized in borosilicate glass vials (16-125 mm) and then irradiated dry under atmospheric O₂ by a Gammacell 220 Excel 60 Co gamma ray irradiator (Ontario, Canada) using doses of 1.0, 10.0 and 25.0 kGy at a rate of 8.25 kGy/h. Each dose was analyzed after irradiation by the following methods.

2.3 Hemagglutinating activity and protein concentration

Hemagglutinating activity (HA), which was defined as the lowest sample dilution that showed hemagglutination, was evaluated as described by Correia & Coelho (1995). Specific HA (SHA) corresponded to the relationship between the HA and protein concentration measured according to Lowry et al. (1951) using bovine serum albumin (BSA) as a protein standard in the range of 0-500 µg/mL. The percentage of the

remaining SHA (%SHA_{REM}) was calculated according to the equation: %SHA_{REM} = (SHA)_{GM} / (SHA)_{G0} x 100, where G_M is the Con-A SHA at each radiation dose (1, 10 and 25 kGy) and G₀ is the SHA of non-irradiated Con-A (control).

2.4 SDS-PAGE

To detect any insoluble aggregates formed, the precipitate was submitted to gel electrophoresis after centrifugation. SDS-PAGE was performed according to Laemmli (1970). Protein samples were resolved on a 10% separating gel and stained with Coomassie blue.

2.5 Chromatography analysis

To detect fragmented protein, the supernatant was separated by RP-HPLC after centrifugation. Irradiated samples were submitted to reverse-phase chromatography on a C-4 column (Vydac-Protein Peptide Ultrasphere) performed on an HPLC system (Shimadzu LC-10AD-Tokyo, Japan) and monitored at 215 nm. The column was equilibrated with 0.1% TFA (solvent A) and eluted using 90% acetonitrile/10% H₂O/0.1% TFA (solvent B) in a non-linear gradient, where B = 0% at t = 5 minutes; B = 45% at t = 10 minutes; B = 50% at t = 30 minutes and B = 100% at t = 35 minutes.

2.6 Fluorescence and light scattering measurements

Intrinsic fluorescence and light scattering assays were performed on a spectrofluorometer (JASCO FP-6300, Tokyo, Japan). The fluorescence emission intensity of tryptophan from irradiated Con-A was measured in a rectangular quartz cuvette with a 1 cm path-length at 0.1 mg/mL in phosphate buffer, pH 7.2 (25°C). For intrinsic fluorescence measurements, the excitation was at 295 nm and emission was recorded from 305 to 450 nm, using 5 nm band pass filters for both excitation and emission. For light scattering measurements, the excitation was at 320 nm and emission was recorded from 300 to 340 nm. The light scattered was measured at 90° for the aggregation assays obtained from the area under the fluorescence spectra. The center of spectral mass (CM) was calculated according to Eq. (1): $CM = \sum I_{\lambda} F_{\lambda} / \sum F_{\lambda}$, where F_{λ} stands for the fluorescence emission at wavelength I_{λ} and the summation was carried out over the range of appreciable values of F .

2.7 Hydrophobic surface analysis

The hydrophobic surface was measured using the same conditions as employed for the intrinsic fluorescence experiment. Samples were transferred to a quartz cuvette and then mixed with 5 μ M bis-ANS. The fluorescence emission spectrum was obtained from 400 to 600 nm, with an excitation at 360 nm (Bhattacharyya et al., 2000).

2.8 Circular dichroism (CD) measurements

CD measurements were carried out on a spectropolarimeter (JASCO J-810, Tokyo, Japan). The instrument was calibrated with D-10-camphorsulfonic acid. The protein concentrations were the following: non-irradiated (5 μ M), 1 (5 μ M) and 25 kGy (11 μ M) in phosphate buffer, pH 7.2. After irradiation, the samples were centrifuged and the measurements were performed with the supernatant. CD spectra were measured in the far-UV range (190-250 nm) in 1 mm path-length quartz cuvette at 25°C. The data were averaged for 8 scans that were performed at a speed of 50 nm/min and collected in 0.5-nm steps. The baselines (buffer alone) were subtracted from the protein spectra. Results were expressed as mean residue ellipticity, $[\theta]$, defined as $[\theta] = \theta_{obs}/(10 \cdot C \cdot l \cdot n)$, where θ_{obs} is the CD in millidegrees, C is the protein concentration (M), l is the path-length of the cuvette (cm) and n is the number of amino acid residues assuming a mean number of 237 residues.

2.9 Differential scanning calorimetry (DSC) analysis

The thermal stability of native and irradiated Con-A was determined by Differential Scanning Calorimetry (DSC). Thermal transitions were monitored by heating using a Microcal VP-DSC micro-calorimeter (Northampton, MA, USA). Prior to all the measurements the buffers and protein solutions were degassed. Thermal transitions were by heating the sample from 5-100°C. A scan rate of 10°C/h was used. The resulting thermograms were analyzed by the ORIGIN DSC software that was provided by Microcal Inc. (Northampton, USA).

