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Effects of gamma irradiation on chemical, microbial quality and shelf life of shrimp

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HIGHLIGHTS

- We examine the combined effect of irradiation and storage low temperatures on shrimp.
- Irradiation and low temperature storage reduce the bacterial growth in shrimp.
- Irradiation with frozen storage extend the shelf-life of shrimp meat to about 90 days.
- The combined application stabilize the chemical characteristics of shrimp meat

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ABSTRACT

In the present study the combined effect of gamma irradiation (1, 3 and 5 kGy) and storage at two temperatures: refrigeration (+4 °C) and frozen (−18 °C), on the shelf-life extension of fresh shrimp meat was investigated. The study was based on microbiological and physicochemical changes occuring in the shrimp samples. Total volatile base nitrogen values and trimethylamine values for irradiated shrimp samples were significantly lower than non-irradiated samples at both storage temperatures, and the rate of decrease was more pronounced in samples irradiated at the higher dose ($p<0.05$). Thiobarbituric acid values for irradiated shrimp samples were significantly higher than non-irradiated samples at both storage temperatures ($p<0.05$). pH values of shrimp samples were affected significantly by both irradiating dose and storage temperatures ($p<0.05$). Microbial counts for non-irradiated shrimp samples were higher than the respective irradiated samples at both storage temperatures ($p<0.05$). The results revealed that irradiation at high dose (5 kGy) might enhance lipid oxidation, although the growth of microorganisms and protein oxidation was inhibited.

1. Introduction

In spite of the availability of cold-chain and better transport facilities, distribution of fresh fishery products among inland consumers still remains a problem. This is due to the highly perishable nature of fishery products, which is accelerated by the ambient conditions present in tropical countries and possibility of microbial contamination from numerous sources (Gram and Huss, 1996; Ward and Baj, 1988). Fish and shellfish are also known to be carriers of several pathogenic microorganisms which are implicated in food-borne diseases (Garrett et al., 1997).

Along with increasing demand of high quality fish, there is an obvious need for the development of new technologies and efficient seafood preservation methods which permit shelf life extension of these products. Conventional methods of seafood preservation have obvious limitations such as the loss of flavor and freshness associated with canning, the expensive equipment needed for refrigeration, freezing and special packaging such as modified atmosphere wrapping, as well as the steadily rising public concern over food additives (Tewfik et al., 2004). Besides traditional methods used to extend the shelf life of fish and fishery products such as rapid chilling and ice storage (Himelbloom et al., 1994), freezing in low temperature, use of organic acids, anti-microbials (Al-Dagal and Bazarra, 1999; Gelman et al., 2001), modified atmosphere packaging (Masniyom et al., 2002) and ionizing radiation (Venugopal et al., 1999; Savvaidis et al., 2002) have been proposed.

Shrimp is a highly perishable product, and postmortem changes occur rapidly compared with fish. The high content of free amino acids and other soluble non-nitrogenous substances, which partly contribute to the desirable, delicate sweet taste of shrimp (Konosu and Yamaguchi, 1982), can also serve as easily digestible nutrients for microbial growth. Its shelf life and wholesomeness during refrigerated storage and shipping is greatly

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influenced by both enzymatic and microbiological changes (Al-Dagal and Bazarra, 1999; Benner et al., 1994). During the last several years, reliable methods have been developed to extend the shelf life of shrimp and to avoid health hazards for consumers (Al-Dagal and Bazarra, 1999).

Irradiation is recognized as an effective, widely applicable food processing technique. The process exposes foods to a carefully controlled amount of energy in the form of high-speed particles or rays that reduce the risk of food poisoning, control food spoilage and extend the shelf life of food without detriment to health and with minimal effect on nutritional or sensory quality. This process has no effect on food taste, color and smell, and it does not leave radioactive residues (ICGFI, 2002). Combination of treatments for food preservation may result in synergistic or cumulative effects of microbiological barriers or hurdles, leading to a reduced level of one or all the treatments (Leistner and Gorris, 1995). Food irradiation, in combination with good refrigeration and handling practices, might provide a means to increase the shelf life of fish products.

Many researchers have recognized and reported that gamma irradiation at low doses (below 10 kGy) kill most organisms without any deterioration of food quality (Javanmard et al., 2006; Lacroix et al., 1991; Mulder, 1982; Olson, 1998; Thayer et al., 1995). Freezing is known to reduce viable cell counts by 1–2 log units, with extended storage causing additional, time dependent reductions (Yammamoto and Harris, 2001). In this study the effects of gamma irradiation of fresh shrimp meat and its effect on extending shelf life in freezing (−18 °C) and refrigeration (+4 °C) conditions as a combination were examined.

2. Experimental

2.1. Materials

Fresh shrimps were collected from Tekirdag bazaar, Turkey and immediaely packed in polyethylene bags and stored at 4 °C until irradiated. The samples were divided into two groups of four levels of exposure to irradiation. One group was exposed to doses of 0.0 (control) 1.0, 3.0, and 5.0 kGy and subsequently stored at 4 °C, while the second group was exposed to the same doses of radiation and stored at −18 °C.

