

Microbial Population, Physicochemical Quality, and Allergenicity of Molluscs and Shrimp Treated with Cobalt-60 Gamma Radiation

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ABSTRACT

Frozen molluscs (squid, octopuses, and cuttlefish) and crustaceans (shrimp) were irradiated using a cobalt-60 gamma source, at different doses, in order to investigate the effects of gamma radiation on their microbial population, organoleptic characteristics, lipid profile, and tropomyosin content. Irradiation of shrimp and squid with either 2.5 or 4.7 kGy reduced mesophilic bacteria contamination to low or nondetectable levels, respectively, whereas irradiation of octopus and cuttlefish with the same doses reduced the bacterial population. Irradiation treatment had no significant ($P > 0.05$) effect on the total lipid content and the major detected classes of polar and neutral lipids, whereas it significantly ($P < 0.05$) increased the contents of neutral lipids in octopus mantle and in shrimp muscle and cephalothorax samples. The total fatty acid content and the ω -3: ω -6 fatty acid ratio was not affected. A dose-dependent significant ($P < 0.05$) decrease in the ratio of polyunsaturated fatty acids:saturated fatty acids was observed. With the increase in radiation dose, redness (a) and yellowness (b) values showed a variation, whereas the lightness (L) value was significantly ($P < 0.05$) decreased in mollusc mantles and shrimp muscle and increased in shrimp cephalothorax. The total of color changes (ΔE) increased ($P < 0.05$) as the dose increased. Significant ($P < 0.05$) changes in textural properties were observed with radiation treatment in octopus tentacles and in squid and cuttlefish mantle. The amount of tropomyosin, which is the major mollusc and crustacean allergen in the irradiated organisms, was reduced by gamma radiation, depending on the dose.

Gamma radiation processing has been demonstrated to be a safe and effective method for controlling, reducing, or eliminating biological hazards in foods (36). Radiation treatment, depending on condition of irradiation and the kind of food, can effectively eliminate potentially pathogenic bacteria, including *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter*, and *Listeria monocytogenes*. This is due to the damage of nucleic acids in the microorganisms from suspected food products, without affecting their nutritional and organoleptic values (10, 11). Radiation dose, processing temperature, and packaging conditions strongly influence the effects of irradiation treatments on both the microbiological and nutritional quality of irradiated foods (34). Gamma radiation in aqueous model solutions leads to a nonenzymatic browning reaction (carbonyl amino reaction) similar to those induced by heating. This reaction may influence the changes of the color in irradiated foods (26).

Proteins, carbohydrates, and fats are not significantly affected by radiation doses up to 10 kGy. Ionizing radiation causes damage to membrane lipids and lipoproteins, mainly through lipid peroxidation of their unsaturated fatty acid constituents (30). The lipid and fatty acid composition is important for nutritional value, flavor, and textural properties of the food and significantly affects the acceptability of a product. There is only a limited amount of information

on the gamma radiation effect on the fatty acid composition of molluscs and crustaceans. Several studies have shown that the lipid profiles of the above items display high concentrations of polyunsaturated fatty acids (PUFA), especially highly unsaturated fatty acids from the ω -3 series (13, 32). Nutritionists have focused on the type of PUFA and the balance in the diet between ω -3 PUFA formed from linolenic acid (18:3) and ω -6 PUFA formed from linoleic acid (18:2) as the high ratio of ω -6: ω -3 PUFA is a risk factor for cancer and heart disease (35).

Control of food allergens is increasingly important in the food industry, and crustaceans such as shrimp and crabs and molluscs such as squid and octopus are among the frequent causes of food allergy mediated by immunoglobulin E (IgE) antibodies. Immunoblotting studies showed that many crustacean-allergic patients are IgE specific only for tropomyosin. Even if tropomyosin is degraded to peptide fragments, some of these fragments may have IgE-binding epitopes. Epitopes on the allergens are structurally altered by gamma radiation, and IgE antibodies do not recognize sufficiently antigen-determinant sites on allergens. Therefore, irradiation processing is a potential technique for the reduction of the allergenicity of foods (5).

The study of the molluscs and crustaceans is of increasing nutritional, commercial, and financial importance, as these seafoods are some of the commonly consumed food. The objective of this project was to evaluate the ef-

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ficacy of ionizing radiation from cobalt-60 to eliminate pathogenic bacteria of molluscs and crustaceans, which are commonly contaminated with some pathogenic and spoilage bacteria (such as *Salmonella* spp. and *Vibrio*) (10), without affecting their organoleptic and nutritional characteristics. Emphasis was given to the analysis of their lipid profile and fatty acid composition, as these seafoods are known to have a favorable ω -3: ω -6 ratio. Moreover, the application of gamma irradiation for the reduction of allergenic activity of tropomyosin was evaluated in order to produce hypoallergenic mollusc and crustacean foods.

MATERIALS AND METHODS

Reagents, chemicals, and sampling. The standard lipids used (purity >98%) were cardiolipine, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, lysophosphatidylcholine sphingomyelin, cholesterol, stearic acid, and triolein, all purchased from Sigma (St. Louis, Mo.). Fatty acid methyl esters used as gas chromatographic standards were lauric acid M-E, L7272, *cis*-5,8,11,14,17-eicosapentaenoic acid M-E, E2012, and *cis*-4,7,10,13,16,19-docosahexaenoic acid M-E, D2659 (purity $\geq$ 98%). In addition, Matreya Bacterial Acid Methyl Esters CP Mix and Supelco 37 Component FAME Mix (both purchased from Sigma) were used. All solvents used for sample preparation were of analytical grade from Merck (Darmstadt, Germany). Double distilled water was used throughout this work, and all reagents used were of analytical grade, purchased from Sigma. For the quantitative analysis of the crustacean tropomyosin residues in the samples, a commercially available assay from ELISA Systems (Brisbane, Australia) was used. The crustacean tropomyosin residue kit (lot number CRURD05-052) is a double-antibody (sandwich) enzyme-linked immunosorbent assay (ELISA), utilizing an anti-tropomyosin inhibitor coated onto microwells. The immunoassays were performed in 96-well microtiter plates, using the Multiskan MK3 ELISA reader (Thermo LabSystems, Waltham, Mass.).

