Effects of gamma irradiation on tropomyosin allergen, proximate composition and mineral elements in giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract Effects of food irradiation on allergen and nutritional composition of giant freshwater prawn are not well documented. Thus, this study aimed to investigate the effects of gamma irradiation on tropomyosin allergen, proximate composition, and mineral elements in *Macrobrachium rosenbergii*. In this study, prawn was peeled, cut into small pieces, vacuum packaged and gamma irradiated at 0, 5, 7, 10 and 15 kGy with a dose rate of 0.5 kGy/h using cobalt-60 as the source, subsequently determined the level of tropomyosin, proximate composition and mineral elements respectively. The results showed that band density of tropomyosin irradiated at 10 and 15 kGy is markedly decreased. Proximate analysis revealed that moisture, protein, and carbohydrate content were significantly

different as compared with non-irradiated prawn. Meanwhile, gamma irradiated *M. rosenbergii* at 15 kGy was observed to be significantly higher in nickel and zinc than the non-irradiated prawn. The findings provide a new information that food irradiation may affect the tropomyosin allergen, proximate composition and mineral elements of the prawn.

Keywords Gamma irradiation · Giant freshwater prawn · Tropomyosin · Proximate composition · Mineral elements

Introduction

In the last few decades the application of food irradiation has been explored widely. It has been used principally as a preservation technique; nevertheless it also has a potential in food operation to produce specific changes in the food matrices (Noramaliza et al. 2016). Among the specific changes is reduction in the allergenicity of allergens. Byun et al. (2002) demonstrated that irradiation could reduce the allergenicity of ovalbumin and β -lactoglobulin; similar findings have been reported to pure tropomyosin in shrimp (Byun et al. 2002). In contrast, Leszczynska et al. (2003) revealed that irradiation lead to an increase in allergenicity of gliadin and wheat flour. This could be due to the differences in transformation paths and the nature of protein damage resulted from irradiation in the solid form and/or in a water environment. Thus, food irradiation processing at several conditions could have unpredictable effects on the allergenicity of allergens in food matrices.

The use of gamma irradiation as a technique of reducing food allergy has opened a new field for irradiation application in which significant work has been done. As such, The Codex General Standard on irradiated food was

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reviewed in 2003 and stated that the maximum absorbed dose to be delivered to a food should not surpass 10 kGy, except when necessary to attain a legitimate technological purpose (EFSA 2011). Development of this technique creates the basic concern that gamma irradiation might reduce the nutritive value and mineral elements of the irradiated food. The entire relationship between radiation dose and possible effects must be identified in order to comprehensively evaluate the acceptability towards radiation-treated food (Khattak and Klopfenstein 1989). The stability of individual food compositions such as carbohydrates, protein, and fats during gamma irradiation has been widely studied, however foods, which are complex systems, possibly will not interact as expected from results with individual constituents (Khattak and Klopfenstein 1989).

Some food constituents have been shown to be vulnerable to destruction by ionizing radiation. Andrade et al. (2014) demonstrated that food composition of mushrooms was affected by gamma irradiation, in particular the dose of 500 Gy, which granted to highest average of fiber and crude protein in the mushrooms. Similarly, Bamidele and Akanbi (2013) reported that gamma irradiation has significant effect on the amino acids profile, but no significant effect on minerals and vitamins content of the pigeon pea flour. Abreu et al. (2010) revealed that gamma irradiation in doses up to 6 kGy positively affect fatty acid profile, cholesterol content, lipid and color stability of frozen and packed shrimp. Furthermore, Sanni et al. (2015) found that gamma irradiation increased the presence of the magnesium, calcium, iron and manganese in the treated samples of sorrel seeds (*Hibiscus sabdariffa*); however, decreased the presence of sodium, potassium, lead and copper when dose of irradiation increased was observed. The present work is part of a series of investigations in relation to some nutrition-associated chemical changes induced in prawn and upon gamma irradiation, with more attention to changes in mineral elements content.

