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Effect of Low-dose Gamma Irradiation on the Quality of Tilapia Fish Muscle with Storage at 0 ℃

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Abstract The effect of gamma irradiation, followed by storage in ice, on Tilapia (*Oreochromis sp*.) fillets was investigated by monitoring microbiological and chemical changes after low-dose irradiation (1 and 3 kGy). Control and irradiated samples were stored in ice, and were analyzed at 7-day intervals. Bacterial counts showed that the shelf-life of 3 kGy irradiated Tilapia was extended to 70 days, and 1 kGy irradiated Tilapia had a shelf-life of 56 days. By comparison, control samples had a shelf-life of 7 days. Coliform bacteria was eliminated by 1 kGy irradiation, and was not detected again during the study. Peroxide and thiobarbituric acid levels increased up to the 28th day of storage, and then underwent reductions. The percentage of free fatty acid increased with the duration of storage in each treatment. Saturated fatty acids were significantly reduced with 1 kGy irradiation, whereas 3 kGy and control samples didn't fluctuate. Levels of monounsaturated fatty acids were significantly increased in irradiated Tilapia, whereas control samples showed reductions with storage. Polyunsaturated fatty acids in Tilapia muscle treated with 1 kGy irradiation showed significant increases over time; however, with a 3 kGy irradiation dose, significant reductions were observed with storage. Palmitic acid (C 16:0), linoleic acid (C 18:2), and docosahexaenoic acid (C 22:6) showed significant reductions with storage. The present study indicates that 1 kGy is the safest irradiation dose for the preservation of Tilapia muscle stored in ice.

Keywords: *gamma irradiation, tilapia, bacterial count, coliform, thiobarbituric acid, fatty acids*

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1. Introduction

Fish is one of the most valuable sources of food available for human consumption, providing significant amount of animal protein, lipids, vitamins and minerals [1]. Omega 3 polyunsaturated fatty acid (n-3 PUFA) in fish has shown beneficial effects on human health such as curative and preventive effects on cardiovascular diseases, mortality and neurodevelopment in infants [2,3,4]. However, fish meat is a highly perishable product that spoils rapidly due to microbiological activity, chemical oxidation of lipids, and autolysis [5]. Polyunsaturated fatty acids (PUFAs) increase susceptibility to lipid oxidation, thus leads to negative effects on quality parameters, such as flavour, colour, texture and nutritive value [6].

With the growth of global population, and issues surrounding the storage and transportation of food items from one place to another, improved preservation techniques are needed to lengthen the shelf-life of food and maintain its nutritional value, texture, and flavor [7]. Fish meat has a relatively short commercial life unless it undergoes an appropriate means of preservation soon after capture [8]. Food irradiation is a process that has proven successful in this regard, not only ensuring the safety of the meat, but also extending its shelf-life because of its effectiveness in inactivating pathogens without a concomitant decrease in product quality [9]. Irradiation is a non-thermal process that is approved for minimizing food-deteriorating and pathogenic microorganisms, and extending shelf life of food in many countries [10]. Gamma irradiation is a process that has the potential to extend refrigerated shelf-life and decontaminate fish products [11,12,13,14]. In a study of Mediterranean Sea bass (*Dicentrarchus labrax L*.) irradiated up to 3 kGy and stored at 1 ℃ were protected from degradation of membrane lipids [15]. Studies conducted to identify the combined effect of low dose irradiation (1 and 3 kGy) and refrigeration on the shelf-life of vacuum-packed sea bream (*Sparus aurata*) fillets by monitoring microbiological, chemical, and sensory changes of control and irradiated samples [16]. The acceptability scores (sensory evaluation) indicated that vacuum-packed sea bream had a shelf-life of 28 days (3 kGy), compared with a shelf-life of 9 to 10 days for non-irradiated samples.

Tilapia is a freshwater fish species that boasts one of the highest commercial values on the world market [17]. Even in Sri Lanka, it is in high demand in rural and urban area for its ability to alleviate malnutrition and poverty,

especially in the inland areas. However, processing of tilapia for national and international marketing is very limited due to limited shelf-life of the product. Even though several processing and preservation techniques such as vacuum packing and freezing are being used, the requirement of novel technology is exists to ensure the quality and safety of the fish product. In this regards, irradiation processing of tilapia can be an excellent treatment for extending the shelf-life, but no information available on the application of the technology on tilapia.

The aim of this study was to evaluate the combined effect of low-dose gamma irradiation followed by storage in ice to preserve Tilapia fish fillets (*Oreochromis sp.*), by assessing the variation of fatty acids and some other microbiological and chemical parameters.

2. Materials and Methods

2.1. Fish Samples

Fresh Tilapia (*Oreochromis sp.*) were collected from local fishermen in the Mahakanadarawa tank, Mihintale, Sri Lanka. Samples were degutted, deheaded, filleted, and packed in sealed polythene bags. Each pack consisted of 80 ± 20 g fish fillet. These packs were divided into three lots; control samples (non-irradiated), and 1- or 3-kGy irradiated samples (n = 12 for each), and packed in polystyrene boxes with ice.

2.2. Gamma Irradiation

Irradiation was conducted at the Sri Lanka Gamma Center, Atomic Energy Board of Sri Lanka, Biyagama, using a split-type source plaque with product overlap geometry that had a source-loading capacity up to 249900 Ci. It was a category IV device, with panaromic wet source storage. Metal double capsule Co-60 were used as a source for irradiation. The dose rate was 0.28 kGy per hour and doses of 1 or 3 kGy were administered. The absorbed dose was monitored by polymethyl methacrylate type dosimeters (Harwell Amber Perspex dosimeter, batch v type 3042, range-1-30 kGy, UK). The absorbance signal was measured using a UV visible spectrophotometer (UV-3600 Shimadzu) at 603 nm. Dose readings were 1.01 ±0.08 kGy and 3.01 ±0.12 kGy for 1 kGy and 3 kGy, respectively. The samples were stored in ice, insulated with polystyrene boxes under refrigerated conditions, and analyzed at 7-day intervals, up to the 77th day of storage.