2.2. Irradiation

Samples were irradiated at the GAMMAPAK Company, Çerkezköy, Tekirdağ, Turkey, using a ^{60}cobalt radiation source (MDS, Nordion, Canada). The doses applied in this study were 1.0, 3.0 and 5.0 kGy. Exposure time was 52, 156 and 260 min (dose rate of 0.02 kGy per min). Samples were maintained at 4 ± 1 °C during irradiation by using sealed ice covering. The absorbed dose was monitored by polymethyl methacrylate dosimeters (Harwell Amber Perspex dosimeter, Batch R Type 3042 Range 1–30 kGy (ISO/ASTM 51276, 2002). The absorbance signal was measured using a Camspec M 201 UV spectrophotometer at 640 nm. The Harwell dosimeters were calibrated using Ceric-Cerous Sulfate dosimeters (ISO/ASTM 51205, 2002) from MDS Nordion that maintains measurement traceability to NIST, USA.

2.3. Storage conditions

After irradiation, the non-irradiated and irradiated shrimps were transported within 1.5 h to the laboratory in packed ice in insulated polystyrene boxes. The first four group of samples were subsequently stored in packed ice in a cold room maintained at 4 °C. The second group of four samples were frozen and stored in frozen ice at −18 °C. The storage of the samples lasted for 90 day.

2.4. Proximate analysis

Moisture, crude protein, lipid and ash contents were measured according to the protocols recommended by AOAC (1990).

2.5. Chemical analyses

2.5.1. Measurement of pH

pH was determined at room temperature on homogenates of filleted samples in distilled water (1/10 w/w) (Vyncke, 1981). pH was monitored using a Hanna instrument pH 211 microprocessor pH meter.

2.5.2. Determination of total volatile basic nitrogen (TVB-N)

TVB-N was determined according to the method of Antonacopoulus and Vyncke (1989). For TVB-N, the sample (10 g) was homogenized with 6% perchloric acid (90 ml) for 1 min in an Ultra-Turrax (IKA T 25 Basic, Staufen, Germany). The homogenates were filtered through a filter paper (Whatman no. 1) and the filtrates were alkalized with NaOH (20%) before distillation. Duplicate filtrates were distilled in a Velp UDK 140 (Milano, Italy) distillation apparatus. The distillate was titrated with 0.01 N HCl. Results of the TVB-N analysis were expressed as mg/100 g sample.

2.5.3. Determination of trimethylamine nitrogen (TMA-N)

TMA-N was determined by the method of AOAC (1998). Ten grams of homogenized samples were weighed, blended with 90 ml of 7.5% trichloracetic acid solution and filtered. The blended solution was fixed with formaldehyde (20%). Four milliliter of extract was transferred into test tubes and 1ml formaldehyde, 10 ml anhydrous toluene, and 3 ml K_2CO_3 solution were added. The tubes were shaken and 5 ml of the toluene layer was pipetted. Five milliliter of picric acid working solution (0.02%) was added. The contents were mixed and transferred to a spectrophotometric cell. Absorbance at 410 nm against a blank was measured. At the same time, standards were prepared and measured. Results of the TMA-N analysis were expressed as mg/100 g sample.

2.5.4. Determination of thiobarbituric acid (TBA)

The distillation method of Weilmeier and Regenstein (2004) was used to determine the degree of lipid oxidation in seafood samples. Lipid oxidation was measured using thiobarbituric acid (TBA values), which are expressed as mg malonaldehyde/kg meat.

2.6. Microbial analysis

Microbial analysis of aerobic mesophilic bacteria, *Staphylococcus aureus*, *Escherichia coli* and coliform count was carried out during storage for shrimp samples as described in the Bacteriological Analytical Manual (AOAC, 1998). Twenty five grams from each sample was cut out aseptically with a sterile pastry cutter and bistoury, diluted 1:10 (w/v), and blended for 2 min with 225 ml of sterile peptone water in a blender. Subsequent dilutions were prepared by mixing a 1 ml of the sample with 9 ml of sterile peptone water. The media and incubations used were plate count agar (PCA, Merck) at 35 °C for 48 h for mesophilic aerobic bacteria; Violet Red Bile Agar (VRBA, Merck) at 37 °C for 48 h for coliform counts; Baird Parker Agar (BPA, Merck) at 37 °C for 48 h for *S. aureus* counts; Tryptone Bile X-glucuronide agar (TBX, Merck) at 44 °C for 24 h for *E. coli* counts. Microbial counts were

expressed as the number of viable bacterial colonies per gram (log CFU/g).

3. Results and discussion

Among the seafood consumed, shrimp, being rich in proteins and minerals, demonstrates an exceptional nutritional value in the human diet. The shrimp used in the study had 23.28% moisture; 10.86% protein; 2.14% crude lipids and 8.13% ash. These values are comparable to those reported in other studies concerning shrimps (Göğüş and Kolsarıcı, 1992; Synowiecki and Al-Khateeb, 2000).

3.1. Chemical analysis of shrimp stored under refrigeration (+4 °C) and ice (−18 °C) storage

3.1.1. pH

TVB-N, TBA, TMA-N contents and pH values of non-irradiated and 1, 3 and 5 kGy irradiated shrimps stored at +4 and −18 °C are shown in Table 1 and Table 2, respectively.