Materials and methods

Chemicals and reagents

Freshwater prawn (*Macrobrachium rosenbergii*) was purchased from wholesale market, Seri Kembangan, Selangor, Malaysia. Nitric acid (HNO₃), hydrochloric acid (HCl) and Fisher BioReagents EZ-Run Prestained Rec Protein Ladder were purchased from Fisher Scientific (M) Sdn Bhd and atomic absorption modifier solutions (copper standard, iron standard, magnesium standard, nickel standard and zinc standard) were purchased from Perkin Elmer, USA. Polyclonal anti-tropomyosin produced in rabbit and goat anti-

rabbit IgG conjugated with horseradish peroxidase (HRP) from Novex, USA. Phosphate Buffer Saline tablet, Tween 20, ammonium persulfate and Bromophenol blue were purchased from Sigma-Aldrich, St. Louis, USA. Crystallized Bovine Serum Albumin (BSA), methanol, *N,N,N',N'*-Tetramethyl ethylenediamine (TEMED), glycerin, 2-Mercaptoethanol and PVDF membrane were purchased from Merck Millipore. Bradford 1× dye reagent, BD Difco Skim Milk, 30% acrylamide, Coomassie Brilliant Blue R-250 Staining Solution and bis-acrylamide solution from Bio-Rad. UltraPure Tris Buffer was purchased from Invitrogen.

Sample preparation

The prawn was washed with deionized water, peeled off and cut into small pieces. Subsequently, divided into 5 groups each weighing 200 g, vacuum packaged in Nylon 11 using vacuum sealer and keep at − 20 °C before being irradiated.

Gamma irradiation set up

The prawn was irradiated using a cobalt-60 gamma irradiator (Gamma Cell 220 Excel, MDS Nordion, Ontario) at Universiti Kebangsaan Malaysia, providing a mean energy of 1.25 MeV in the dose range between 0.0 and 15.0 kGy (Zhenxing et al. 2007) and a dose rate of 0.5 kGy/h. The 0 kGy was served as the control. After irradiation, the sample was stored at − 20 °C until used for further analysis.

Freshwater prawn protein extracts

Extraction of protein from freshwater prawn was performed as described by Yadzir et al. (2012) with some modification. Irradiated and non-irradiated freshwater prawns were homogenized in phosphate buffered saline (PBS), pH 7.4 at ratio 2:10 (weight/volume) using food processor (Tefal, France) in duplicate. The homogenized samples were constantly stirred for overnight at 4 °C prior to protein extraction. The samples were then centrifuged at 4500 rpm for 30 min at 4 °C followed by 10,000 rpm for 20 min at 4 °C, the supernatant was collected and transferred into 50 ml centrifuge tube and stored at − 20 °C until used. Protein concentration in the samples was determined using the Bradford assay (Bradford 1976).

Protein electrophoresis and immunoblotting

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970). Each sample was dissolved in loading buffer in the presence of 5% 2-mercaptoethanol and heated

at 95 °C for 5 min, electrophoresed in 12% polyacrylamide separating gel and 5% stacking gels employing Mini-PROTEAN Tetra System (Bio-Rad, USA) at 110 V for 45–60 min. Gels were either stained with Coomassie Brilliant Blue R-250 or transferred to a polyvinylidene fluoride (PVDF) membrane (0.2 µm, Merck Millipore). For immunoblotting, proteins were transferred from the gels onto PVDF membrane using Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, USA) by employing a constant current of 23 V for 30 min at room temperature, according to the method of Towbin and Gordon (1984). Blots were blocked with 5% skimmed milk that was diluted in PBS-T (PBS with 0.05% Tween 20) for 1 h at room temperature with a gentle shaking. The blots were then washed with PBS-T and incubated with polyclonal anti-tropomyosin produced in rabbit (1:5000) for overnight at 4 °C. The blots were washed again and incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (Novex, USA) (1:5000) in PBS-T for 30 min at room temperature. Finally, immunoreactive bands were visualized using Novex HRP Chromogenic Substrate (TMB). Dried membranes were scanned and the intensity of dark bands was measured by scanning densitometry (Syngene, UK).