2.3. Microbiological Analysis

Bacteriological changes were estimated using the total plate count (TPC) technique, following the ISO 4833:2003 standard. A 10-g fish sample was aseptically withdrawn and suspended in 90 mL of sterile maximum-recovery diluent (MRD). The sample was homogenized using a laboratory ~~blender~~ stomacher blender. Further decimal dilutions were made, and then plates were prepared using the pour-plate method in duplicate. The plates were incubated for 2 days at 37 ℃. The coliform bacteria count was carried out using same dilution series, via the most-probable number (MPN) technique. MacConkey broth, brilliant green bile broth and eosin methylene blue agar were used for analysis.

2.4. Lipid Extraction

A 25-g sample was homogenized with 75 mL of a methanol:chloroform mixer (50:25) for 2 minutes [18]. The homogenate was added to 25 mL of chloroform and homogenized for 30 seconds. Then, 25 mL of distilled water was added and the sample was homogenized again for 30 seconds. The homogenate was centrifuged at 3000 rpm for 15 minutes. The chloroform phase was drained off into a 50-mL volumetric flask and topped up with chloroform.

2.5. Peroxide Value

The peroxide value (PV) was determined according to methods outlined by the Association of Official Analytical Chemists [19]. The chloroform extract of lipids was mixed with acetic acid and saturated potassium iodide, and then titrated with standard sodium thiosulphate solution in the presence of starch.

2.6. Thiobarbituric Acid Reactive Substances Value

A 1-g muscle sample was homogenized with 10 mL of 0.15 KCl and 0.1 mM of butylated hydroxy toluene (BHT). Then, 0.5 mL of homogenate was incubated with 1% (w/v) 2-thiobarbituric acid in 0.25 mL of 50 mM NaOH and 2.8% (w/v) trichloroacetic acid (0.25 mL) in a boiling water bath for 10 minutes [20]. After cooling at room temperature for 20 minutes, the pink chromogen was extracted using 2 mL of n-butanol. Absorbance was measured at 532 nm against a blank of n-butanol. Concentrations were calculated using 1, 1, 3, 3-tetraethoxypropane (0-0.8 μM) as the standard. Results were expressed in terms of malonaldehyde (MA) mg per kg of meat (Thiobarbituric Acid, TBA; 1 mg of MA per kg of meat = 1 TBA unit).

2.7. Free Fatty Acid Value

Free fatty acid (FFA) values were determined using the titration method, with standard NaOH solution, according to the method described in Association of Official Analytical Chemists [21].

2.8. Fatty Acid Composition

Fatty acid composition was determined by gas chromatography. The fatty acids were converted to fatty acid methyl esters (FAME), which were analyzed using a Agilent 7890B gas chromatograph equipped with a DB WAX (Agilent 122-7032) capillary column (30 m x 250 μm internal diameter, 0.25-μm film thickness) and flame ionization detector (FID). The injector and the detector temperatures were 240 ℃ and 250 ℃, respectively. The operating conditions for gas chromatography were as follows: initial oven temperature, 160 ℃ for 10 minutes, rising to 190 ℃ at a rate of 3 ℃/min and held for 5 minutes, rising to 230 ℃ at 8 ℃/min and a final hold time of 12 minutes. The flow rate was 25 mL/min. The carrier gas

was helium. The eluted peaks were identified via comparison with commercial standards (Qualimix 89-5560, Larodane Fine Chemicals, Sweden).

2.9. Statistical Analysis

To evaluate the effects of irradiation and storage time, data were subjected to a Type III sum of squares analysis of variance (ANOVA). The analysis was performed using the general linear model (GLM) procedure of SPSS software, version 23. Significant differences between the means were evaluated using Tukey HSD post hoc tests. In addition, a linear discriminant analysis (LDA) was carried out to assess the influence of storage time or irradiation dose on different parameters (total plate count, PV, thiobarbituric acid value, FFA value, fatty acid composition).

3. Results and Discussion

3.1. Effect of Gamma Irradiation on Microbial Load of Tilapia Stored in Ice

Total microbial counts in the muscle of Tilapia are presented in Figure 1. After irradiation, Tilapia muscle showed a reduction in microbial count.

The non-irradiated samples showed rapid growth of microbes with storage duration compared to irradiated samples. The irradiated samples showed a steady increase for part of the storage period, followed by a sharp increase in microbial growth. At the 1-kGy dose, the duration reached 49 days of storage and at 3 kGy it extended to 56 days of storage in ice.

According to the International Commission on Microbiological Specifications for Food of the International Union of Microbiological Societies (ICMSF), the acceptable limit of microbes in fish flesh is 5 x 10^5 cfu g^{-1}. This value was exceeded in the control sample at 7 days of storage in ice. Of the irradiated samples, 3-kGy irradiated muscle had the longest time period of acceptability, according to the criterion for acceptable microbial counts, which was 70 days of storage in ice. The low-dose 1-kGy irradiation treatment also showed reduction for microbial growth and extended the duration of acceptable storage up to 56 days. Similar results have been reported for Atlantic horse mackerel (*Trachurus trachurus*) after irradiation [22]. Control samples, and samples irradiated at 1 and 3 kGy, were stored on ice for 23 days. The control lot had a microbiological shelf-life of 13 days whereas the irradiated samples reached 23 days of storage [22]. In another study, Chinese pomfret (*Pampus chinensis*) was exposed to 3, 5, and 8 kGy of irradiation and stored at low temperature (-20 ℃) for 90 days. The results showed that the irradiated samples remained acceptable after 90 days at -20 ℃ [13]. In a study of climbing perch assessed during storage at -20 ℃ for microbial quality remained acceptable after 60 days [14]. Irradiation at 3 and 5 kGy has also been reported to significantly decrease microbial loads and improve microbiological safety in dry, salted ribbon fish over a 9-month period of storage [23]. In another study, vacuum-packed refrigerated sea bream reached the criterion of acceptability after 9, 18, and 26 days, following irradiation with 0, 1, or 3 kGy doses, respectively [16].

Coliform bacteria were found only in non-irradiated samples. Both irradiation doses eliminated coliform bacteria from the samples. At 14 days of storage, the coliform bacteria count was higher in non-irradiated samples but it was reduced with prolonged storage time (Table 1). After 42 days of storage, coliform bacteria were reduced to zero, potentially due to increases in other bacterial species under favorable conditions.