At the first day of refrigerated storage, the pH value was 7.15 for the control samples and 7.33, 7.43 and 7.41 for 1, 3 and 5 kGy irradiated samples, respectively (Table 1). Increasing the applied radiation dose resulted in a concomitant increase in the pH value. During the course of refrigerated storage, the pH values showed an increase for all shrimp samples ($p<0.05$). pH values for non-irradiated and irradiated shrimp samples were in the range of 7.15–7.90 during refrigerated storage.

For shrimp samples stored at −18 °C, the pH values of both control and irradiated shrimp increased significantly ($p<0.05$) between days 0 and 1, with the pH value of the the irradiated sample increasing more than the non-irradiated samples (Table 2). At the end of the storage period of 90 day at −18 °C, statistically significant differences ($p<0.05$) were determined between the control group and the irradiated samples (1, 3 and 5 kGy). pH value of the non-irradiated sample was 7.27 at the begining and 8.33 at the end of the storage period of 90 day, thereby showing an increase in pH with respect to time. Increases in pH indicate the accumulation of alkaline compounds, such as ammonia compounds and trimethylamine, mainly derived from the microbial flora (Schormüller, 1968).

Table 1
Chemical evaluation of control and irradiated shrimp during storage at +4 °C.

	Dose	Storage days	
		1	3
pH	Control	7.15 ± 0.06^{bB}	7.83 ± 0.11^{aA}
	1 kGy	7.33 ± 0.04^{abB}	7.87 ± 0.03^{aA}
	3 kGy	$7,43\pm0.10^{aB}$	7.89 ± 0.27^{aA}
	5 kGy	7.41 ± 0.05^{aB}	7.90 ± 0.05^{aA}
TVB-N	Control	12.19 ± 0.38^{aB}	35.17 ± 2.20^{aA}
	1 kGy	11.38 ± 0.04^{bB}	27.67 ± 0.94^{bA}
	3 kGy	11.33 ± 0.02^{bB}	27.20 ± 0.61^{bA}
	5 kGy	10.75 ± 0.27^{bB}	26.05 ± 0.85^{bA}
TBA	Control	1.21 ± 0.07^{bB}	7.28 ± 0.17^{bA}
	1 kGy	1.34 ± 0.12^{bB}	7.45 ± 0.19^{bA}
	3 kGy	1.75 ± 0.16^{aB}	7.51 ± 0.22^{bA}
	5 kGy	2.04 ± 0.09^{aB}	8.36 ± 0.31^{aA}
TMA-N	Control	2.29 ± 0.08^{aB}	7.71 ± 0.08^{aA}
	1 kGy	2.11 ± 0.02^{bB}	7.50 ± 0.03^{bA}
	3 kGy	2.12 ± 0.01^{bB}	7.55 ± 0.01^{bA}
	5 kGy	2.16 ± 0.01^{bB}	7.62 ± 0.02^{abA}

Values with different letters (A–B) within a row differ significantly ($p<0.05$); values with different letters (a–b) within a column differ significantly ($p<0.05$).

Table 2
Chemical evaluation of control and irradiated shrimp during storage at −18 °C.