2.10 Sensitization and chronic antigen exposure

Female BALB/c mice (5 weeks old) obtained from Laboratório de Imunopatologia Keizo Asami (LIKA) of the Universidade Federal de Pernambuco were maintained under specific pathogen-free conditions and were given *ad libitum* access to food and water. The animals were kept in an environmentally controlled room, temperature $21 \pm 2^\circ\text{C}$, under a light/dark cycle of 12 hours. Requirements for care and handling of experimental animals according to international and Brazilian regulations (CEEA /

UFPE n° 23076.033254/2010-14) were met. All test substances were administered intragastrically by tube. Con-A was dissolved in 0.5 mL of 0.9% sterile saline.

BALB/c mice were immunized subcutaneously on day 0, 15 and 30 using 0.5 ml Con-A (10 µg/mL) dissolved in saline without use of an adjuvant (eight mice per group). Control animals were treated subcutaneously with 0.5 mL saline. Three days before starting the oral challenge in the mice, they were stimulated with the same dose intraperitoneally. During 28 days, mice were treated as follows: group A, immunized mice were treated with 1 mL saline/day; group B, immunized mice were treated with non-irradiated Con-A; group C and D immunized mice were treated with irradiated Con-A at 1 and 25 kGy, respectively. The dose of bean lectin (27 mg/kg body weight/day) was according to total dietary intake of lectins in human subjects consuming vegetarian diets - calculations based on data from Peumans and van Damme (1996).

2.11 Body weight and leukocyte evaluation

Body weight was determined before and after immunization and after oral challenge. The final body weight of each group was obtained from the means of the individual values and expressed in grams (g). Blood samples were obtained and placed into micro-blood tubes containing the anticoagulant ethylenediamine tetra-acetic acid (EDTA). Hematological indices were determined by an automated particle counter, random-access clinical hematologic analyzer Coulter STK-S (Hospital das Clínicas, UFPE, Recife, Brazil). Hematological indices were confirmed by optical microscopy, in which the morphology of leukocytes stained by the Giemsa method was observed.

2.12 Multiplex cytokine analysis

Multiple cytokine analysis kits were obtained by Genese Produtos Diagnósticos Ltda (São Paulo, SP, Brazil). Millipore multiscreen, 96-well filter plates (Bedford, MA, USA) were used for all multiplex cytokine kits. Assays were run in triplicate according to the manufacturers' protocols. Data were collected using the Milliplex Analyser 200 version 2.3 (Luminex, Austin, USA). Data analysis was performed using the Analyst software, version 3.1. A four-parameter regression formula was used to calculate the sample concentrations from the standard curves.

2.13 Histology

After 28 days of oral challenge, the mice were sacrificed by decapitation. The histopathological evaluation of organs (jejunum) of animals was performed with an optical microscope. After mounting, the preparations were evaluated using a video-microscopy system (MOTIC BA200 Microscope, digital camera with Motic 1000-1.3M Pixel USB 2.0).

2.14 Statistical analysis

Differences between treatment groups of *in vivo* experiments were performed using nonparametric test (Kruskal-Wallis test) followed by the *post hoc* Dunns test in GraphPrism® (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was established at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Structural analysis of food protein after irradiation

Low doses of ionizing radiation (up to 1 kGy) are usually applied to control food-borne pathogens, reduce microbial load and insect infestation, inhibit the germination of root crops, and extend the shelf-life of perishable produce for commercial purposes (FDA, 1997). It is possible, however, that the types of modification that food proteins may undergo during irradiation, including protein unfolding and aggregation, may not be observed at a low-dose range. Thus, the potential to affect structural stability, and hence the form in which allergens are presented to the immune system after a low dose of radiation compared with a high dose was investigated. The SHA of Con-A in its irradiated forms revealed major changes in activity at a high dose (Fig. 1a). At 1 kGy, the SHA was reduced by around 20%. The change in structural basis for selective sugar recognition and binding was affected at a high dose. Irradiation inhibits cell agglutination (Vaz et al., 2012) which may affect allergen binding to the gut and reduce the allergic effect.

In order to evaluate alteration in the structure of Con-A after irradiation, the samples were analyzed by SDS-PAGE. Non-irradiated Con-A showed protein bands at approximately 25, 13 and 11 kDa. As shown by Wang et al. (1971), N-terminal sequence determination of the lighter components indicated that they corresponded to the N-terminal portion (13 kDa) and the carboxyl terminal portion (11 kDa) of the intact protein. SDS-PAGE profiles show that a high dose of γ -radiation induces peptide bond cleavages which later undergo aggregation (Fig. 1b). At low-dose, even in the

denaturing conditions of SDS-PAGE, fragmentation and molecular aggregation were not observed. Non-irradiated Con-A was separated by RP-HPLC into three components, A1, A2 and A3 (intact protein). The reverse phase chromatography analysis revealed a profound change in the elution profile at 25 kGy, as indicated in Fig. 1c. No significant change was detected to Con-A after exposure to a low dose. As shown in Fig. 1d, the scattering spectra of irradiated Con-A showed a shape characteristic of proteins with a high degree of aggregation. This aggregation frequently occurs through inter-protein cross-linking reactions, and hydrophobic and electrostatic interactions after irradiation (Moon and Song, 2001; Lee and Song, 2002).