Frozen adult octopus (*Octopus vulgaris*), squid (*Todarodes sagittatus*), cuttlefish (*Sepia officinalis*), and shrimp (*Penaeus monodon*) (30 adult specimens per species, six composite samples, and five organisms per composite) were bought during 2005. They were weighed and stored in the freezer (-18°C) until the day of irradiation. The water content of mollusc mantles and shrimp muscle and cephalothorax parts was measured by drying in an oven at 105°C until constant weight was reached.

Radiation process. For gamma radiation, a cobalt-60 source irradiator was used. The strength of the source was 150 kCi, with a dose rate capacity of 1 kGy/h. The samples were vacuum packaged in low-density polyethylene bags, kept in packed ice, placed in polyethylene plates (22 by 22 by 4 cm), and irradiated under air atmosphere. Dosimetry was performed using a polymethylmethacrylate dosimeter (PMMA Instruments, Harwell, UK) placed on the irradiated samples surface. The doses applied were 2.5 and 4.7 kGy. The temperature during the irradiation treatment was maintained at approximately $0 \pm 1^{\circ}\text{C}$. Immediately after irradiation, the treated samples were stored with the controls at $0 \pm 1^{\circ}\text{C}$. Four composite samples (two per dose), each including five adult organisms, were irradiated, and two composite samples were taken as controls.

Microbiological analysis. All samples were mixed and homogenized in a blender (Ultra-Turax, T-25, IKA-VERGE, Germany). Eleven grams of the mixture was further homogenized in a Lab-blender 400 stomacher (Laboratory Equipment, London

UK) for 2 min in a stomacher bag containing 99 ml of sterile (buffered) peptone water. Eleven milliliters of each sample mixture was serially diluted in 99 ml of (buffered) peptone water (Merck), and then 1 ml of each dilution was plated on the appropriate medium.

Molluscs and shrimp were pour plated on plate count agar (Difco, Becton Dickinson, Sparks, Md.) and analyzed for aerobic mesophilic bacteria. The plates were incubated at 30°C for 48 h. The enumeration of mesophilic bacteria was expressed as CFU per gram.

Lipid extraction and separation of neutral from polar lipids. After the measurement of the total weight of wet tissues, the mollusc mantles and the shrimp muscle and cephalothorax parts were homogenized separately in a mixture of chloroform and methanol (2:1, vol/vol) and were extracted for total lipids according to the Bligh and Dyer method (2). After phase equilibration, the lower chloroform layer (total lipids) was removed and dried in a rotary evaporator. The extracted lipids were weighed to determine the total lipid, then redissolved in chloroform-methanol (9:1, vol/vol), and finally stored at a temperature of 0°C . Afterward, total lipids were separated in neutral and polar lipids on prewashed 500-mg silicic acid columns (Kieselgel 60, Merck) by solid-phase extraction, using the modified method of Mastronicolis et al. (24). Neutral lipid fractions were quantitated by weight after being eluted from solid-phase extraction columns.

HPTLC of total, neutral, and polar lipids. The total lipid as well as the polar and neutral lipid (separated by solid-phase extraction) composition was determined by single-dimensional high-performance thin-layer chromatographic analysis (1D-HPTLC), carried out on precoated silica gel 60-G HPTLC plates (size: 10 by 10 cm, layer thickness: 0.25 mm; Merck). The following development solvent systems were used: solvent A (for total lipids), chloroform-methanol-water (65:25:4, vol/vol/vol); solvent B (for polar lipids), chloroform-methanol-glacial acetic acid-water (50:25:6:2, vol/vol/vol/vol); and solvent C (for neutral lipids), petroleum ether-diethylether-glacial acetic acid (70:30:4, vol/vol/vol). Visualization of spots was effected by exposure to iodine vapor, followed by spraying with the phosphomolybdenum blue reagent-detecting phospholipids (7). Lipid spots were also visualized with copper sulfate spray and heating at 180°C for 5 min (29).

Gas chromatographic analysis of fatty acid methyl esters. Methyl esters of the fatty acids contained in the total lipid fractions of the studied mollusc mantles and shrimp muscle and cephalothorax parts were prepared by the procedure described by Sinanoglou and Miniadis-Meimaroglou (32). Quantitative and qualitative analysis were performed on an HRGC Mega 2 Series 8560 MFC 800 (Fisons Instruments, San Carlos, Calif.) gas chromatograph equipped with an EL 980 CE Instruments FID (flame ionization detector) (Hellenic Labware, Athens, Greece). A fused silica capillary column of high polarity was used (60 m by 0.32-mm inside diameter and 0.2- μm film thickness; Supelco SP 2340, Supelco, Bellefonte, Pa.). This polymeric stationary phase was a nonbonded poly(biscyanopropylsiloxane). The carrier gas was hydrogen at a pressure of 15 lb/in². The make-up gas was atmospheric air at a pressure of 15 lb/in², and hydrogen was supplied to the FID at a pressure of 9 lb/in². The temperature of the injector was 230°C and of the detector was 250°C . The temperature was programmed at 150°C for 15 min, raised from 150 to 170°C at a rate $2^{\circ}\text{C min}^{-1}$, held constant at 170°C for 10 min, raised from 170 to 215°C at $4^{\circ}\text{C min}^{-1}$, and held at 215°C for 15 min. The duration of the analysis was 62 min. In the gas chromatographic

TABLE 1. The total indigenous mesophilic aerobic microflora (CFU/g)^a of mollusc mantles and crustacean muscle treated with gamma radiation