	Dose	Storage time (day)							
		1	3	5	7	14	21	30	60	90
pH	Control	7.27 ± 0.09^{bE}	7.69 ± 0.01^{aD}	7.74 ± 0.02^{aE}	7.78 ± 0.01^{aCD}	7.88 ± 0.03^{aC}	7.89 ± 0.01^{aC}	7.91 ± 0.01^{aC}	8.11 ± 0.01^{aB}	8.33 ± 0.04^{aA}
	1 kGy	7.32 ± 0.12^{bC}	7.77 ± 0.13^{aB}	7.72 ± 0.07^{aC}	7.80 ± 0.09^{aB}	7.90 ± 0.06^{aAB}	7.86 ± 0.05^{aB}	7.87 ± 0.03^{aB}	7.94 ± 0.02^{aAB}	8.05 ± 0.05^{bA}
	3 kGy	7.53 ± 0.01^{aD}	7.72 ± 0.08^{aC}	7.75 ± 0.06^{aD}	7.76 ± 0.06^{aC}	7.89 ± 0.05^{aBC}	7.95 ± 0.06^{aAB}	8.03 ± 0.04^{aAB}	8.03 ± 0.06^{aAB}	8.07 ± 0.05^{bA}
	5 kGy	7.61 ± 0.04^{aE}	7.72 ± 0.09^{aDE}	7.75 ± 0.07^{aCDE}	7.77 ± 0.05^{aDE}	7.89 ± 0.05^{aCD}	7.92 ± 0.04^{aABC}	8.02 ± 0.02^{aAB}	8.05 ± 0.03^{aAB}	8.08 ± 0.03^{bA}
TVB-N, mg/100 g	Control	9.41 ± 0.06^{aBC}	9.09 ± 0.05^{aC}	9.19 ± 0.01^{aBC}	9.27 ± 0.07^{aBC}	9.38 ± 0.04^{aBC}	9.46 ± 0.04^{aBC}	9.35 ± 0.07^{aBC}	9.70 ± 0.05^{aB}	12.33 ± 0.44^{aA}
	1 kGy	9.42 ± 0.05^{aB}	9.37 ± 0.12^{aB}	9.23 ± 0.06^{aB}	9.14 ± 0.15^{aB}	9.27 ± 0.06^{aB}	8.98 ± 0.07^{abB}	8.97 ± 0.09^{aB}	9.40 ± 0.18^{abB}	11.27 ± 0.12^{bA}
	3 kGy	9.36 ± 0.04^{aB}	9.20 ± 0.14^{aBC}	9.15 ± 0.13^{aBC}	9.02 ± 0.17^{aBC}	8.78 ± 0.18^{abBC}	8.65 ± 0.32^{bcC}	8.85 ± 0.09^{aBC}	8.95 ± 0.16^{bcBC}	10.73 ± 0.09^{bcA}
	5 kGy	9.16 ± 0.14^{aB}	8.46 ± 0.06^{bC}	8.87 ± 0.04^{aBC}	8.35 ± 0.46^{bC}	8.42 ± 0.41^{bC}	8.30 ± 0.44^{cC}	8.76 ± 0.09^{aBC}	8.73 ± 0.23^{cBC}	10.24 ± 0.05^{cA}
TBA, mg/kg	Control	0.62 ± 0.23^{bB}	0.32 ± 0.15^{cB}	1.08 ± 0.03^{aA}	0.39 ± 0.10^{bB}	0.21 ± 0.06^{aB}	0.24 ± 0.05^{aB}	0.34 ± 0.06^{bB}	0.19 ± 0.06^{aB}	0.27 ± 0.05^{aB}
	1 kGy	0.62 ± 0.06^{bB}	0.65 ± 0.11^{bcB}	1.19 ± 0.04^{aA}	0.49 ± 0.09^{abB}	0.23 ± 0.07^{aB}	0.26 ± 0.05^{aB}	0.36 ± 0.05^{bB}	0.25 ± 0.10^{bB}	0.25 ± 0.03^{aB}
	3 kGy	1.14 ± 0.30^{aAB}	0.80 ± 0.13^{abBC}	1.27 ± 0.09^{aA}	0.52 ± 0.09^{abCD}	0.26 ± 0.07^{aD}	0.30 ± 0.07^{aD}	0.48 ± 0.07^{bCD}	0.30 ± 0.13^{bD}	0.29 ± 0.02^{aD}
	5 kGy	1.21 ± 0.31^{aAB}	1.12 ± 0.18^{aAB}	1.41 ± 0.14^{bA}	0.85 ± 0.08^{aBC}	0.45 ± 0.08^{aC}	0.45 ± 0.07^{aC}	1.07 ± 0.57^{aAB}	0.53 ± 0.30^{bC}	0.51 ± 0.04^{bC}
TMA-N, mg/kg	Control	0.40 ± 0.04^{aI}	0.57 ± 0.03^{aH}	0.88 ± 0.03^{aG}	1.56 ± 0.04^{aF}	1.73 ± 0.04^{aE}	3.08 ± 0.06^{aD}	3.62 ± 0.36^{aC}	3.97 ± 0.06^{aB}	4.41 ± 0.09^{aA}
	1 kGy	0.31 ± 0.04^{aG}	0.46 ± 0.01^{aG}	0.75 ± 0.02^{aF}	1.34 ± 0.04^{bE}	1.49 ± 0.04^{bE}	2.69 ± 0.03^{bD}	3.01 ± 0.10^{cC}	3.33 ± 0.07^{cB}	3.56 ± 0.09^{bA}
	3 kGy	0.32 ± 0.05^{aH}	0.48 ± 0.01^{aG}	0.78 ± 0.02^{aF}	1.39 ± 0.04^{bE}	1.54 ± 0.03^{bE}	2.73 ± 0.04^{bD}	3.14 ± 0.08^{bcC}	3.42 ± 0.07^{bcB}	3.63 ± 0.11^{bA}
	5 kGy	0.35 ± 0.06^{aH}	0.51 ± 0.02^{aG}	0.83 ± 0.02^{aF}	1.40 ± 0.04^{bE}	1.61 ± 0.03^{abD}	2.75 ± 0.04^{bC}	3.27 ± 0.05^{bB}	3.50 ± 0.06^{bA}	3.62 ± 0.09^{bA}

Values with different letters (A–I) within a row differ significantly ($p<0.05$); values with different letters (a–c) within a column differ significantly ($p<0.05$).

3.1.2. TBA

An increase in the TBA value was seen in all the samples during refrigerated storage (Table 1). The initial TBA value of the control sample was 1.21 mg/kg with the value increasing to 7.28 mg/kg on the third day of storage. It was observed that the TBA values of the irradiated groups were higher than the control group during the 3-day-storage period suggesting that lipid oxidation was initiated by gamma irradation and that the TBA values of the shrimps increased in direct proportion to the irradiation dose. According to the statistical analysis results, it was found that the difference among the groups (control, 1, 3 and 5 kGy irradiation dose) was statistically significant ($p<0.05$). In addition, the increase in the TBA value at the end of the 3-day-storage at 4 ± 1 °C was also found to be statistically significant ($p<0.05$). According to Connell (1990), the ideal TBA value should be less than 3 mg malonaldehyde/kg. The proposed TBA limit of 3 mg/kg was exceeded at the end of refrigerated storage (at 4 °C) for both irradiated and non-irradiated samples.