Proximate analysis

Moisture, crude fat and ash content of the irradiated and non-irradiated freshwater prawn were measured according to the protocols established by AOAC (1990). The crude protein content was determined by the method of Micro-Kjeldahl (Pearson 1999), with the value for nitrogen conversion factor was 6.25 (Chang 2003). Meanwhile, carbohydrate content was determined by subtracting moisture, ash, protein and fat content from 100%.

Mineral elements analysis

To analyse the mineral elements content, sample preparation and analysis were carried out according to the procedure described by AOAC (1980) as well as dry-ashing method was used as the digestion procedures (Akinyele and Shokunbi 2015). In brief, 2.5 g of wet irradiated and non-irradiated freshwater prawn tissues were weighed into a crucible and placed in oven at 135 °C for 2 h. Subsequently, the crucible was removed, placed in a cool furnace and temperature was raised slowly to 450–500 °C and left overnight. The crucible was removed to a desiccator to allow cooling to room temperature and 0.2 mL of nitric acid (HNO₃) was added and swirled slowly to facilitate the process. The crucible was then placed on a warm hot plate for evaporation to dry the sample and again placed in a cool furnace and the temperature was raised slowly to

450–500 °C for 1 h. The crucible was then removed and allowed to cool at room temperature. One ml of 1 N HCL was added to the crucible to dissolve the ash, transferred to 25 ml volumetric flask and diluted with pure water. The samples were analysed for 5 elements (zinc at 213.9 nm, copper at 324.8 nm, magnesium at 285.2 nm, nickel at 232.0 nm and iron at 248.3 nm) using atomic absorption spectroscopy (AAS 3300 by PerkinElmer, US) with a double beam and deuterium background corrector. All digested samples were analysed three times for each element. The concentration of the element in each sample was determined from their constructed standard calibration curve. The curve was prepared by plotting absorbance of standard versus their concentration.

Statistical analysis

Data obtained are presented as mean ± standard deviations of triplicate experiments. Statistical evaluation was carried out using the Statistical Package for Social Sciences (SPSS) version 17 by one-way ANOVA followed by the LSD test. The significant level was accepted at $p < 0.05$.

Results and discussion

Total protein concentration of prawn protein extracts

Table 1 illustrates the total protein concentration of the irradiated and non-irradiated prawn. The protein concentration of *M. rosenbergii* irradiated at 5 and 7 kGy shows a significant increment as compared to non-irradiated sample (control). Similarly, Moon and Song (2001) has reported that at low dose of gamma irradiation, polypeptide chain is broken down and provide more binding site for the dye to react, resulting in slightly increase in the protein concentration. In contrast, there were significant reduction in total

Table 1 Total protein concentration of *M. rosenbergii*

Irradiation dose (kGy)	Total protein concentration (mg/ml) <i>M. rosenbergii</i>
0 (control)	1.0362 ± 0.0325 ^b
5	1.2036 ± 0.0278 ^a
7	1.1871 ± 0.0151 ^a
10	0.8596 ± 0.0252 ^a
15	0.7551 ± 0.0147 ^a

The value is presented as mean ± SD of triplicate samples

^{a,b}Significant difference between results at $p < 0.05$ if the row not sharing the same letter

protein concentration of the sample irradiated at 10 and 15 kGy as compared with control in concordance with Byun et al. (2000).

Protein electrophoresis and immunoblotting

In reference to Fig. 1, the selected band was expected to be the band of interest i.e. tropomyosin with molecular weight 36–38 kDa (Margaret et al. 2015). There were several bands appeared above and below of the expected band. The appearance of these bands may be due to the use of polyclonal antibody. As mentioned by Bordeaux et al. (2010), polyclonal antibody represented a pool of antibodies against the immunogen and typically showed a higher probability for detection in a range of different conditions and might contain superior affinity clones. Apart from that, a study by Zhenxing et al. (2007) suggested that crosslink with free amino acids or protein-to-protein augmentation can exist in muscle system leading to formation of new proteins, these too can be the possible reason for appearance of several bands. In term of band density of immunoblot analysis, at low doses (5–7 kGy), prawn muscles may contain other ingredient such as lipid that protects allergen from being destroyed by free radicals (Zhenxing et al. 2007) resulting in an increase in the band density and thus, marked reduction can be observed only at 15 kGy (Fig. 1). Our findings in concordance with the study reported by Byun et al. (2000) as dose of irradiation increases, the density of tropomyosin decreases; however, irradiation of more than 15 kGy may not be safe for human consumption.