Radiation dose (kGy)	<i>Todarodes sagittatus</i> (squid)	<i>Octopus vulgaris</i> (octopus)	<i>Sepia officinalis</i> (cuttlefish)	<i>Penaeus monodon</i> (shrimp)
0	$5.01 \times 10^3 \pm 0.12$	$1.00 \times 10^3 \pm 0.23$	$1.00 \times 10^3 \pm 0.17$	$2.51 \times 10^3 \pm 0.19$
2.5	$5.00 \times 10^1 \pm 0.15$	$1.25 \times 10^2 \pm 0.08$	$1.00 \times 10^2 \pm 0.22$	NG ^b
4.7	NG ^b	$5.00 \times 10^1 \pm 0.06$	$5.00 \times 10^1 \pm 0.07$	NG ^b

^a All experiments were performed in triplicate, and the data are given in mean values.

^b NG, no growth at detection limit (<10 CFU/g).

method, the fatty acid methyl esters were identified by comparison with the standard mixture of fatty acid methyl esters. The total fatty acid content was found by the use of lauric acid methyl ester as the internal standard.

Texture measurement. Instrumental texture analysis was performed using a Texture-Analyzer, model TA-XT2i (Stable Micro Systems, Blackdown Rural Industries, Surrey, UK) with a load cell (Heavy Duty Platform/90) of 25 kg. A blade (60° knife-edge) probe with a thickness of 0.75 mm and width of 70 mm was used at a velocity of 2 mm/s. The force-time graphs were recorded by computer and analyzed using Stable Micro System software designed for use with the TA-XT2. The force (newtons) required to cut the sample, the firmness (g per second), and the slope (newtons per millimeter) were determined.

Color measurement. Color values such as *L* (lightness), *a* (redness), and *b* (yellowness) values were measured with a tristimulus chromameter (Minolta, Tokyo, Japan, model CR-210) calibrated with a red standard (no. 482, Bureau Communautaire de Reference). Three random readings per sample were taken and averaged. Color change, ΔE , which represents the comprehensive contributions from the variations of three color indexes, was calculated according to the equation $\Delta E = [(a - a_c)^2 + (b - b_c)^2 + (L - L_c)^2]^{1/2}$. The values of nonirradiated samples were used as control values.

The values *a* (red-green) and *b* (yellow-blue) were used to calculate the metric chroma (*C*), according to the equation $C = (a^2 + b^2)^{1/2}$, which provides information about the color index of the samples (25).

Sandwich ELISA. After the extraction of tropomyosin from the samples, 100 μ l of the sample extract was added in each well and incubated for 30 min at room temperature. A wash step (ELISA system ready to use wash buffer) was repeated five times, and then 100 μ l of enzyme conjugate was added in the wells for an incubation of 15 min at room temperature. After a second wash cycle, 100 μ l of the substrate solution was added in each well and incubated for 10 min at room temperature. After the completion of the final incubation, 100 μ l of stop solution was added in each well, and the absorbance was measured at 450 nm with the ELISA reader (Thermo LabSystems).

Statistical analysis. All measurements were obtained (at least) in triplicate, and values were averaged and reported along with the standard deviation. Data were analyzed with one-way analysis of variance post hoc tests, and pairwise multiple comparisons were conducted with the Tukey's honestly significant difference test. Linear trends were tested with one-way analysis of variance contrasts. All data were analyzed with the SPSS 13.1 statistical software (SPSS, Chicago, Ill.).

RESULTS AND DISCUSSION

Microbial quality of irradiated molluscs and crustaceans. Data on the microbiological quality of mollusc mantles and crustacean muscle treated with radiation are shown in Table 1. The initial mesophilic microbial population in the molluscs and crustaceans was about 10^3 CFU/g. Radiation doses up to 4.7 kGy reduced the mesophilic microorganisms in squid mantle and shrimp muscle to non-detectable levels, although these doses did not completely eliminate aerobic microorganisms in octopus and cuttlefish mantles. The results indicated that the microorganisms present in the studied molluscs and shrimp might have a difference in radiation sensitivity; therefore, the radiation resistance of mesophilic microflora may depend on the irradiation dose and the kind of the species. A radiation dose of 4.7 kGy to frozen shrimp and squid decreased the bacterial population from 10^3 CFU/g to nondetectable levels, whereas the same radiation dose to octopus and cuttlefish resulted in a 1.3-log cycle reduction (from 10^3 to 5×10^1 CFU/g). Irradiation treatment at doses of 4 and 6 kGy in frozen Malaysian shrimp had been proved effective in reduction of the aerobic psychrotrophic and mesophilic colony counts by about 3 and 4 log cycles, respectively (27), which is in agreement with the above results. Irradiation of frozen precooked shrimp (*P. monodon*) from Thailand at doses 1.8 to 3.6 kGy reduced the indigenous microflora, resulting in shelf-life extension of more than 14 days (19). Radiation microbiology studies in fishery products indicated that gamma radiation with doses up to 4 kGy to control and eliminate pathogenic population could be very effective with frozen fishery products such as molluscs and crustaceans originating from polluted aquatic environments (10). A dose as low as 1 kGy is sufficient to eliminate *Vibrio parahaemolyticus*, the leading causative agent of bacterial gastroenteritis from fishery products and frozen seafood (10). Therefore, irradiation of fish and shellfish can be considered as part of good manufacturing practices or hazard analysis critical control point processing.