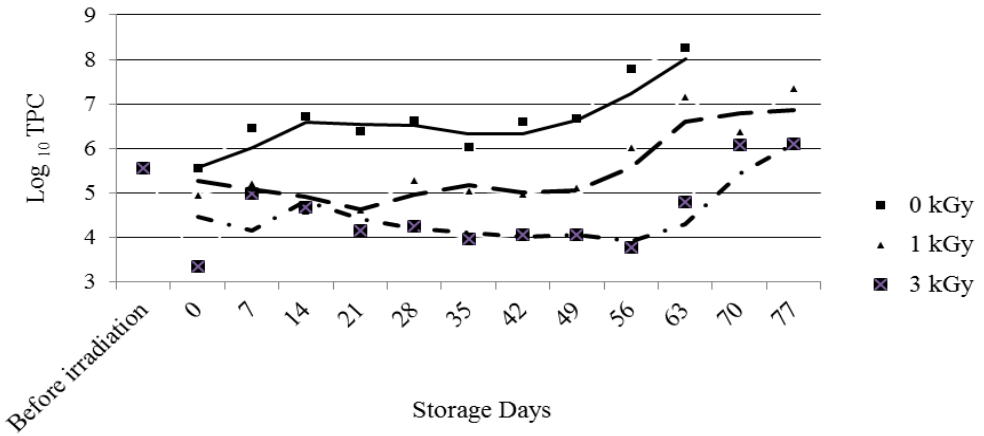

Figure 1. Variation of total bacterial count in irradiated tilapia during the storage

Table 1. Total Coliform Count in Tilapia Fish Muscle Stored on Ice

Duration (Days)	Control (MPN/g)	1 kGy (MPN/g)	3 kGy (MPN/g)
0	93	ND	ND
14	1100	ND	ND
28	11	ND	ND
42	3.6	ND	ND
56	ND	ND	ND
70	ND	ND	ND

ND-Not Detected.

That is, the environment may not have been favorable for proliferation of coliform bacteria. The results revealed that coliform bacteria are highly sensitive to irradiation, which can eliminate coliform from fish muscle at low doses. In a study of gamma-irradiated, frozen chicken meat, 46.6% of samples irradiated at a dose of 0.75 kGy were positive for *E.coli*. No *E.coli* was observed in samples irradiated at doses of 3 and 5 kGy, and no growth was observed in samples irradiated at doses above 2 kGy [24].

3.2. Effect of Gamma Irradiation on Lipid Oxidation and Hydrolysis

The effects of 1- and 3-kGy irradiation doses on PV are shown in Figure 2.

There was no significant effect of dose on PV (Table 2). According to the Figure 2, PV increased with storage time up to 28 days and fluctuated with time and dose. The GLM-ANOVA revealed a significant storage day x dose interaction (Table 2).

Between 0 days of storage vs 14 or 28 days, there were significant differences in PV (p = 0.002 and 0.000, respectively). After 28 days of storage, PV showed significant reductions with respect to day of storage. For example, between 28 days vs 42, 56, or 70 days, there were significant reductions in PV (p = 0.011, 0.020, and 0.021, respectively).

The results of this study are in agreement with the findings of other studies that have reported increases in oxidation activity and lipid peroxidation as a result of both radiation treatment and storage time on meat and meat products [25,26,27,28]. In a study of ground beef irradiated at 1, 2.5, or 5 kGy and monitored over a storage period of 16 days at 4 ℃, increases in peroxides were resulted from irradiation [29]. However, a study of gamma irradiated, frozen chicken meat in Iran revealed that there was no significant difference between irradiated and control groups [24].

Figure 3 illustrates variation in TBARS with storage of irradiated and non-irradiated Tilapia muscle. The TBARS values of samples irradiated at 1 and 3 kGy decreased just after irradiation. Then, the values gradually increased over time.

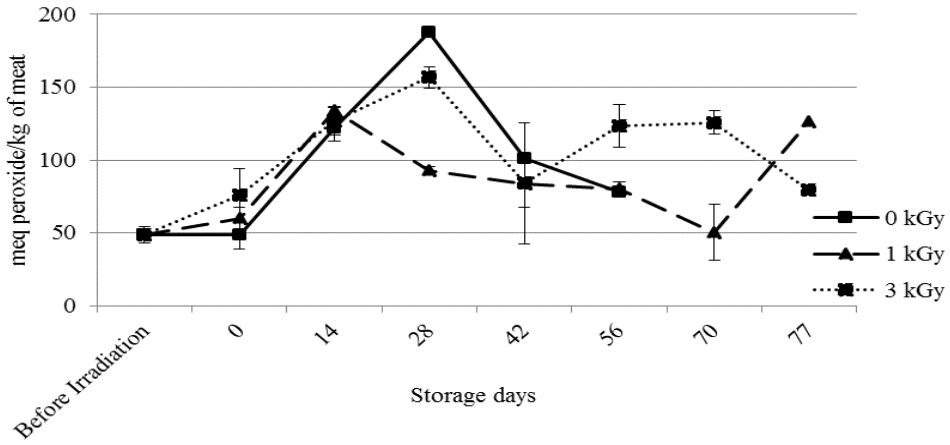

Figure 2. Variation of Peroxide Value in gamma irradiated tilapia during the storage

Table 2. GLM-ANOVA Results of Bacterial and Lipid Deterioration Parameters

	TPC	PV	TBA	FFA
Storage days (SD)	0.004	0.000	0.000	0.000
Irradiation dose (ID)	0.004	0.086	0.000	0.000
SD * ID	0.002	0.016	0.000	0.000