The phenomenon of the conformational stability of irradiated versus non-irradiated Con-A was investigated by fluorescence spectroscopy, circular dichroism and DSC. Since the intensity of the intrinsic fluorescence spectra of proteins is easily affected by many environmental factors, the center of spectral mass (CM) was used to reflect the structural integrity of the protein. The typical spectra are presented in Fig 2a. After irradiation at 1 kGy, no significant change was observed in the CM value. For samples irradiated at 25 kGy, the intrinsic fluorescence spectra had a red-shift of about 8 nm. These observations suggested that the inactivation of Con-A at a high dose was caused by irreversible changes in Con-A tertiary structure. Bis-ANS fluorescence was weakly observed in the non-irradiated Con-A. After high-dose irradiation, bis-ANS fluorescence increased, with its peak shifted to 497 nm and its intensity increased (Fig. 2b).

The CD spectrum recorded at 25°C of non-irradiated Con-A exhibited a typical shape of β proteins (Fig 3a). At a high dose, the shape of the CD spectra revealed that irradiated protein was dominated by a lack of β -sheet structures. This was reflected by the lack of single negative peak at around 222 nm. To elucidate the profile of non-native oligomers that still have β -sheet structures after low-dose irradiation, differential scanning calorimetry (DSC) experiments were performed. As shown in Fig. 3b, the heat flow versus temperature profile showed only one endothermic peak, suggesting that the thermal denaturation of non-irradiated Con-A, a tetrameric protein at pH 7.2; follows the dissociative mechanism of denaturation as $2N_2 \leftrightarrow D \rightarrow U$ (Lumry and Eyring, 1954). The main peak was centered at about 63°C. For Con-A irradiated at 1 kGy, the main peak was centered at about 57°C with a pre-transition at around 37°C. For samples irradiated at 25 kGy, the DSC was not run due to higher protein aggregation.

The occurrence of red-shift in the intrinsic fluorescence spectra and lack of native β -rich secondary structures in irradiated Con-A, indicates the accumulation of completely unfolded, fragmented peptides that strongly bind to bis-ANS and have a tendency to form insoluble amorphous aggregates after a high dose of irradiation. However, what is the real effect of aggregation on managing allergen risks? And if there is a risk, what kind of reaction is triggered? To this end, we decided to evaluate the effect of food irradiation on food allergens using a chronic food allergy model.

3.2 Treatment schedule

Currently, little is known about how irradiation may alter food allergens, and hence there is a need to systematically investigate the relationship between food protein allergenicity and the effect of food irradiation. However, we know that the decrease or increase of allergenicity can be caused by protein unfolding, mis-folding as well as by aggregation. Sanchez and Frémont (2003) have extensively reviewed the effects of food processing on the structural stability of food allergens and concluded that effects of protein-protein interactions (especially aggregation) are virtually unknown in attempting to reduce/eliminate food allergens. To investigate these effects, we describe a BALB/c mouse model of chronic food allergy that were given non-irradiated and irradiated Con-A. We monitored morphological changes and lymphocyte infiltration in the small intestine as well as body weight, blood leukocytes, cytokine secretions of IL-4, IL-5, eotaxin and rantes.

Table 1 shows the significant weight loss ($p < 0.05$) for non-irradiated and irradiated Con-A at 1 kGy of BALB/c mice after oral administration by 28 days. However, despite a significant difference ($p < 0.05$) for non-irradiated and control groups, there was no weight loss after sensitization to mice treated with Con-A irradiated at 25 kGy. The analysis of cytokines and chemokines revealed a significant increase ($p < 0.05$) for eotaxin but not significant for IL-4 and IL-5 in mice treated with non-irradiated Con-A when compared to the control group (Table 1). To our surprise, mice treated with Con-A irradiated at 1 kGy showed a significant increase ($p < 0.05$) in levels of eotaxin and IL-4 compared to animals treated with non-irradiated Con-A. While mice treated with Con-A irradiated at 25 kGy showed normal levels of cytokines compared to control animals.