Fig. 1 Changes in tropomyosin band intensity of non-irradiated (0 kGy) and irradiated (5–15 kGy) samples of *M. rosenbergii*. The immunoblot analysis was carried out by exposure of prawn protein extracts to polyclonal anti-tropomyosin produced in rabbit followed by the appropriate peroxidase-labelled secondary antibody. The change in band density was measured using scanning densitometry (Syngene, UK). Each lane was loaded with 1.04 mg/ml of total protein

Proximate analysis

The variation in the results of moisture, ash, protein, crude fat, and carbohydrate content of gamma irradiated and non-irradiated freshwater prawn (*M. rosenbergii*) is presented in Table 2. The results showed moisture content of the prawn irradiated at 7 kGy shows significant difference, with the lowest content amongst the samples. These findings were comparable to those found in irradiation of rawa (Sudha Rao et al. 1994). There were no significant differences between non-irradiated and different doses of irradiation in the case of ash and fat content; in contrast to Bhat et al. (2008) whom reported a decrease in ash content of gamma irradiated velvet bean seed at a dose range of 0–30 kGy. These results may be due to the reason of low ash and fat content in the prawn. Protein content at 5 kGy was significantly difference and higher than other doses, but at 10 and 15 kGy was significantly lower than 0, 5 and 7 kGy. According to Partmann and Keskin (1979), majority of amino acids in chicken breast muscle and minced lean beef are stable up to a dose of 5 kGy. Changes in protein fraction may be associated to aggregation or some cross-linking of protein as a consequence of gamma irradiation affecting nitrogen solubility (Ciesla et al. 2000). Abdelwhab et al. (2009) also reported a decrease in the protein content of gamma irradiated dry bean at doses 1, 1.5, and 2 kGy. Furthermore, in the present study carbohydrate content at 5 kGy was the lowest and significantly difference amongst the samples; however at 7, 10 and 15 kGy was increased irregularly and significantly difference than untreated dose. An increase in the carbohydrate content of irradiated samples was also reported by other investigators (Abdelwhab et al. 2009; Bhat et al. 2008; Mohammadzai et al. 2010). Our findings revealed that the effect of gamma irradiation on proximate composition of freshwater prawn (*M. rosenbergii*) is dose-specific; similar findings were reported by Pezzutti et al. (2005) on imported onion flakes when they were exposed to various doses of irradiation from 5 to 25 kGy.

Mineral elements analysis

Table 3 shows the results of the effect of gamma irradiation on the mineral elements i.e. nickel, copper, zinc, iron and magnesium in freshwater prawn *M. rosenbergii*. The result showed that gamma irradiated *M. rosenbergii* has significantly higher in nickel and zinc than the non-irradiated and recorded the highest value at 15 kGy. Copper was significantly higher than control when irradiated at 5, 7 and 15 kGy. Iron was significantly higher than control at 5 kGy, but later was significantly lower than the non-irradiated prawn at 7, 10 and 15 kGy. For magnesium, the concentration of irradiated prawn is decreased irregularly

Table 2 Proximate composition of gamma irradiated freshwater prawn (*Macrobrachium rosenbergii*)