Lipid content and polar and neutral lipid profile of irradiated molluscs and crustaceans. Following the irradiation, lipids were extracted from the mollusc mantles and shrimp muscle and cephalothorax parts separately, to evaluate the radiation effect. Total lipids of the cephalopod mollusc *T. sagittatus* (squid), *O. vulgaris* (octopus), and *S. officinalis* (cuttlefish) nonirradiated mantle constituted 1.80,

TABLE 2. Total lipid (TL), neutral lipid (NL) and fatty acid (FA) composition (wt/wt, %) of molluscs and crustaceans stored at $0 \pm 1^\circ\text{C}^a$

Samples	Parameters	0 kGy	2.5 kGy	4.7 kGy
<i>Todarodes saggitatus</i> (squid)	TL % of wt/wt	$1.80 \pm 0.18 \text{ A}^b$	$1.76 \pm 0.14 \text{ A}$	$1.70 \pm 0.26 \text{ A}$
	NL % of TL	$28.9 \pm 0.27 \text{ A}$	$29.2 \pm 0.31 \text{ A}$	$29.7 \pm 0.45 \text{ A}$
	% FA of TL	$46.5 \pm 0.55 \text{ A}$	$46.4 \pm 0.62 \text{ A}$	$46.1 \pm 0.73 \text{ A}$
<i>Octopus vulgaris</i> (octopus)	TL % of wt/wt	$2.32 \pm 0.11 \text{ A}$	$2.24 \pm 0.09 \text{ A}$	$2.16 \pm 0.21 \text{ A}$
	NL % of TL	$45.6 \pm 0.28 \text{ A}$	$46.3 \pm 0.31 \text{ B}$	$47.4 \pm 0.19 \text{ C}$
	% FA of TL	$54.4 \pm 0.38 \text{ A}$	$54.3 \pm 0.47 \text{ A}$	$54.0 \pm 0.64 \text{ A}$
<i>Sepia officinalis</i> (cuttlefish)	TL % of wt/wt	$1.55 \pm 0.17 \text{ A}$	$1.52 \pm 0.11 \text{ A}$	$1.48 \pm 0.13 \text{ A}$
	NL % of TL	$36.8 \pm 0.26 \text{ A}$	$37.1 \pm 0.09 \text{ A}$	$37.7 \pm 0.15 \text{ A}$
	% FA of TL	$46.9 \pm 0.74 \text{ A}$	$46.7 \pm 0.41 \text{ A}$	$46.3 \pm 0.56 \text{ A}$
<i>Penaeus monodon</i> (shrimp muscle)	TL % of wt/wt	$1.30 \pm 0.09 \text{ A}$	$1.30 \pm 0.12 \text{ A}$	$1.22 \pm 0.18 \text{ A}$
	NL % of TL	$25.4 \pm 0.29 \text{ A}$	$25.7 \pm 0.15 \text{ AB}$	$26.3 \pm 0.32 \text{ B}$
	% FA of TL	$71.1 \pm 0.49 \text{ A}$	$70.8 \pm 1.08 \text{ A}$	$70.2 \pm 0.62 \text{ A}$
<i>P. monodon</i> (shrimp cephalothorax)	TL % of wt/wt	$2.75 \pm 0.28 \text{ A}$	$2.70 \pm 0.13 \text{ A}$	$2.59 \pm 0.25 \text{ A}$
	NL % of TL	$53.8 \pm 0.53 \text{ A}$	$54.5 \pm 0.27 \text{ AB}$	$55.4 \pm 0.36 \text{ A}$
	% FA of TL	$82.0 \pm 1.12 \text{ A}$	$81.7 \pm 0.77 \text{ A}$	$81.2 \pm 0.33 \text{ A}$

^a Data reported as means $\pm$ standard deviations of the determinations in triplicate of two composite samples per radiation dose, each including five adult organisms.

^b Means in the same row with unlike letters differ significantly ($P < 0.05$).

2.32 and 1.55% of the wet tissue, respectively. The lipid contents of *P. monodon* nonirradiated muscle and cephalothorax was 1.30 and 2.75% respectively. At doses of 4.7 kGy, mollusc mantle showed a loss of the total lipid content of 4.5 to 6.9%, and in shrimp muscle and cephalothorax, the decrease was 6.2 and 5.8% respectively, in comparison with the nonirradiated samples (Table 2), but all changes were not statistically significant ($P > 0.05$). This reduction of total lipid content could be due to a decrease of the phospholipids and cholesterol levels, because gamma-radiation processing leads to their oxidation as described by Kanatt et al. (15). Neutral lipid components of the octopus mantle and of the shrimp muscle and cephalothorax showed a significant ($P < 0.05$) increase after irradiation treatment, at the dose of 4.7 kGy (Table 2).

The total of polar and neutral lipids from nonirradiated and irradiated mollusc mantles and shrimp muscle and cephalothorax were determined on analytical 1D-HPTLC to investigate if there was any change in the lipid profile due to irradiation. The identified polar lipid classes were cardiolipine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine sphingomyelin and lysophosphatidylcholine, and the neutral lipid classes were triglycerides, free fatty acids, and sterols. In irradiated samples, a dose-dependent decrease in the intensity of the phospholipid and cholesterol spots was observed. At 4.7 kGy a considerable increase in the intensity of the free fatty acid spots was obtained, and this is in agreement with the increase of the neutral lipid proportion. Previous studies have shown that irradiation of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol, containing either saturated or unsaturated fatty acid chains at 9.66 kGy and 0 to 4°C in aqueous suspension, resulted in phospholipid disappearance and production of two radiolytic products, phosphatidic acid and the lysophospholipid (30, 34). Similar studies in food items

with high lipid content, like egg yolk, showed that irradiation caused a significant ($P < 0.01$) increase in the contents of free fatty acids, indicating the liberation of some free fatty acids due to irradiation (1).