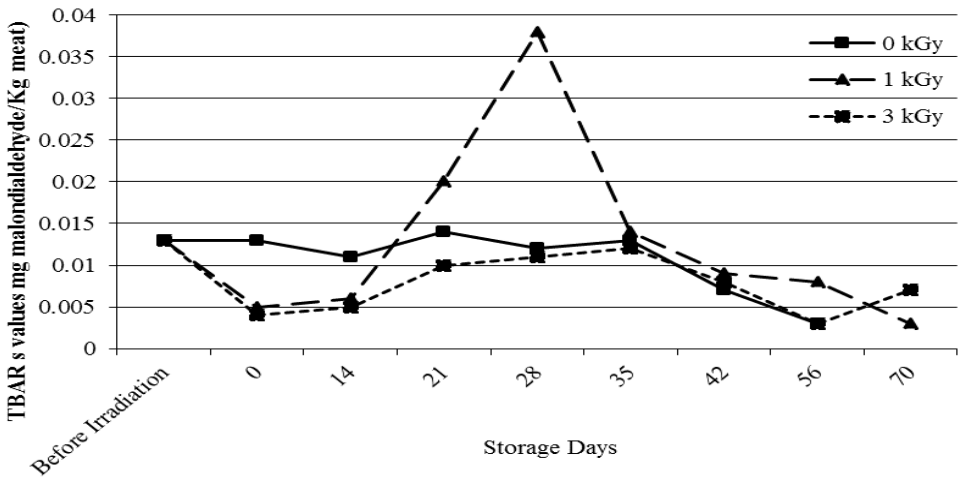

Figure 3. Changes in the thiobarbituric acid value of gamma-irradiated Tilapia stored in ice

The initial TBARS value of 0.013 mg MA kg^{-1} muscle rose to maximum value of 0.014 mg MA kg^{-1} muscle at 21 days of storage in non-irradiated samples. It was followed by a change from 0.005 mg MA kg^{-1} muscle to 0.038 mg MA kg^{-1} at 28 days of storage for samples irradiated at a dose of 1 kGy, and a change from 0.004 mg MA kg^{-1} muscle to 0.012 mg MA kg^{-1} muscle at 35 days of storage for samples irradiated at a dose of 3 kGy. After this period of storage, the TBARS values for both non-irradiated and irradiated samples decreased gradually to reach final values of 0.003, 0.003, and 0.007 mg MA kg^{-1} muscle at day 56 for non-irradiated samples, and day 70 for irradiated samples (1-kGy and 3-kGy doses). This finding could be due to the loss of volatile oxidative compounds from fish muscles that have been stored for longer periods of time. Irradiation has ability to initiate the normal process of lipid oxidation [30]. Highly unsaturated fatty acids are more readily oxidized than less unsaturated fatty acids. In general, radiolytic decomposition occurs in lipids containing those unsaturated fatty acids and induces the formation of some volatile compounds. The GLM-ANOVA indicated that TBARS values were significantly affected by all three factors (Table 2). The results indicated that TBARS values significantly increased at 21 and 28 days of storage ($p < 0.05$) compared with 0 days of storage, and then significantly declined with further storage time (between 21 days and 42, 56, and 70 days of storage, TBARS values differed significantly with $p<0.01$ respectively. Between 28 days and 25, 42, 56, and 70 days of storage, TBARS values differed significantly with $p = 0.014$, 0.000, 0.000, and 0.000 respectively).

Previous researchers have also shown increases in TBARS values in various meat and meat products during irradiation and storage [16,31,32,33,34,35]. Animal species, irradiation dose, storage time, and packaging method significantly influenced the TBARS values of meats [36]. However, in a study of chicken breasts found that no significant lipid oxidation occurred during the storage period in chicken breasts irradiated with 3 kGy gamma rays [37]. Similar observations were obtained by researcher, who found no significant differences in TBARS values with increased irradiation dose or increased storage duration in chicken breasts [38].

The effect of irradiation on the lipid hydrolysis of Tilapia fish muscle during storage in ice was monitored using FFA %, as depicted in Figure 4.

Before being irradiated, the FFA% value of the Tilapia muscle was 9.15%. Irradiated samples had higher FFA% compared with control samples. During storage, the FFA% value in all samples increased continuously. The highest FFA% was observed in Tilapia irradiated at 1 kGy (27.83%) after storage for 77 days. Non-irradiated samples showed peak FFA% after 56 days of storage, and 3-kGy irradiated samples showed peak values after 70 days of storage. The GLM-ANOVA indicated that FFA was significantly affected by storage duration and dose (Table 2).

3.3. Effect of Gamma Irradiation on Fatty Acid Composition of Tilapia during Storage

There was a dose-dependent significant effect ($p < 0.05$) on saturated fatty acids (SFAs), and a significant interaction was observed between storage x dose. Control samples did not show significant fluctuations over the storage time period. However, SFAs in the 1-kGy irradiated sample were significantly reduced with storage time (42.595% at 0 days, decreasing to 32.441% at 77 days of storage). The 3-kGy irradiated sample didn't fluctuate over the course of the study (Table 3).

Palmitic acid (C 16:0) was found to be most predominant SFA in Tilapia muscle, followed by stearic acid (C 18:0), and myristic acid (C 14:0). The initial percentages of palmitic acid were 26.530%, 34.613%, and 34.050% in control, 1-kGy irradiated and 3-kGy irradiated Tilapia, respectively. At 77 days of storage on ice, there were higher significant reductions in 1-kGy irradiated samples than 3 kGy irradiated ones. At 77 days of storage, the percentage of palmitic acid obtained in 1-kGy irradiated Tilapia muscle was 18.470%, whereas the 3-kGy irradiated sample was significantly reduced to 27.039%.