The extent of lipid oxidation in the shrimp during storage at −18 °C is shown in Table 2. The highest TBA value was 1.41 mg/kg after 5 day of storage at −18 °C for samples irradiated at 5 kGy dose. TBA values for all the samples stored at −18 °C were within acceptable limits. The formation of thiobarbituric acid reactive substances (TBARS) did not show a consistent trend during frozen storage for both control and irradiated groups. These variations can be explained as a result of the different phases of decomposition of peroxides, formation of carbonyls and the interaction compounds with nucleophilic molecules present in the shrimp (Aubourg et al., 2004). Similar results have been reported for irradiated sea bass, anchovy and treadfin bream (Özden et al., 2007; Lakshmanan et al., 1999; Jeevanandam et al., 2001; Chouliara et al., 2004).

In the case of the non-irradiated shrimp, the TBA value increased to a maximum during storage up to the 5th day and then decreased. A similar trend was observed in the irradiated shrimp samples as well. Significant differences ($p<0.05$) in the TBA values were found between the control and each irradiated sample during the storage period. This indicates that lipid oxidation in the shrimp increased due to gamma radiation. Seafoods such as saury and mackerel, containing large content of high unsaturated fatty acids, are rapidly oxdized in oxygen and natural light. The decrease in TBA values after day 7 of storage may represent the breakdown of the malonaldehyde to tertiary degradation products.

3.1.3. TVB-N

During the period of refrigerated storage (4 °C), the TVB-N value of shrimp was significantly ($p<0.05$) higher in the non-irradiated control samples than in the irradiated samples. As shown in Table 1, the TVB-N value of the irradiated and non-irradiated shrimps increased significantly ($p<0.05$) between days 1 and 3. The initial TVB-N value in non-irradiated shrimp was 12.19 mg/100 g; the value increased to 35.17 mg/100 g within 3 day of refrigerated storage. On the other hand, irradiation at 1, 3 and 5 kGy suppressed the formation of TVB-N during storage; and values reached 27.67, 27.20 and 26.05 mg/100 g, respectively, after 3 day. This corroborates to data published previously by other groups (Al-Kahtani et al., 1996; Von Amin et al., 1978; Jo et al., 2004).

At the begining of storage at −18 °C, the TVB-N value was 9.41 mg/100 g for the control sample and 9.42, 9.36 and 9.16 mg/100 g, respectively, for the samples irradiated 1, 3 and 5 kGy. However, the TVB-N values showed a different trend with the progression of the storage period and actually increased at the end of storage for 90 day (−18 °C). The TVB-N values were 12.33, 11.27, 10.73 and 10.24 mg/100 g for non-irradiated and 1, 3 and 5 kGy irradiated shrimp samples, respectively, at the end of the storage period of 90 day (−18 °C). The TVB-N value of the irradiated samples was lower than control samples after storage for 7 day.

The statistical analysis of the TVB-N data showed that significant differences were found between control and each irradiated sample stored at −18 °C after 90 day of storage ($p<0.05$).

TVB-N levels for non-irradiated and irradiated shrimp samples did not exceed 35 mg/100 g at both storage temperatures (except the refrigerated control sample after 3 day), which is considered to be the maximum acceptable level for fish (Huss 1988). This result is in aggrement with TVB-N levels of irradiated Chinese pomfret, *Pampus chinensis*, stored in ice as reported by Ahmed et al. (2009). However, Çaklı et al. (2006a), (2006b) reported TVB-N levels of 39.89 and 35 mg/100 g, respectively, for sea bass after 18 day and 14 day of storage on ice. Chouliara et al. (2005) reported that the initial TVB-N levels of vacuum packed irradiated (1–3 kGy) sea bream samples stored under refrigeration were 27.5, 27.3 and 25.1 mg/100 g, reaching the acceptable limits at day 10 in control samples, and at day 21 and 28 for 1 and 3 kGy irradiated samples. Van Cleemput et al. (1980) reported a lower TVB-N level in irradiated shrimp than the non-irradiated samples during the storage at 4 °C. Similarly, lower TVB-N levels in the irradiated samples compared to control non-irradiated samples were reported by Jo et al. (2004) in irradiated fish and Al-Kahtani et al. (1996) in irradiated tilapia and mackerel. Mendes et al. (2005) reported that the initial TVB-N level of 15.6 mg/100 g in chilled horse mackerel reached the limit levels of 30–35 mg/100 g at day 12 in the control unirradiated samples whereas the 1 and 3 kGy irradiated samples reached TVB-N levels of 13.6, and 12.7 mg/100 g, respectively, at day 20.

It can be said that the combination of irradiation and low temperature preservation used in the current study resulted in very low levels of TVB-N even after a considerable period of 90 day storage at −18 °C and after 3 day of refrigerated storage at 4 °C.

3.1.4. TMA-N

TMA-N is considered as a valuable tool in the evaluation of fish quality because of its rapid accumulation in muscle under refrigerated conditions (Gökodlu et al.,1998). In this study, initial average values were below 1 mg TMA-N/100 g, indicating the freshness of the initial product. TMA-N levels increased in all samples ($p<0.05$) but the values in the irradiated groups remained lower than that in the non-irradiated samples both during storage at −18 °C for 90 day and at 4 °C for 3 day.