The treatment of sensitized animals subjected to intragastric administration of non-irradiated Con-A showed a lymphocytic infiltrate in the stroma of the microvilli

and in the colon zone, where the crypts and the villi are mixed, when compared with untreated immunized mice (Fig. 4 a-b). High levels of leukocyte infiltration with eosinophils can also be found (Fig. 4 c-d). However, the jejunum from immunized animals treated with irradiated Con-A (1 kGy) showed a lymphocytic infiltrate filling completely the stroma of microvilli without a real definition of the tubular glands and submucosa (Fig. 5 a-b). On the other hand, in immunized animals treated with irradiated Con-A (25 kGy), infiltration with less intensity was observed (Fig. 5 c-d).

Classic findings of food allergy were observed in mice treated with native Con-A (non-irradiated). The weight loss, increase in cytokines IL-4, IL-5, eotaxin and leukocyte infiltration after administration on the gut has been previously observed (Gutierrez-Ramos et al., 1999; Sampson et al., 2001; Bischoff and Crowe, 2005). Food processing may help inactivate certain conformational epitopes on some, but not all, allergens and is unlikely to eliminate linear epitopes (Sathe et al., 2005). Food irradiation can be effective in eliminating allergens after a high dose, since fragmentation of the molecular structure was observed.

To our knowledge, this is the first direct demonstration of effects from irradiated food protein on a chronic food allergy model. The development of this phenotype shows lymphocytic infiltration, and it also leads to the persistence of silent inflammation within the gut. Moreover, all phenotypes mapped in this study were not expressed in allergens irradiated at high-dose. On the other hand, a low dose of radiation exacerbates the phenotype and can cause severe allergic inflammatory responses. This effect may be due to increased exposure of conformational and linear epitopes resulting from the formation of partially unfolded and aggregated species after irradiation. Further investigations into the impact of food irradiation on allergen structure and allergenic potential are still warranted. However, two areas that had been previously neglected were clarified in our study: 1) impact that food processing procedures have on allergenic potential 2) the way in which food irradiation may alter allergic reactions in sensitized animals. Yet, the exact mechanism, including interactions between chemokines and cytokines as well as conformational and linear epitope modifications, remains to be further investigated.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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Figure captions

Fig. 1 - Effect of γ -radiation on Con-A structure. (a) The percentage of remaining specific hemagglutination activity, %SHA_{REM}. (b) SDS-PAGE profile from irradiated Con-A. (MW) Molecular weight; (C) non-irradiated; (1S) precipitate 1kGy; (1P) soluble 1kGy; (25S) precipitate 25kGy; (25P) soluble 25kGy. Arrows indicate high-molecular-weight molecules. (c) Reverse phase chromatography in an HPLC system. (—) Non-irradiated and irradiated Con-A at (—) 1 and (—) 25 kGy. (d) Light scattering for the aggregation assays; excitation (320 nm) and emission (300–340 nm).

Fig. 2 - Intrinsic and bis-ANS fluorescence. (a) Center of spectral mass of intrinsic fluorescence; Lectin excitation at 295 nm and emission at 305-450 nm. (b) Center of spectral mass of bis-ANS fluorescence; excitation at 360 nm and emission at 400-600 nm.

Fig. 3 - Far-UV CD spectra and DSC thermograms. (A) CD spectra were measured in the far-UV range (190-250 nm) in 1 mm path-length quartz cuvette. () is given in degree squared centimeters per decimole. (■) Non-irradiated and irradiated Con-A at (Δ) 1 and (○) 25 kGy. (B) Con-A thermal denaturation monitored by DSC. The heating rate was 10°C/hour. The protein concentration was 1 mg/mL. (—) Non-irradiated and irradiated Con-A at (—) 1 kGy.

Fig. 4 - Photomicrograph of jejunal mucosa of untreated mice and mice treated with native Con-A. (a) Jejunum of immunized animals treated with saline: Note the lymphocytic infiltrate filling the stroma of microvilli (mv) and also around the tubular glands or crypts (cr). (b) High numbers of enterocytes (e) with a prominent striated cuticle. Central stroma with evident lymphocytic infiltration. (c) Jejunum of animal treated with non-irradiated Con-A: there is lymphocytic infiltrate in the stroma of the microvilli (mv) and in the colon zone (cz), where the crypts and the villi are mixed. (d) Leukocyte infiltration with numerous eosinophils (eo) in animals treated with non-irradiated Con-A.

Fig. 5 - Photomicrograph of jejunal mucosa of animals treated with irradiated Con-A. (a) Jejunum of animals treated with Con-A irradiated at a low dose (1 kGy): lymphocytic infiltrate is observed filling completely the stroma of microvilli (mv) and no definition of tubular glands (tb) and submucosa (sm) can be seen because the same infiltration (b). (c) Jejunum of animals treated with Con-A irradiated at a high dose (25 kGy) where the same infiltration already mentioned for the 1 kGy dose can be observed but with less intensity (d).