Fatty acid composition of total lipids. The data on the main fatty acids for total lipids of the studied cephalopod molluscs and crustaceans are given in Table 3. Fatty acid profiles had a difference in the proportions rather than in the variety of fatty acids. The major saturated fatty acids found in all the organisms' total lipid fractions were palmitic (C16:0) and stearic (C18:0) acid, and the most abundant monounsaturated fatty acid was oleic acid (C18:1, ω -9). Eicosapentaenoic acid (C20:5, ω -3) and docosahexaenoic acid (C22:6, ω -3) were identified as the major polyunsaturated ω -3 fatty acids, whereas arachidonic acid (20:4, ω -6) was detected in higher amounts in shrimp muscle and cephalothorax. Myristic (C14:0), hydroxy myristic (2-OH-C14:0), myristoleic (C14:1, ω -5), palmitoleic (C16:1, ω -7), heptadecanoic (C17:0), branched heptadecanoic (iso C17:0), cyclo heptadecanoic (17:0 cyclo), heptadecenoic (C17:1, ω -7), linoleic (C18:2, ω -6), α -linolenic (C18:3, ω -3), γ -linolenic (C18:3, ω -6), eicosanoic (C20:0), eicosanoic (C20:1, ω -9), eicosadienoic (C20:2, ω -6), docosapentaenoic acid (C22:5, ω -3), and tetracosenoic (C24:1, ω -9) acids were also found in lower or modest amounts. Similar results were also reported for cephalopod mollusc and crustacean total lipid fatty acids in previous studies (13, 33). The main results observed between nonirradiated and irradiated mollusc mantle and shrimp muscle and cephalothorax total lipid fatty acids were (i) the proportion of saturated fatty acids was significantly ($P < 0.05$) increased at 2.5 and 4.7 kGy, along with a significant ($P < 0.05$) linear decrease of PUFA; (ii) the monounsaturated fatty acid content showed no significant ($P > 0.05$) variation; (iii) the PUFA:saturated fatty acids ratios were significantly ($P <$

TABLE 3. Major fatty acid (FA) composition (wt/wt, %) of the total lipids (TL) extracted from molluscs and crustaceans^a

Radiation dose	C16:0	C18:0	C18:1, ω -9	C20:4, ω -6	C20:5, ω -3	C22:6, ω -3	$\Sigma n:0$ (SFA)	$\Sigma n:1$ (MUFA)	$\Sigma \omega:3$	$\Sigma \omega:6$	PUFA/ SFA	ω -3: ω -6
<i>Todarodes sagittatus</i> (squid)												
0 kGy	19.33 $\pm$ 1.21	8.29 $\pm$ 0.37	4.78 $\pm$ 0.29	3.21 $\pm$ 0.15	14.94 $\pm$ 0.64	35.16 $\pm$ 0.92	33.64 ^a	10.81 A	51.42	4.13	1.65 ^a	12.45 A
2.5 kGy	20.09 $\pm$ 0.86	9.07 $\pm$ 0.51	4.95 $\pm$ 0.46	2.87 $\pm$ 0.32	14.10 $\pm$ 0.57	33.72 $\pm$ 0.80	36.11 B	11.08 A	48.94	3.87	1.46 B	12.65 A
4.7 kGy	21.12 $\pm$ 1.23	9.68 $\pm$ 0.62	4.71 $\pm$ 0.43	2.50 $\pm$ 0.68	13.72 $\pm$ 0.79	31.90 $\pm$ 0.54	38.86 C	10.97 A	46.47	3.70	1.29 C	12.56 A
<i>Octopus vulgaris</i> (octopus)												
0 kGy	16.34 $\pm$ 0.70	7.12 $\pm$ 0.36	7.26 $\pm$ 0.68	6.94 $\pm$ 0.25	17.18 $\pm$ 0.97	26.22 $\pm$ 1.13	28.15 A	18.95 A	44.12	8.78	1.88 A	5.03 A
2.5 kGy	17.11 $\pm$ 0.84	7.45 $\pm$ 0.40	7.28 $\pm$ 0.91	6.29 $\pm$ 0.57	16.28 $\pm$ 0.73	25.13 $\pm$ 0.82	30.17 B	19.29 A	42.29	8.25	1.67 B	5.13 A
4.7 kGy	18.22 $\pm$ 0.83	8.65 $\pm$ 0.77	7.71 $\pm$ 0.36	5.83 $\pm$ 0.50	15.16 $\pm$ 0.39	23.48 $\pm$ 0.74	33.25 C	19.56 A	39.52	7.67	1.42 C	5.15 A
<i>Sepia officinalis</i> (cuttlefish)												
0 kGy	18.15 $\pm$ 0.46	7.89 $\pm$ 0.78	5.12 $\pm$ 0.31	4.17 $\pm$ 0.69	17.56 $\pm$ 0.66	29.58 $\pm$ 0.48	31.12 A	13.42 A	48.59	6.87	1.78 A	7.07 A
2.5 kGy	18.72 $\pm$ 0.53	8.56 $\pm$ 0.61	5.29 $\pm$ 0.44	3.70 $\pm$ 0.38	17.12 $\pm$ 0.47	28.31 $\pm$ 0.98	33.79 B	13.65 A	46.35	6.21	1.55 B	7.46 B
4.7 kGy	19.98 $\pm$ 0.72	9.30 $\pm$ 0.81	5.11 $\pm$ 0.25	3.23 $\pm$ 0.37	16.61 $\pm$ 0.50	27.40 $\pm$ 0.62	36.82 C	13.42 A	43.95	5.81	1.35 C	7.56 B
<i>Penaeus monodon</i> (shrimp muscle)												
0 kGy	17.89 $\pm$ 0.87	10.93 $\pm$ 0.56	15.57 $\pm$ 0.73	12.64 $\pm$ 0.51	16.18 $\pm$ 0.39	12.35 $\pm$ 0.44	34.87 A	21.63 A	30.28	13.22	1.25 A	2.29 A
2.5 kGy	18.93 $\pm$ 0.47	12.21 $\pm$ 0.62	16.04 $\pm$ 0.85	11.25 $\pm$ 0.38	15.53 $\pm$ 0.41	11.49 $\pm$ 0.67	37.25 B	22.12 A	28.32	12.31	1.09 B	2.30 A
4.7 kGy	19.23 $\pm$ 0.74	11.65 $\pm$ 0.42	15.80 $\pm$ 0.86	10.90 $\pm$ 0.60	15.05 $\pm$ 0.32	10.51 $\pm$ 0.37	39.96 C	21.88 A	26.38	11.78	0.95 C	2.24 A
<i>P. monodon</i> (shrimp cephalothorax)												
0 kGy	24.54 $\pm$ 0.96	10.44 $\pm$ 0.28	16.62 $\pm$ 0.62	10.85 $\pm$ 0.34	10.70 $\pm$ 0.18	9.48 $\pm$ 0.59	43.70 A	23.16 A	21.19	11.95	0.76 A	1.77 A
2.5 kGy	25.77 $\pm$ 1.02	11.68 $\pm$ 0.45	17.05 $\pm$ 0.88	9.73 $\pm$ 0.57	9.81 $\pm$ 0.38	8.55 $\pm$ 0.47	45.98 B	23.45 A	19.71	10.86	0.66 B	1.81 A
4.7 kGy	27.14 $\pm$ 0.74	12.93 $\pm$ 0.65	17.32 $\pm$ 0.50	8.91 $\pm$ 0.61	8.74 $\pm$ 0.42	7.61 $\pm$ 0.59	48.72 C	23.62 A	17.88	9.78	0.57 C	1.83 A