The initial TMA-N level in whole shrimp was as low as 2 mg per 1000 g; subsequently TMA-N levels rose significantly ($p<0.05$) in both the control and irradiated samples after 3 day of refrigerated storage period, but remained lower than the rejection limit. TMA-N levels of irradiated shrimps were lower than the control samples (Table 1).

In the samples that were stored at −18 °C, the initial average value of TMA-N was found to be 0.4 mgN/kg in the control samples and 0.31, 0.32 and 0.35 mg/kg in the 1, 3 and 5 kGy irradiated shrimps, respectively. The final TMA-N values at the end of the 90 day storage period were significantly higher ($p<0.05$) at 4.41 mg/kg (control), 3.56 mg/kg (1 kGy), 3.63 mg/kg (3 kGy) and 3.62 mg/kg (5 kGy). However, these concentrations are within the acceptable limits for both frozen (90 day storage) and refrigerated (3 day storage) irradiated and control samples. Similarly, low TMA-N values have been reported for whole fresh fish stored in ice (Papadopoulos et al., 2003; Kyrana and Lougovois, 2002; Tejada and Huidobro, 2002). The suppression of TMA-N production in sea bream and horse mackerel following low dose irradiation reported by Chouliara et al. (2005) as well as the lower TMA-N values for

irradiated samples as compared with control samples stored at 4 °C reported by Mendes et al. (2005) are both in agreement with the findings of the current study.

Spinelli and Pelroy (1969) and Ahmed et al. (1997) reported that the rate of formation of TMA-N and TVB-N was reduced in irradiated fish compared to non-irradiated samples because of the radiation sensitivity of *Pseudomonas* and *Shewenellas*, which are the microorganisms responsible for the decomposition of trimethylamine oxide. Conell (1975) and Huss (1986) suggested that the TMA-N values between 10–15 mg N/100 g of fish muscle were at the upper limit of acceptability while according to Yanamura (1938), the acceptable limit of TMA-N was assigned as 30 mg N/100 g for marine fish.

Microbial activities are also implicated in the formation chemical compounds (TVB-N, TMA-N, etc.), which are suggested as indicators of fish quality (Debevere and Boskou, 1996; Gram and Huss, 1996). The amounts of TMA-N and TVB-N in fish are considered by many workers as the most promising indices of spoilage of fresh marine fish (Conell, 1975). TMA-N content varies with species, season and type of storage (Ababouch et al., 1996). TVB-N and TMA-N values for irradiated shrimp samples were significantly lower than non-irradiated samples in both storage temperatures, which may be attributed to the reduction of microbial populations and the rate of decrease was more pronounced in samples irradiated at higher dose ($p<0.05$). The amounts of these compounds are correlated with increasing viable microbial counts.

From the present investigation it was found that the irradiated shrimps when stored at −18 °C for 90 day showed acceptable quality indices, which is most likely due to the synergistic effect of the two preservation methods.

Table 3
Change in microbial counts of shrimp after gamma irradiation and storage at 4 °C ($\log_{10}$ cfu/g).

	Dose	Day of storage	
		1	3
Total mesophiles	Control	6.33 ± 0.08^{aB}	7.18 ± 0.07^{aA}
	1 kGy	$4.34\pm0,05^{bB}$	4.74 ± 0.08^{bA}
	3 kGy	3.51 ± 0.28^{cA}	4.32 ± 0.14^{cA}
	5 kGy	1.73 ± 0.89^{dB}	3.43 ± 0.16^{dA}
Total coliformes	Control	3.56 ± 0.04^{aB}	4.40 ± 0.06^{aA}
	1 kGy	2.53 ± 0.15^{aB}	3.61 ± 0.15^{bA}
	3 kGy	0.77 ± 0.77^{bB}	2.68 ± 0.20^{cA}
	5 kGy	0.00 ± 0.00^{bA}	0.00 ± 0.00^{dA}
E. coli	Control	3.86 ± 0.04^{aB}	4.20 ± 0.04^{aA}
	1 kGy	2.48 ± 0.09^{bB}	3.07 ± 0.07^{aA}
	3 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}
	5 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}
S. aureus	Control	3.45 ± 0.07^{aA}	4.49 ± 0.40^{aA}
	1 kGy	3.02 ± 0.10^{bA}	$2,20\pm0.14^{bB}$
	3 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
	5 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}

Values with different letters (A–B) within a row differ significantly ($p<0.05$); values with different letters (a–c) within a column differ significantly ($p<0.05$).

3.2. Microbiological analysis of shrimp stored under refrigeration (+4 °C) and ice (−18 °C) storage

The present study focused on the monitoring of the following microorganisms: mesophilic aeorobic bacteria, total coliforms, *E. coli* and *S. aureus* (Table 3 and Table 4). The initial counts of mesophilic bacteria, coliforms, and *E. coli* in non-irradiated shrimps were 6.22 log cfu/g, 3.45 log cfu/g and 3.30 log cfu/g, respectively, (day 0), while those of *S. aureus* were 2.50 log cfu/g.