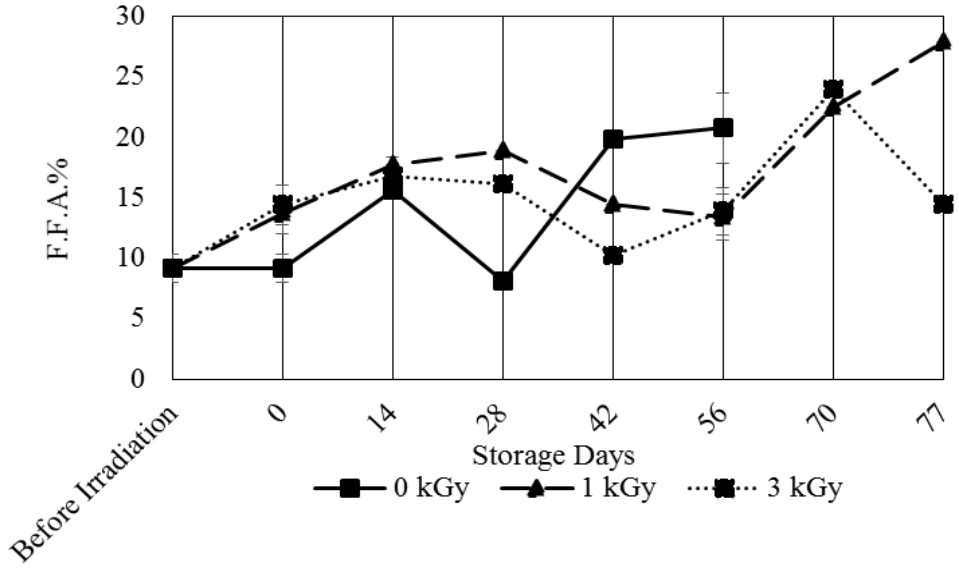

Figure 4. Variation of FFA% of gamma irradiated tilapia fish muscles stored in ice

Table 3. Variation in total fatty acid composition of gamma-irradiated Tilapia muscle stored on ice (% of total fatty acids)