Dose (kGy)	Proximate composition (%w/w)				
	Moisture	Ash	Protein	Fat	Carbohydrate
0	78.23 ± 0.24 ^a	1.20 ± 0.03 ^a	18.06 ± 0.77 ^{ab}	0.21 ± 0.02 ^a	2.31 ± 0.68 ^{bc}
5	78.22 ± 0.17 ^a	1.34 ± 0.05 ^a	18.71 ± 0.33 ^a	0.21 ± 0.02 ^a	1.53 ± 0.39 ^c
7	77.10 ± 0.15 ^b	1.32 ± 0.17 ^a	17.85 ± 0.11 ^{ab}	0.23 ± 0.03 ^a	3.50 ± 0.28 ^a
10	78.00 ± 0.19 ^a	1.28 ± 0.02 ^a	17.52 ± 0.52 ^b	0.22 ± 0.03 ^a	2.98 ± 0.40 ^{ab}
15	77.83 ± 0.25 ^a	1.32 ± 0.07 ^a	17.47 ± 0.25 ^b	0.21 ± 0.03 ^a	3.17 ± 0.30 ^{ab}

Data are expressed as mean ± standard deviation of three replicates measurement. Values in the same column with different superscripts denote significant different by one-way ANOVA followed by the LSD test at the level of $p < 0.05$

Table 3 Mineral elements of gamma irradiated freshwater prawn (*Macrobrachium rosenbergii*)

Dose (kGy)	Mineral elements (mg/L)				
	Nickel	Copper	Zinc	Iron	Magnesium
0	1.28 ± 0.10 ^b	3.06 ± 0.12 ^{cd}	12.54 ± 0.45 ^b	10.21 ± 0.01 ^b	528.83 ± 6.64 ^a
5	7.23 ± 1.45 ^a	3.21 ± 0.08 ^c	13.35 ± 0.35 ^b	14.87 ± 0.07 ^a	341.50 ± 2.65 ^c
7	6.60 ± 0.15 ^a	4.10 ± 0.17 ^b	16.58 ± 0.40 ^a	8.88 ± 0.16 ^c	464.30 ± 35.2 ^b
10	6.90 ± 0.50 ^a	2.74 ± 0.05 ^d	15.89 ± 0.21 ^a	7.94 ± 0.01 ^d	253.33 ± 10.25 ^d
15	7.30 ± 1.02 ^a	4.59 ± 0.15 ^a	16.87 ± 0.6 ^a	8.437 ± 0.32 ^c	265.667 ± 1.29 ^d

Data are expressed as mean ± standard deviation of three replicates measurement. Values in the same column with different superscripts denote significant different by one-way ANOVA followed by the LSD test at the level of $p < 0.05$

and significantly lower than control; however, the values were no observable trends with increasing irradiation doses. Any changes in mineral elements composition after irradiation can be ascribed to doses delivered which might have influenced the extractability (Kilcast 1994). On the other hand, the ash content is measured the total amount of minerals exist within a sample, while the mineral content is measured of the amount of specific inorganic components, such as iron, zinc and magnesium present within a sample. Wet ashing is mainly used in the preparation of samples for subsequent analysis of specific minerals. It breaks down and removes the organic matrix surrounding the minerals via the use of both heat and acids, so that minerals are left in an aqueous solution or vaporized resulting in variation level of mineral elements (Palma et al. 2015). The same observation was reported by Hussein et al. (2014) during the investigation on the effect of gamma irradiation on physicochemical properties of Malaysian honey. The findings from this study corroborate with the data reported by Asaikkutti et al. (2016); Sadiq et al. (1982); Tu et al. (2008) on magnesium and iron, copper and zinc, and nickel; respectively.

Conclusion

In sum, this work contributes to the knowledge of the beneficial application of gamma irradiation on giant freshwater prawn (*M. rosenbergii*). Many reports are made available on the effects of gamma irradiation on microbiological qualities though almost no or limited reports are found to assess the effects of gamma irradiation on tropomyosin allergen, proximate composition and mineral content of seafood namely giant freshwater prawn. Therefore, this study will help to give an overview which exhibited that the gamma irradiation technique at dose as low as 5 kGy offered minimal loss of nutrients. This study showed a minimal effect of gamma irradiation on essential minerals content of giant freshwater prawn. Further study is proposed to investigate the changes in other factors like sensory, taste, texture, and shelf-life of giant freshwater prawn.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

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