^a Data reported as means $\pm$ standard deviations of the determinations in triplicate of two composite samples per radiation dose, each including five adult organisms. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

^b For each species, means in the same column with unlike letters differ significantly ($P < 0.05$).

^c For each species, means in the same column with unlike letters decrease in a linear trend ($P < 0.05$).

TABLE 4. Moisture (wt/wt, %) of molluscs and crustaceans retained at $0 \pm 1^\circ\text{C}^a$

Samples	0.0 kGy	2.5 kGy	4.7 kGy
<i>Todarodes sagittatus</i> (squid)	0.82 ± 0.09 A ^b	0.93 ± 0.05 A	0.84 ± 0.11 A
<i>Octopus vulgaris</i> (octopus)	0.80 ± 0.06 A	0.81 ± 0.04 A	0.82 ± 0.07 A
<i>Sepia officinalis</i> (cuttlefish)	0.83 ± 0.11 A	0.84 ± 0.12 A	0.85 ± 0.08 A
<i>Penaeus monodon</i> (shrimp muscle)	0.74 ± 0.13 A	0.75 ± 0.10 A	0.76 ± 0.11 A
<i>P. monodon</i> (shrimp cephalothorax)	0.70 ± 0.05 A	0.71 ± 0.04 A	0.73 ± 0.09 A

^a Data reported as means $\pm$ standard deviations of the determinations in triplicate of two composite samples per radiation dose, each including five adult organisms.

^b Means in the same row with unlike letters differ significantly ($P < 0.05$).

0.05) decreased in a linear trend with the irradiation treatment; and (iv) the proportion of ω -3: ω -6 fatty acids showed no significant differentiation in all the cases, with the exception of cuttlefish muscle at the higher dose (significant increase, $P < 0.05$), so irradiation did not affect the positive impact of high ω -3: ω -6 ratios (Table 3). Previous studies have shown that the decrease of unsaturated fatty acids after irradiation treatment was mainly due to oxidative decay (14). Kanatt et al. (15) reported that fatty acid profiles of total lipids from the rib and leg regions of lamb meat did not show significant changes, whereas they also found a significant decrease in the ratio of PUFA to saturated fatty acids of phospholipids with the irradiation treatment at 2.5 and 5.0 kGy. Dogan et al. (8) reported that the unsaturated acyl chain concentration for high-dose irradiated samples of hazelnut tissues decreased. Badr (1) reported that gamma radiation at a 3.0-kGy dose had no significant effects on

fatty acid profiles of liquid egg yolk lipids, which mainly consisted by saturated fatty acids (35%), monounsaturated fatty acids (45.2%), and linoleic acid (C18:2, ω -6; 16.8%).

Organoleptic characteristics of irradiated molluscs and crustaceans. Irradiation at levels of 2.5 and 4.7 kGy caused no significant ($P > 0.05$) change in the moisture of irradiated molluscs and crustaceans (Table 4). The polyethylene-packaging material used retained the moisture content of the product (16). Changes in textural properties were observed after radiation treatment. Cutting strength and firmness of organisms subjected to radiation processing were lower ($P < 0.05$) only for molluscs as compared with that of nonirradiated samples (Table 5). Kanatt et al. (16) reported the reduction in cutting strength in irradiated marinating shrimp, whereas Lacroix et al. (20) reported that irradiation at 1.8 to 3.6 kGy did not impair the firmness of shrimp. Gamma radiation of crustaceans in Thailand with 2.2 kGy did not significantly ($P > 0.05$) affect the texture of the organisms (10). During radiation treatment, breakage and polymerization of macromolecules was observed, which influenced the mechanical properties of the food (16). Since slope is related to the apparent elastic properties and expressed the measure of stiffness (6), it is demonstrated that the cuttlefish muscle and octopus tentacle slope had a 44% diminution at the higher dose (4.7 kGy), whereas squid slope represented a 27% diminution. Kye et al. (18) observed that the textural change is clearly related to the myofibrillar protein degradation, which may be promoted by the irradiation treatment. Firmness also depends on the distribution of fat and protein and the structural deterioration of the tissue (6).