The effects of irradiation and refrigerated storage (4 °C) on microbial counts in the shrimp samples are presented in Table 3. The number of aerobic plate counts (APC), total coliforms, *E. coli* and *S. aureus* decreased with increase of irradiation dose but increased following 3 day of storage at 4 °C (Table 3). The level of viable microorganisms decreased immediately after irradiation, depending on the absorbed dose. *E. coli* and *S. aureus* were not observed in 3 and 5 kGy irradiated shrimp immediated after irradiation as well as during storage. Coliform was also not detected in 5 kGy irradiated shrimps. Irradiation doses of 1, 3 and 5 kGy produced immediate reduction of 2, 3 and 5 log units of APCs, respectively, in shrimp. Irradiation doses ranging from 1 to 3 kGy have been suggested for shelf life extension of

Table 4
Change in microbial counts of shrimp after gamma irradiation and storage in ice (−18 °C) ($\log_{10}$ cfu/g).

	Dose	Storage time (day)								
		1	3	5	7	14	21	30	60	90
TMB	Control	6.33 ± 0.08^{aB}	6.45 ± 0.09^{aB}	6.53 ± 0.06^{aAB}	5.51 ± 0.07^{aC}	5.74 ± 0.11^{aBC}	5.87 ± 0.07^{aBC}	6.09 ± 0.06^{aBC}	6.39 ± 0.07^{aB}	7.26 ± 0.12^{aA}
	1 kGy	4.34 ± 0.05^{bA}	3.29 ± 0.10^{bC}	4.28 ± 0.07^{bAB}	3.41 ± 0.17^{bC}	$3.49\pm0.14B^{bC}$	4.28 ± 0.12^{bAB}	3.49 ± 0.08^{bBC}	3.60 ± 0.10^{bABC}	3.85 ± 0.14^{bABC}
	3 kGy	3.17 ± 0.60^{cA}	3.91 ± 0.14^{bA}	3.45 ± 0.18^{cA}	3.16 ± 0.09^{bA}	3.11 ± 0.16^{bA}	3.26 ± 0.14^{cA}	3.05 ± 0.10^{bcA}	3.14 ± 0.08^{bA}	3.27 ± 0.07^{bcA}
	5 kGy	1.74 ± 0.89^{dB}	1.69 ± 0.55^{cB}	1.63 ± 0.81^{dB}	1.71 ± 0.85^{cB}	2.72 ± 0.07^{bA}	2.69 ± 0.21^{dA}	2.33 ± 0.20^{cAB}	2.20 ± 0.10^{cAB}	2.59 ± 0.06^{cA}
TCB	Control	3.51 ± 0.05^{aA}	3.56 ± 0.07^{aA}	3.63 ± 0.09^{aA}	3.40 ± 0.07^{aA}	3.56 ± 0.07^{aA}	3.59 ± 0.06^{aA}	3.61 ± 0.06^{aA}	3.76 ± 0.07^{aA}	3.82 ± 0.07^{aA}
	1 kGy	2.49 ± 0.25^{bA}	2.52 ± 0.04^{bA}	2.49 ± 0.06^{bA}	2.10 ± 0.10^{bB}	2.00 ± 0.00^{bB}	2.15 ± 0.67^{bB}	2.28 ± 0.08^{bB}	2.57 ± 0.07^{bA}	2.64 ± 0.11^{bA}
	3 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
	5 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
E. coli	Control	3.38 ± 0.04^{aCD}	3.46 ± 0.03^{aCD}	3.94 ± 0.07^{aABC}	3.78 ± 0.04^{aBC}	3.58 ± 0.02^{aAB}	3.17 ± 0.08^{aD}	3.41 ± 0.05^{aCD}	3.77 ± 0.06^{aBCD}	4.44 ± 0.04^{aA}
	1 kGy	0.00 ± 0.00^{bC}	2.10 ± 0.10^{bAB}	2.35 ± 0.27^{bAB}	2.16 ± 0.16^{bAB}	2.69 ± 0.05^{bA}	2.10 ± 0.10^{bAB}	2.06 ± 0.06^{bB}	2.38 ± 0.14^{bAB}	2.64 ± 0.20^{bAB}
	3 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
	5 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}	0.00 ± 0.00^{cA}
S. aureus	Control	2.55 ± 0.17^{aCD}	2.29 ± 0.21^{aD}	3.46 ± 0.24^{aA}	3.01 ± 0.43^{aABC}	3.16 ± 0.35^{aAB}	3.09 ± 0.08^{aABC}	2.89 ± 0.06^{aBC}	2.75 ± 0.11^{aBCD}	2.33 ± 0.20^{aD}
	1 kGy	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}
	3 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}
	5 kGy	0.00 ± 0.00^{cA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}

Values with different letters (A–D) within a row differ significantly ($p<0.05$); values with different letters (a–c) within a column differ significantly ($p<0.05$).

fresh fish (Venugopal et al., 1999; Molins et al., 2001; Jo et al., 2004). Chen et al. (1996), Ouattara et al. (2001), Mendes et al. (2005) reported that mesophilic bacteria count of irradiated shrimp, crab and fish were lower than those in non-irradiated samples during the storage at 4 °C.