Compound	Notation	0 Days			28 Days		
		0 kGy	1 kGy	3 kGy	0 kGy	1 kGy	3 kGy
Unidentified 1	--	ND	0.245 ± 0.000	ND	ND	ND	ND
Unidentified 2	--	ND	1.261 ± 0.000	ND	0.073 ± 0.103	0.146 ± 0.000	ND
Tetradeconoic acid	C 14:0	3.188^{a} ± 0.000	3.867^{b} ± 0.000	3.180^{a} ± 0.000	4.671^{c} ± 0.185	4.540^{c} ± 0.000	3.043^{a} ± 0.000
Pentadecanoic acid	C 15:0	1.027^{a} ± 0.000	1.098^{b} ± 0.000	1.084^{a} ± 0.000	1.457^{c} ± 0.069	1.409^{c} ± 0.000	1.324^{d} ± 0.000
Palmitic acid	C 16:0	26.530^{a} ± 0.000	34.613^{b} ± 0.000	34.050^{b} ± 0.000	34.366^{b} ± 4.991	30.837^{b} ± 0.000	31.824^{b} ± 0.000
Palmitoleic acid	C 16:1	8.593^{a} ± 0.000	8.115^{a} ± 0.000	9.250^{a} ± 0.000	10.695^{b} ± 1.815	9.411^{ab} ± 0.000	9.256^{ab} ± 0.000
Hexadecadienoic acid	C 16:2	0.337^{a} ± 0.000	1.534^{b} ± 0.000	ND	0.607^{c} ± 0.148	0.502^{c} ± 0.000	0.771^{d} ± 0.000
Hexadecatrienoic acid	C 16:3	0.975^{a} ± 0.000	ND	0.853^{a} ± 0.700	1.567^{b} ± 0.249	1.391^{ab} ± 0.000	1.306^{ab} ± 0.000
Heptadecanoic acid	C 17:0	1.333^{a} ± 0.000	ND	0.853^{a} ± 0.700	0.725^{a} ± 1.025	1.450^{ab} ± 0.000	1.533^{ab} ± 0.000
Octadecanoic acid	C 18:0	7.862^{a} ± 0.000	3.017^{b} ± 0.000	1.986^{bc} ± 0.000	0.945^{c} ± 1.336	ND	1.039^{bc} ± 0.000
Oleic acid (C 9)	C 18:1	9.223^{a} ± 0.000	9.632^{a} ± 0.000	10.459^{a} ± 0.000	12.198^{ab} ± 4.102	15.099^{b} ± 0.000	12.114^{ab} ± 0.000
Vaccenic acid (C 11)	C 18:1	3.188^{a} ± 0.000	3.099^{a} ± 0.000	2.405^{a} ± 0.000	3.282^{a} ± 0.666	3.753^{ab} ± 0.000	2.465^{ac} ± 0.000
Linoleic acid	C 18:2	10.859^{a} ± 0.000	10.250^{b} ± 0.000	11.290^{c} ± 0.000	9.055^{d} ± 0.182	8.927^{d} ± 0.000	15.408^{e} ± 0.000
Octadecadienoic acid	C 18:2	ND	ND	ND	ND	ND	ND
Linolenic acid	C 18:3	8.420^{a} ± 0.000	2.130^{b} ± 0.000	3.067^{c} ± 0.000	2.957^{bc} ± 0.896	3.590^{cd} ± 0.000	5.155^{d} ± 0.000
Gamma-linolenic acid	C 18:3	0.876^{a} ± 0.000	ND	0.848^{a} ± 0.000	0.344^{b} ± 0.487	0.688^{ab} ±0.000	ND
Octadecatetraenoic acid	C 18:4	3.328^{a} ± 0.000	ND	2.211^{ab} ± 0.000	1.127^{bc} ± 1.594	1.248^{bc} ± 1.424	2.414^{ab} ± 0.000
Eicosenoic acid	C 20:1	0.439^{a} ± 0.000	ND	ND	0.191^{ab} ± 0.270	0.382^{a} ± 0.000	ND
Arachidonic acid (ω 6)	C 20:4	2.715^{a} ± 0.000	4.738^{b} ± 0.000	3.849^{c} ± 0.000	3.051^{d} ± 0.149	2.946^{de} ± 0.000	2.676^{a} ± 0.000
Omega 3-Arachidonic acid (ω 3)	C 20:4	1.395^{a} ± 0.000	ND	2.024^{b} ± 0.000	1.888^{bc} ± 0.095	1.821^{cd} ± 0.000	ND
Heneicosapentaenoic acid	C 21:5	0.959^{a} ± 0.000	1.302^{b} ± 0.000	0.886^{ac} ± 0.000	0.897^{ac} ± 0.152	0.790^{cd} ± 0.000	1.028^{a} ± 0.000
Unidentified 3	--	1.113^{a} ± 0.000	1.482^{b} ± 0.000	1.010^{ac} ± 0.000	1.059^{ac} ± 0.272	0.867^{cd} ± 0.000	0.783^{cd} ± 0.000
Docosapentaenoic acid	C 22:5	2.267^{a} ± 0.000	3.702^{b} ± 0.000	3.250^{c} ± 0.000	2.840^{d} ± 0.161	2.726^{d} ± 0.000	2.441^{e} ± 0.000
Unidentified 4	--	ND	3.231 ± 0.000	ND	ND	ND	ND
Docosahexaenoic acid (ω 6)	C 22:6	5.371^{a} ± 0.000	4.881^{b} ± 0.000	7.445^{c} ± 0.000	5.532^{d} ± 0.005	5.528^{d} ± 0.000	5.420^{e} ± 0.000
Unidentified 5	--	ND	1.801^{a} ± 0.000	ND	ND	ND	ND
Unidentified 6	--	ND	0.901 ± 1.274	ND	ND	ND	ND
SFAs		39.940^{a} ± 0.000	42.595^{ab} ± 0.000	41.153^{ab} ± 0.700	42.164^{ab} ± 5.555	38.236^{ac} ± 0.000	38.764^{ac} ± 0.000
MUFAs		21.443^{a} ± 0.000	20.846^{a} ± 0.000	22.114^{ab} ± 0.000	26.366^{b} ± 3.223	28.645^{bc} ± 0.000	23.835^{ab} ± 0.000
PUFAs		37.503^{a} ± 0.000	28.537^{a} ± 0.000	35.723^{ac} ± 0.700	29.865^{a} ± 1.835	30.157^{a} ± 1.424	36.619^{ac} ± 0.000
Total unsaturated		58.945^{a} ±0.000	49.383^{b} ± 0.000	57.837^{a} ± 0.700	56.231^{a} ± 5.058	58.802^{a} ± 1.424	60.454^{a} ± 0.000
Omega 3		22.716^{a} ± 0.000	10.949^{b} ± 1.506	17.517^{c} ± 0.700	14.195^{d} ± 1.418	14.192^{d} ± 1.424	12.609^{bd} ± 0.000
Omega 6		14.450^{a} ± 0.000	14.988^{b} ± 0.000	15.986^{c} ± 0.000	12.450^{d} ± 0.565	12.561^{d} ± 0.000	18.084^{e} ± 0.000
Total omega		37.165^{a} ±0.000	25.937^{b} ± 1.506	33.503^{c} ± 0.700	26.646^{b} ± 1.574	26.752^{b} ±1.424	30.693^{d} ± 0.000
Total unidentified		1.113^{a} ±0.000	4.430^{b} ± 1.274	1.01^{a} ± 0.000	1.059^{ac} ±0.272	0.867^{ac} ± 0.000	0.783^{ac} ±0.000
Compound	Notation	56 Days			77 Days		
		0 kGy	1 kGy	3 kGy	0 kGy	1 kGy	3 kGy
Unidentified 1	--	ND	ND	ND	--	ND	ND
Unidentified 2	--	ND	ND	ND	--	ND	ND
Tetradeconoic acid	C 14:0	2.529^{d} ± 0.000	2.844^{e} ± 0.000	4.036^{b} ± 0.000	--	2.322^{f} ± 0.003	3.610^{g} ± 0.001
Pentadecanoic acid	C 15:0	1.049^{a} ± 0.000	1.156^{a} ± 0.000	1.356^{d} ± 0.000	--	0.981^{a} ± 0.002	1.339^{d} ± 0.000
Palmitic acid	C 16:0	35.786^{b} ± 0.000	28.906^{ab} ± 0.000	35.531^{b} ± 0.000	--	18.470^{c} ± 0.000	27.039^{a} ± 0.002
Palmitoleic acid	C 16:1	6.132^{c} ± 0.000	6.459^{c} ± 0.000	9.221^{ab} ± 0.000	--	5.532^{c} ± 0.003	9.641^{ab} ± 0.002
Hexadecadienoic acid	C 16:2	0.775^{d} ± 0.000	1.116^{e} ± 0.000	1.030^{d} ± 0.000	--	ND	0.772^{d} ± 0.003
Hexadecatrienoic acid	C 16:3	ND	ND	ND	--	ND	ND
Heptadecanoic acid	C 17:0	1.670^{ab} ± 0.000	1.496^{ab} ± 0.000	1.452^{ab} ± 0.000	--	1.529^{ab} ± 0.001	1.932^{ab} ± 0.003
Octadecanoic acid	C 18:0	2.122^{bc} ± 0.000	ND	1.776^{bc} ± 0.000	--	9.139^{d} ± 0.002	8.262^{ad} ± 0.002
Oleic acid (C 9)	C 18:1	7.640^{a} ± 0.000	23.060^{c} ± 0.000	7.919^{a} ± 0.000	--	9.150^{a} ± 0.000	12.231^{ab} ± 0.001
Vaccenic acid (C 11)	C 18:1	2.848^{a} ± 0.000	4.579^{d} ± 0.000	2.792^{a} ± 0.000	--	3.900^{b} ± 0.000	4.488^{b} ± 0.003
Linoleic acid	C 18:2	10.144^{b} ± 0.000	5.768^{f} ± 0.000	9.170^{d} ± 0.000	--	7.999^{g} ± 0.001	9.691^{h} ± 0.001
Octadecadienoic acid	C 18:2	ND	ND	ND	--	ND	0.690 ± 0.000
Linolenic acid	C 18:3	0.886^{e} ± 0.000	5.814^{f} ± 0.000	1.498^{g} ± 0.000	--	6.889^{af} ± 0.001	8.292^{a} ± 0.003
Gamma-linolenic acid	C 18:3	ND	ND	ND	--	ND	ND
Octadecatetraenoic acid	C 18:4	3.022^{a} ± 0.000	ND	2.805^{ab} ± 0.000	--	ND	ND
Eicosenoic acid	C 20:1	0.329^{a} ± 0.000	1.085^{c} ± 0.000	0.557^{ab} ± 0.000	--	1.111^{c} ± 0.002	0.651^{ad} ± 0.002
Arachidonic acid (ω 6)	C 20:4	6.066^{f} ± 0.001	4.265^{g} ± 0.000	4.678^{b} ± 0.000	--	5.450^{h} ± 0.000	1.700^{i} ± 0.001
Omega 3-Arachidonic acid (ω 3)	C 20:4	2.286^{e} ± 0.000	1.136^{f} ± 0.000	1.866^{cd} ± 0.000	--	0.731^{g} ± 0.001	ND
Heneicosapentaenoic acid	C 21:5	1.579^{e} ± 0.000	1.779^{f} ± 0.000	1.465^{e} ± 0.000	--	2.610^{g} ± 0.002	ND
Unidentified 3	--	ND	1.902^{e} ± 0.000	1.550^{b} ± 0.000	--	2.749^{f} ± 0.001	0.648^{g} ± 0.003
Docosapentaenoic acid	C 22:5	3.580^{b} ± 0.000	2.498^{e} ± 0.000	3.859^{f} ± 0.000	--	3.912^{f} ± 0.003	1.110^{g} ± 0.000
Unidentified 4	--	ND	ND	ND	--	ND	ND
Docosahexaenoic acid (ω 6)	C 22:6	9.095^{f} ± 0.000	5.032^{g} ± 0.000	7.069^{h} ± 0.000	--	7.021^{i} ± 0.002	1.340^{j} ± 0.002
Unidentified 5	--	0.665^{b} ± 0.000	1.105^{c} ± 0.000	ND	--	ND	ND
Unidentified 6	--	ND	ND	ND	--	ND	ND
SFAs		43.156^{ab} ± 0.000	34.402^{cd} ± 0.000	44.520^{abe} ± 0.000	--	--	42.182^{ab} ± 0.004
MUFAs		16.949^{d} ± 0.000	35.183^{e} ±0.000	20.489^{ad} ± 0.000	--	--	27.011^{bc} ± 0.002
PUFAs		37.433^{ac} ± 0.001	27.408^{a} ± 0.000	33.44^{a} ± 0.000	--	--	23.595^{b} ± 0.008
Total unsaturated		54.382^{ab} ± 0.001	62.591^{ac} ± 0.000	53.929^{b} ± 0.000	--	54.305^{ab} ± 0.007	50.607^{b} ±0.010
Omega 3		19.562^{ce} ± 0.000	10.445^{b} ± 0.000	17.064^{c} ± 0.000	--	14.274^{d} ± 0.004	2.45^{f} ± 0.001
Omega 6		16.210^{f} ± 0.001	10.033^{g} ± 0.000	13.849^{h} ± 0.000	--	13.450^{h} ± 0.001	11.392^{i} ± 0.003
Total omega		35.772^{ce} ± 0.001	20.479^{f} ± 0.000	30.914^{d} ± 0.000	--	27.722^{b} ± 0.003	13.841^{g} ± 0.001
Total unidentified		0.665^{ac} ±0.000	3.007^{b} ± 0.000	1.55^{ab} ± 0.000	--	2.749^{b} ±0.001	0.648^{ad} ±0.003