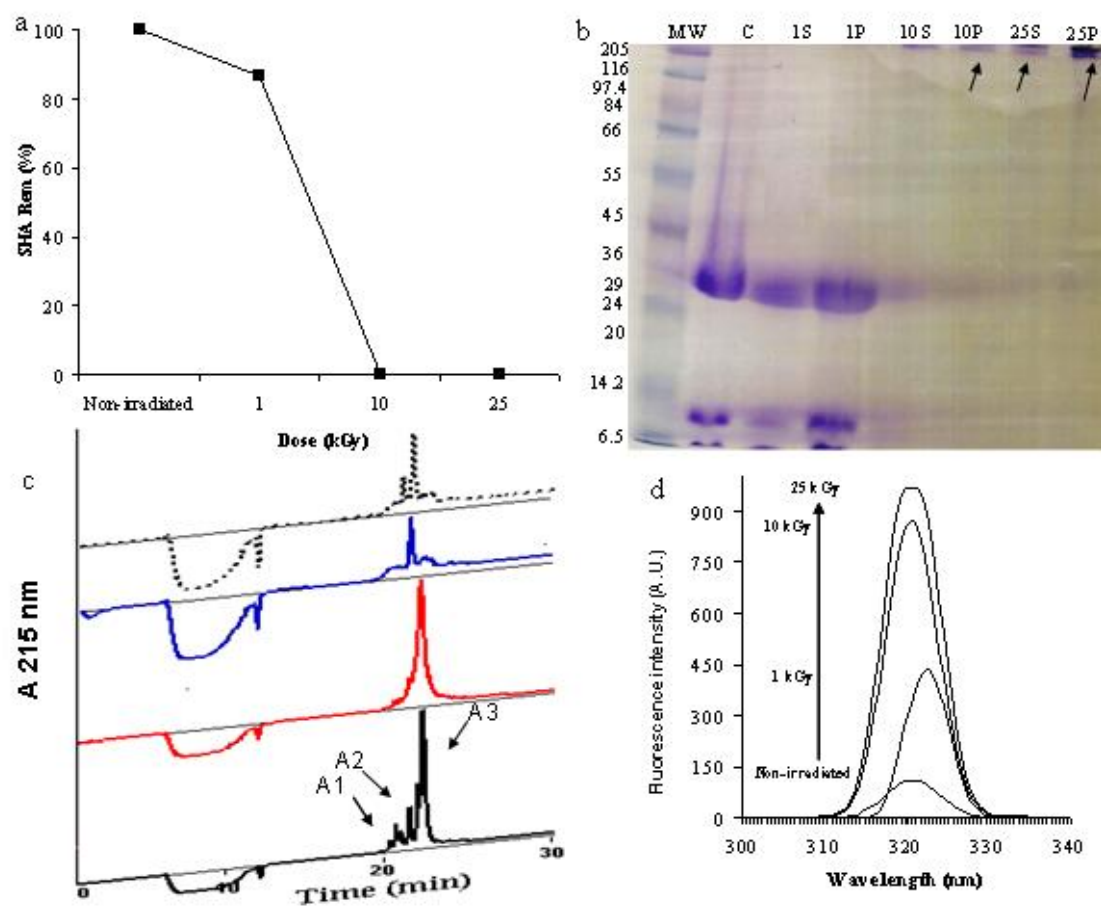


Figure 1

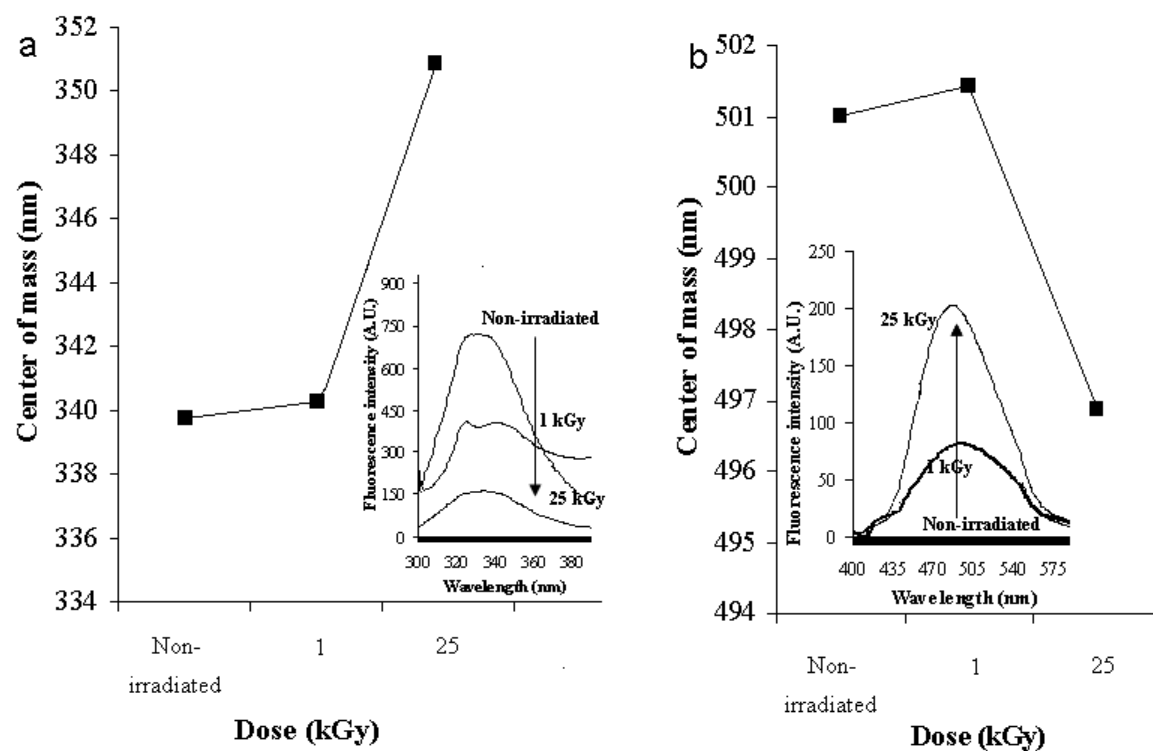


Figure 2

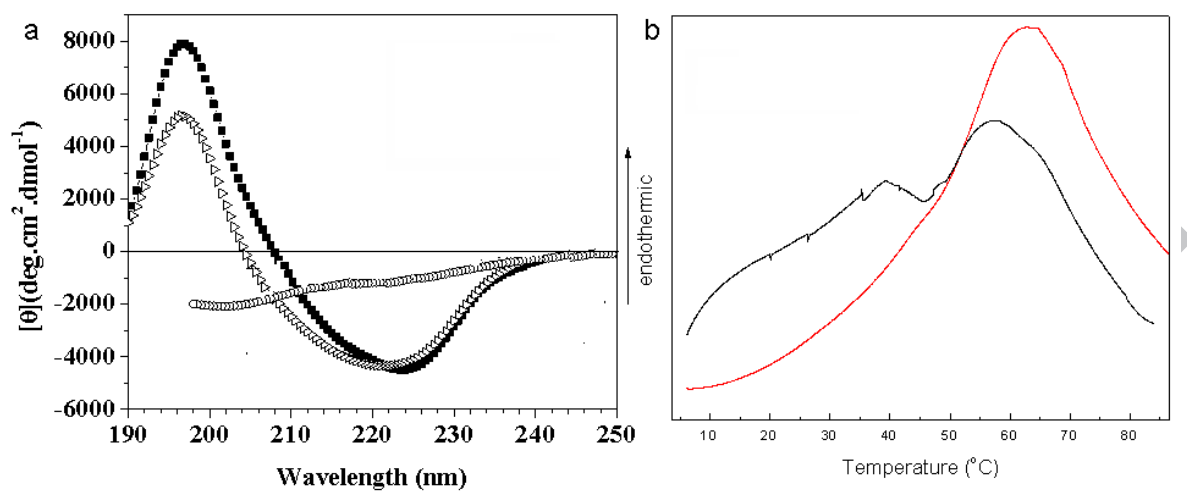


Figure 3

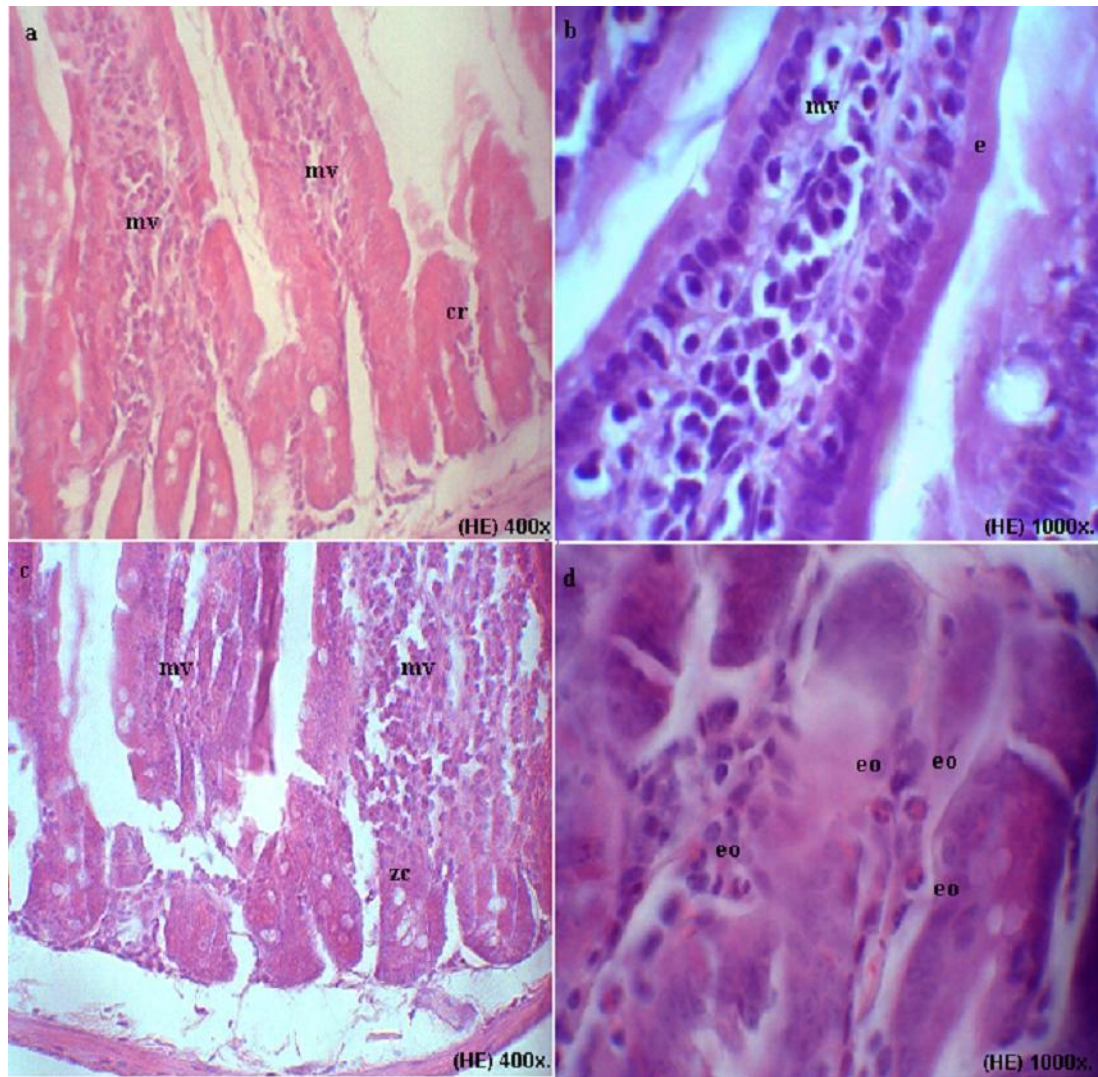


Figure 4

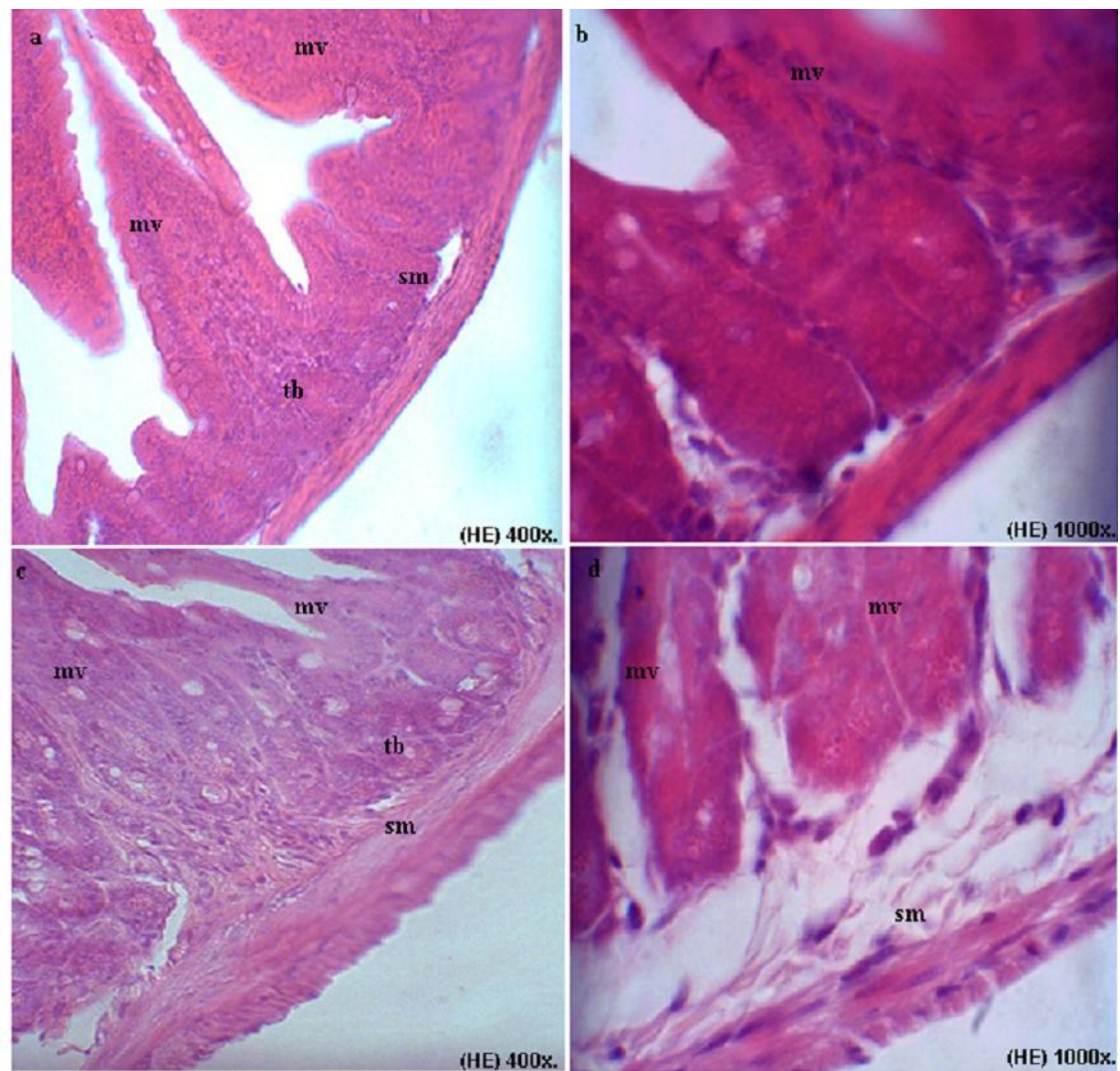


Figure 5

Table 1 - Body weight, leukocytes and cytokine profiles of chronic allergic mice model.

Weight (g)	Groups			
	Go	G1	G2	G3
Initial	34.7 ± 1.0	28.1 ± 2.1	28.8 ± 1.1	27.6 ± 0.3
Post immunization	41.0 ± 0.8	31.1 ± 3.2*	32.2 ± 1.7* [#]	36.3 ± 0.7* [#]
Post treatment	43.1 ± 1.0	27.6 ± 3.7*	29.6 ± 2.3* [#]	36.4 ± 0.7* [#]
White blood count				
WBC ^a	1.5 ± 0.3	4.2 ± 2	4.0 ± 1.1	3.8 ± 0.4
Large lymphocytes ^b	80 ± 4.8	91.7 ± 1.0*	80.8 ± 16.4	86.2 ± 4.4
monocytes ^b	4.2 ± 2.7	1.9 ± 0.4	2.3 ± 1.4	1.4 ± 0.5