The effects of the radiation treatment and frozen storage on the survival of total bacterial count, total coliforms, *E. coli* and *S. aureus* in shrimp are shown in Table 4. In this study, irradiation and frozen storage were more effective than either treatment alone in decreasing total, coliform, *S. auresus* and *E. coli* counts. Irradiation reduced the bacterial population in a dose-dependent manner (Table 4).

Total aerobic plate count (APC) in fishery products is a useful tool for quality evaluation of shelf-life and post-processing contamination. Psychrotropic bacteria are especially a major group of microorganisms responsible for spoilage of fresh seafood (Huss, 1994). Total bacterial count (TBC) presented in Table 4 shows that at the beginning of the storage period, bacterial growths were affected by the radiation. The level of viable microorganisms decreased immediately after irradiation, depending upon the absorbed dose. The initial bacterial load of the control sample was 6.33 log cfu/g whereas the values for 1, 3 and 5 kGy irradiated shrimps were 4.34, 3.17, 1.74 log cfu/g, respectively. After a storage period of 90 day, this value increased to 7.26 log cfu/g in the control sample, 3.85 log cfu/g in 1 kGy, 3.27 log cfu/g in 3 kGy and 2.59 log cfu/g in 5 kGy samples stored at −18 °C.

The total microbial count limit recommended by the ICMSF—International Commission of Microbiological Specifications for Foods Bulletin, ICMSF (1986) is 5.70–6.00 $\log_{10}$ cfu/g for frozen shellfishes. Hence, the TBC values reported in the present investigation suggest that the irradiated samples remain within acceptable limits after storage for 90 day at −18 °C.

The initial coliform bacterial load of the control was 3.51 log cfu/g followed by the 1 kGy irradiated shrimps 2.49 log cfu/g. After storage for 90 day at −18 °C, this value increased to 3.82 log cfu/g in the control sample and to 2.64 log cfu/g in the 1 kGy irradiated sample. At 3 and 5 kGy radiation the samples were completely sterilized resulting no coliform bacterial growth just after irradiation and during the storage at −18 °C for 90 day.

E. coli counts in the non-irradiated control samples were 3.38 log cfu/g, but irradiation at 1, 3 and 5 kGy showed no viable cell growth of *E. coli* just after irradiation at the detection limit of this study (10^1 cfu/g). During the storage, *E. coli* counts were 2.1 log cfu/g for the 1 kGy irradiated samples after 3 day of storage and reached to 2.64 cfu/g after 90 day of storage. However *E. coli* counts were not detected throughout the storage period for the 3 and 5 kGy irradiated samples.

Analysis of *S. aureus* counts indicated the presence of this microorganism initially only in the non-irradiated samples. However no *S. aureus* counts were detected in the irradiated samples during the storage period. Irradiation at 1 kGy was enough for the complete elimination of *S. aureus* in shrimps making *S. aureus* more sensitive to radiation than *E. coli*.

Irradiation was found to inhibit microbial proliferation in fish and seafood (Radomyski et al., 1994). Bayizit et al. (2003) found the *S. aureus* number to be between 2.88 and 3.28 log cfu/g in their study on frozen shrimps. Cozzo-Siqueira et al. (2003) did not find *S. aureus* in the irradiated and non irradiated samples of the Tilapia (*Oreochromis niloticus*) fish which were irradiated at different doses (1.0; 2.2 and 5 kGy) and stored for 20–30 day at 0.5 °C and −2 °C.

In the irradiated samples, there was a dose-dependent reduction in the viable cells immediately after irradiation. No viable cells of *S. aureus*, *E. coli* and total coliform were detected immediately after irradiation at 3 and 5 kGy as well as during ice storage at −18 °C. The results indicated that irradiation at 3 kGy or above was effective in securing the microbial safety of the shrimps.

Generally, just after irradiation, at doses of 1, 3 and 5 kGy, the microbial charge was significantly reduced ($p < 0.05$) and irradiated samples showed a good microbiological quality and *E. coli* (except 1 kGy) and *S. aureus* were absent during the storage period.

4. Conclusion

The results obtained from this study showed that the combination of irradiation and refrigated or frozen storage resulted in a significant reduction of bacterial growth and irradiation at 3 and 5 kGy dose with frozen (−18 °C) or refrigerated (+4 °C) storage could inhibit pathogen growth completely. Results showed that the employed radiation dose (1–5 kGy) in conjunction with frozen storage extended the shelf-life of shrimp meats to about 90 day. As chemical quality parameters, the levels of pH, TBA, TVB-N and TMA-N in irradiated and non-irradiated shrimp samples were also examined. Irradiated samples of shrimp had significantly lower concentrations of TVB-N and TMA-N during their refrigerated and ice storage as compared with the controls, which may be attributed to the reduction of microbial populations. These parameters were within acceptable limits until the end of both ice and refrigerated storage in irradated and control samples. The results revealed that irradiation at high dose (5 kGy) might enhance lipid oxidation, although the growth of microorganisms and protein oxidation was inhibited. In conclusion, the results demonstrated that combination of irradiation and low temperature storage resulted in a significant reduction of bacterial growth and stabilized the chemical characteristics of shrimp meat.

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