ND, not detected. All values are the mean ± standard deviation (n=4). Superscript letters in the same line indicate significant differences at $p < 0.05$.

These findings are consistent with several previous studies. In a study on the influence of irradiation on chemical components of Tilapia and Spanish mackerel [39]. Irradiation of Tilapia at doses of 1.5 to 10 kGy caused decreases in some fatty acids (C 14:0, C 16:0 and C 16:1). In case of Spanish mackerel, C 16:0 and C 16:1 fatty acids also decreased after irradiation at doses of 1.5 to 10 kGy. In a study of broiler chicken carcasses reported that palmitic acid decreased at irradiation doses of 0.5 to 3.0 kGy [40].

Monounsaturated fatty acids (MUFAs) decreased over the storage time period in the control samples. The initial MUFA content of 21.443% in control samples was significantly reduced to 16.949% over 56 days of storage. In the 1-kGy irradiated Tilapia samples, the initial MUFA content of 20.846% increased significantly over time, showing the highest value of 35.183% at 56 days of storage before declining significantly to 19.693% at 77 days of storage. The 3-kGy samples showed significant increases in MUFA over time; in these samples, the initial MUFA content of 22.114% increased to 27.011% at 77 days of storage.

Oleic acid was the primitive MUFA found in Tilapia muscle. The initial oleic acid content of 9.632% in 1-kGy irradiated fish increased significantly to 23.060% at 56 days of storage, before undergoing subsequent reductions. Similarly reported a significant increase in oleic acid in irradiated chicken meat [40].

Polyunsaturated fatty acids (PUFAs) didn't fluctuate significantly in control samples over time. However, the initial value of PUFA (28.537%) significantly increased with storage in 1-kGy irradiated Tilapia muscle (34.612 % at 77th day). The initial PUFA content of 35.723% was significantly reduced to 23.595% at 77 days of storage in samples irradiated at a dose of 3 kGy. Linoleic acid (C 18:2), linolenic acid (C 18:3), and docosahexaenoic acid (DHA) (22:6) were the predominant PUFA found in Tilapia muscle. Linoleic acid showed significant reductions over time in all samples. Initial values of 10.859%, 10.250%, and 11.290% linoleic acid declined to the lowest values of 10.144%, 7.999%, and 9.691% at 56 days in control samples, at 77 days in 1-kGy and 3-kGy irradiated muscles, respectively. In 3-kGy irradiated Tilapia, the initial value of 7.445% DHA was significantly reduced to 1.340% at 77 days of storage. Linolenic acid was reduced in irradiated samples, as initially control samples had a value of 8.420% of linolenic acid, which was reduced to 2.130% and 3.067% in 1- and 3-kGy irradiated samples, respectively. The control sample showed a significant reduction in linolenic acid with storage time. At 28 days of storage, the value was 2.957%, and it was 0.886% at 56 days of storage. However, gamma-irradiated Tilapia muscles showed a significant increase in linolenic acid with respect to the irradiation dose over the storage time period. Initially, the 1-kGy irradiated Tilapia samples contained 2.130% of linolenic acid (at day 0), which gradually increased to 3.590%, 5.814%, and 6.889% after 28, 56, and 77 days of storage, respectively. These findings are in agreement with some findings [34]. However, in Spanish mackerel, irradiation using doses between 1.5 and 10 kGy caused decreases in some fatty acids (16:0 and 16:1), and increases in others (18:0 and 18:1). These changes, detected immediately after irradiation, were not consistent with those occurring during storage at 2 ± 2˚C for 20 days [39]. In a study of irradiated minced beef samples had significantly less PUFA on day 8 than day 0 [31]. However, there was no significant differences in saturated (C 14:0, C 16:0, C 17:0, and C 18:0) and unsaturated (C 18:1 and C 18:2) fatty acids between lipids extracted from irradiated and non-irradiated camel meat [26]. Changes of fatty acid profile during gamma irradiation of Rainbow Trout (*Oncorhynchus mykiss*) fillets showed that gamma irradiation influenced the fatty acid composition especially polyunsaturated fatty acids in Rainbow trout fillet significantly [41].