With the increase in irradiation dose, the *a* (redness) values of squid and cuttlefish mantles and shrimp muscle were not significantly ($P > 0.05$) affected, whereas *a* values of octopus muscle and shrimp cephalothorax showed a significant increase ($P < 0.05$). As doses increased, the *b* (yellowness) value of the shrimp cephalothorax significantly increased ($P < 0.05$). *L* (lightness) values of the organism muscles showed a significant ($P < 0.05$) decrease, except

TABLE 5. Values of texture parameters measured retained at $0 \pm 1^\circ\text{C}^a$

Samples	Parameter	0.0 kGy	2.5 kGy	4.7 kGy
<i>Todarodes sagittatus</i> (squid mantle)	Cutting strength (kg)	2.55 ± 0.21 A	2.07 ± 0.13 B	1.85 ± 0.12 B
	Slope (N/mm)	8.62	6.97	6.24
	Firmness	1,754	1,421	1,270
<i>Octopus vulgaris</i> (octopus tentacles)	Cutting strength (kg)	13.12 ± 0.55 A	11.27 ± 0.53 B	8.53 ± 0.89 C
	Slope (N/mm)	16.34	13.95	9.15
	Firmness	3,268	2,810	2,125
<i>Sepia officinalis</i> (cuttlefish mantle)	Cutting strength (kg)	5.91 ± 0.41 A	4.04 ± 0.10 B	3.30 ± 0.26 C
	Slope (N/mm)	9.65	6.60	5.39
	Firmness	984	673	549
<i>P. monodon</i> (shrimp muscle)	Cutting strength (kg)	1.79 ± 0.34 A	1.73 ± 0.12 A	1.38 ± 0.32 A
	Slope (N/mm)	2.24	2.16	1.72
	Firmness	454	437	350

^a Data reported as means $\pm$ standard deviations of the determinations in triplicate of two composite samples per radiation dose, each including five adult organisms.

^b Means in the same row with unlike letters differ significantly ($P < 0.05$).

TABLE 6. Color values of molluscs and crustacean retained at $0 \pm 1^\circ\text{C}^a$

	Value	0 kGy	2.5 kGy	4.7 kGy
<i>Todarodes sagittatus</i> (squid)	<i>a</i>	$1.24 \pm 0.57 \text{ A}^b$	$1.65 \pm 0.23 \text{ A}$	$2.26 \pm 0.35 \text{ A}$
	<i>b</i>	$-0.57 \pm 0.15 \text{ A}$	$-1.49 \pm 0.21 \text{ B}$	$-0.76 \pm 0.43 \text{ AB}$
	<i>L</i>	$52.95 \pm 0.65 \text{ A}$	$49.57 \pm 0.79 \text{ B}$	$46.83 \pm 0.32 \text{ C}$
	ΔE	0 A	$3.57 \pm 0.60 \text{ B}$	$6.23 \pm 0.26 \text{ C}$
	<i>C</i>	$1.36 \pm 0.58 \text{ A}$	$2.22 \pm 0.16 \text{ B}$	$2.42 \pm 0.19 \text{ B}$
<i>Octopus vulgaris</i> (octopus)	<i>a</i>	$1.87 \pm 0.45 \text{ A}$	$2.26 \pm 0.24 \text{ A}$	$4.08 \pm 0.32 \text{ B}$
	<i>b</i>	$-2.15 \pm 0.55 \text{ AB}$	$-3.43 \pm 0.42 \text{ A}$	$-1.77 \pm 0.76 \text{ B}$
	<i>L</i>	$50.08 \pm 1.65 \text{ A}$	$41.84 \pm 1.37 \text{ B}$	$40.77 \pm 0.86 \text{ B}$
	ΔE	0 A	$8.34 \pm 1.50 \text{ AB}$	$9.59 \pm 0.73 \text{ B}$
	<i>C</i>	$2.89 \pm 0.13 \text{ A}$	$4.11 \pm 0.31 \text{ B}$	$4.49 \pm 0.32 \text{ B}$
<i>Sepia officinalis</i> (cuttlefish)	<i>a</i>	$0.96 \pm 0.52 \text{ A}$	$1.17 \pm 0.39 \text{ A}$	$1.66 \pm 0.91 \text{ A}$
	<i>b</i>	$-2.63 \pm 0.14 \text{ A}$	$-3.69 \pm 0.46 \text{ AB}$	$-3.89 \pm 0.68 \text{ B}$
	<i>L</i>	$47.38 \pm 1.27 \text{ A}$	$43.64 \pm 1.09 \text{ B}$	$41.90 \pm 1.41 \text{ B}$
	ΔE	0 A	$3.93 \pm 0.11 \text{ B}$	$5.74 \pm 1.18 \text{ C}$
	<i>C</i>	$2.77 \pm 0.06 \text{ A}$	$3.89 \pm 0.32 \text{ B}$	$4.26 \pm 0.60 \text{ B}$
<i>Penaeus monodon</i> (shrimp muscle)	<i>a</i>	$3.14 \pm 1.30 \text{ A}$	$1.65 \pm 0.21 \text{ A}$	$1.47 \pm 0.52 \text{ A}$
	<i>b</i>	$4.90 \pm 0.88 \text{ A}$	$2.64 \pm 0.52 \text{ B}$	$3.11 \pm 0.59 \text{ B}$
	<i>L</i>	$50.26 \pm 1.64 \text{ A}$	$46.41 \pm 1.29 \text{ B}$	$44.95 \pm 1.11 \text{ B}$
	ΔE	0 A	$4.83 \pm 1.61 \text{ B}$	$5.91 \pm 0.81 \text{ B}$
	<i>C</i>	$5.87 \pm 1.24 \text{ A}$	$3.09 \pm 0.53 \text{ B}$	$3.44 \pm 0.76 \text{ B}$
<i>P. monodon</i> (shrimp cephalothorax)	<i>a</i>	$2.26 \pm 0.30 \text{ A}$	$4.82 \pm 0.26 \text{ B}$	$4.98 \pm 0.98 \text{ B}$
	<i>b</i>	$4.91 \pm 0.67 \text{ A}$	$7.13 \pm 0.34 \text{ B}$	$10.16 \pm 0.74 \text{ C}$
	<i>L</i>	$48.12 \pm 0.33 \text{ A}$	$48.58 \pm 1.00 \text{ A}$	$53.19 \pm 1.35 \text{ B}$
	ΔE	0 A	$3.50 \pm 0.21 \text{ B}$	$7.81 \pm 0.80 \text{ C}$
	<i>C</i>	$5.41 \pm 0.73 \text{ A}$	$8.61 \pm 0.43 \text{ B}$	$11.31 \pm 0.94 \text{ C}$

^a Data reported as means $\pm$ standard deviations of the determinations in triplicate of two composite samples per radiation dose, each including five adult organisms.