Neutrophils ^b	13.8 ± 3.2	3.5 ± 0.8*	12.6 ± 12.8	7.9 ± 3.7
Eosinophils ^b	1.8 ± 1.0	2.9 ± 1.1	4.1 ± 3.5	0.5 ± 0.2
Cytokines and chemokines				
Eotaxin (pg/mL)	435.4 ± 37.6	1086.2 ± 61.8*	1438.6 ± 776.5*	595.5 ± 116.2*
IL-4 (pg/mL)	< 0.31	15.3 ± 2.8	27.5 ± 17.1*	16.7 ± 9.4
IL-5 (pg/mL)	15.9 ± 2.8	211.6 ± 63.8	21.3 ± 9.6 [#]	17.2 ± 15.9 [#]
Rantes (pg/mL)	41.4 ± 3.1	76.1 ± 15	67.8 ± 27.9	26.9 ± 10.0 [#]

Group Go, immunized control animals; group G1, immunized mice were treated with non-irradiated Con-A; group G2 and G3 immunized mice were treated with irradiated Con-A at 1 and 25 kGy, respectively. ^aWhite blood count in thousand per cubic millimeter. ^bMean per cent of total leukocytes. * p < 0.05 compared to immunized control animals; # p < 0.05 compared to immunized mice treated with non-irradiated Con-A; \$ p < 0.05 compared to immunized mice treated with irradiated Con-A at low-dose.

► Effective methods to reduce or eliminate food allergenicity has been investigated ►
Few reports have summarized information on the progress made with food irradiation
► The relationship between allergenicity and physical processing was measured ► Our
model revealed the impact that irradiation procedures have on allergenic potential

ABSTRACT

Few chronic food protein models have described the relationship between allergenicity and the molecular structure of food protein after physical processing. The effect of γ -radiation on the structure of food protein was measured by fluorescence, circular dichroism and microcalorimetry. BALB/c mice were intraperitoneally sensitized and then given non-irradiated and irradiated Con-A by daily gavage for 28 days. The tendency to form insoluble amorphous aggregates and partially unfolded species was observed after irradiation. The administration of non-irradiated and irradiated samples at low-dose significantly increased weight loss as well as plasma levels of eotaxin in animals repeatedly exposed to Con-A. Significant lymphocytic infiltrate filling completely the stroma of microvilli and tubular glands was observed in the small intestinal of the group given Con-A irradiated at a low dose. This phenotype was not observed in animals treated with Con-A irradiated at a high dose.

Keywords: food allergy; food protein; food irradiation; Con-A.