^b Means in the same row with unlike letters differ significantly ($P < 0.05$).

for shrimp cephalothorax; the *L* value was higher ($P < 0.05$) at 4.7 kGy (Table 6). ΔE values (total color change) compared with nonirradiated organisms, had a significant ($P < 0.05$) increase at the higher dose. *C*, chroma, was significantly ($P < 0.05$) higher after the irradiation treatment in mollusc mantles as well as in shrimp cephalothorax, whereas the opposite was observed in shrimp muscle ($P < 0.05$). Farkas (10) reported that radiation treatment of crustaceans in Thailand with 2.2 kGy did not significantly ($P > 0.05$) affect the color and flavor of the organisms, but had an effect ($P < 0.05$) on odor. The changes in *a*, *L*, and ΔE revealed that as dose was increasing, mollusc mantles increased in redness and decreased in lightness. However, there are few scientific reports of the mechanism of the change of the color in irradiated foods (26). The changes in *a*, *L*, and ΔE values in the studied invertebrates might be influenced by the vacuum packaging as well as the packaging material. Du et al. (9) reported that vacuum packaging was helpful in preserving meat color. Luchsinger et al. (23) reported that irradiated vacuum-packaged pork chops appeared redder and were more stable during storage. It is also observed that as radiation dose increased, the color intensity of foods increased (17, 26) due to the formation of colored compounds, assisted by the Maillard reaction. Therefore, irradiation leads to nonenzymatic browning reactions similar to those induced by heat treatment of foods. Du et al. (9) reported that as radiation dose is increasing, the *a* values of irradiated chicken meat appeared redder

when compared with the nonirradiated samples. Liu et al. (22) reported that *a* and ΔE values increased in chicken breasts as a result of a 4.57-kGy dose of ionizing radiation. These reports are in line with the results of mollusc mantles and shrimp cephalothorax.

Allergenicity of irradiated molluscs and crustaceans. Most allergens are reported as resistant to processes. However, food-irradiation technology has been found to alter the structure of epitopes on certain food allergens such as egg, shrimp, and milk, therefore reducing their allergenicity (4, 5, 21). The major mollusc and crustacean allergen is tropomyosin, a muscle protein of about 35 kDa (31), characterized by thermal stability and resistance to proteolysis. The latter is enhanced by an ability to bind ligands, such as metal ions, lipids, or steroids (3). Quantification of allergens in irradiated molluscs and shrimp showed that the amount of tropomyosin and its degraded peptide fragments with the ability to bind IgE was reduced by gamma radiation, depending on the dose of treatment (Table 7). The initial concentration of allergen of the nonirradiated shrimp sample was 0.10 ppm (mg/kg). After treatment with 2.5 and 4.7 kGy, the allergen could no longer be detected (values were below detection limit of 0.05 ppm). Food-irradiation technology was recently applied to shrimp and shown to be considerably effective in reducing its allergenicity (4). Zhenxing et al. (37) reported that irradiated shrimp (*Penaeus vannamei*) protein extracts exhibited a decrease in al-

TABLE 7. Concentration ppm (mg/kg) of tropomyosin detected by sandwich ELISA-based with IgE^a

Radiation dose (kGy)	<i>Todarodes sagittatus</i> (squid)	<i>Octopus vulgaris</i> (octopus)	<i>Sepia officinalis</i> (cuttlefish)	<i>Penaeus monodon</i> (shrimp)
0	0.25 ± 0.02 A ^b	0.13 ± 0.01 A	0.41 ± 0.03 A	0.10 ± 0.01
2.5	0.15 ± 0.01 B	0.10 ± 0.01 B	0.41 ± 0.03 A	— ^c
4.7	— ^c	— ^c	0.31 ± 0.02 B	— ^c

^a Data reported as means ± standard deviations of the determinations in triplicate of two composite samples per radiation dose, each including five adult organisms.

^b Means in the same column with unlike letters differ significantly ($P < 0.05$).

^c Below detection limit (<0.05 ppm).

lergenicity, measured by immunoblot and Ci-ELISA, when the irradiation dose was increased. Shrimp muscle exhibited an increase of allergenicity at a low radiation dose. Moreover, when the dose reached 10 kGy, the allergenicity of shrimp muscle began to decrease. In this study, after treatment with 4.7 kGy, the concentration of allergen decreased to nondetectable levels in irradiated squid and octopus mantles and significantly ($P < 0.05$) reduced but still to detectable levels in irradiated cuttlefish mantle. The results of both the shrimp and the molluscs suggested that there was a definite impact of food irradiation on the detectability of food allergens and indicated that epitopes on allergens were structurally altered by gamma radiation. IgE did not seem to recognize sufficiently the antigen-determinant sites on allergens. This can be explained by the fact that gamma irradiation may either alter the structural conformation of an allergenic protein or it may break it down to smaller molecules, therefore deactivating some of the epitopes on its surface (4, 21). However, it should be noted that some consumers may be sensitive to allergens below the detection limit of current testing methods, and therefore a test result cannot be taken as an indication that food is definitely free from a specific allergen (12).

As a general conclusion, a 4.7-kGy dose of gamma radiation can be used as an effective dose for treating molluscs and crustaceans to ensure their safety and retain their quality characteristics.

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