3.4. GLM-ANOVA for the Fatty Acid Composition of Gamma-Irradiated Tilapia Muscle Stored in Ice

An ANOVA with Type III sums of squares was performed using the GLM procedure of SPSS to identify whether irradiation dose, storage duration, or the interaction of these two factors affected variation in the fatty acid profiles of Tilapia muscle. The identified fatty acids were divided into five groups according to their number of structural double bonds and other similar characteristics and analyzed. The groups were SFA, MUFA, PUFA, Omega-3, and Omega-6 fatty acids.

The GLM-ANOVA results (Table 4) showed that the fatty acid composition of Tilapia fish was affected by all three factors (storage duration, dose, and storage duration x dose), except in SFA.

Table 4. Summary of the Results of GLM-ANOVA for the Fatty Acid Groups with Different Factors in Tilapia Muscle

	Storage Days (SD)	Irradiation Dose (ID)	SD * ID
SFA	0.064	0.000	0.001
MUFA	0.000	0.000	0.000
PUFA	0.000	0.000	0.000
Omega-3	0.000	0.000	0.000
Omega-6	0.000	0.000	0.000

3.5. Linear Discriminant Analysis of the Fatty Acid Composition of Tilapia Stored in Ice

An LDA was carried out to verify the results of the GLM-ANOVA. Identified fatty acids of Tilapia muscle were specified for the LDA, with storage day and dose specified as grouping variables for the analysis.

Regarding storage duration, the LDA resulted in a discriminant model with three significant discriminant functions. The first function explained 95.2% of the variance, the second function explained 4.3% of the variance, and the third explained 0.6% of the variance (Figure 5).

The first function primarily separated days 0 and 77 days (mean of the canonical variance [MCV]: 0 days = 32.771; 28 days = -7.545; 56 days = -7.893; 77 days = -26.00) and was more strongly correlated with pentadecanoic acid (C 15:0) and palmitoleic acid (16:1). The second function supported the separation of 28 days and 77 days (MCV: 0 days = -2.151; 28 days = 5.883; 56 days = 0.985;

77 days = -7.075). The second function was correlated with palmitoleic acid (C 16:1), hexadecatrienoic acid (C 16:3), and pentadecanoic acid (C 15:0). The third function demonstrated very weak discriminant power and poorly classified stearidonic acid (C 18:4), hexadecatrienoic acid (C 16:3), and palmitoleic acid (C16:1). The model showed a satisfactory classification performance, correctly classifying 100.0% of the samples for the original groups (Table 5).

Table 5. Classification Results of a DFA of Fatty Acid Composition of Tilapia Muscle with Storage

		Predicted Group %				Total
	Storage Days	0	28	56	77	
Original group %	0	100	0	0	0	100
	28	0	100	0	0	100
	56	0	0	100	0	100
	77	0	0	0	100	100

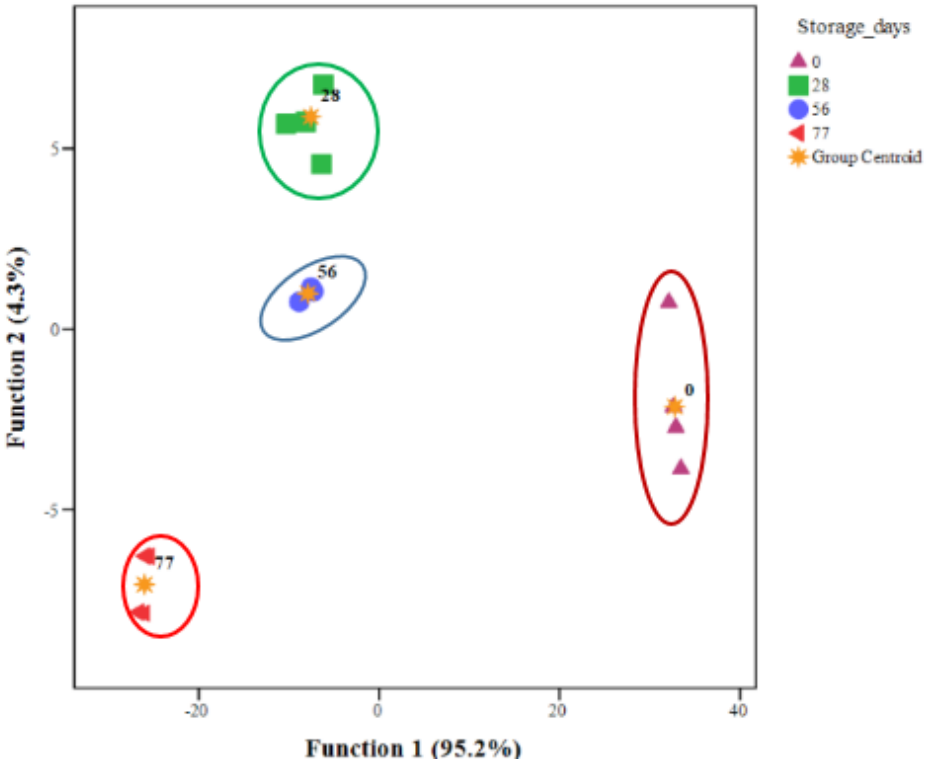

Figure 5. Canonical discriminant function analysis relationship between fatty acids and storage days of gamma irradiated tilapia fish muscles

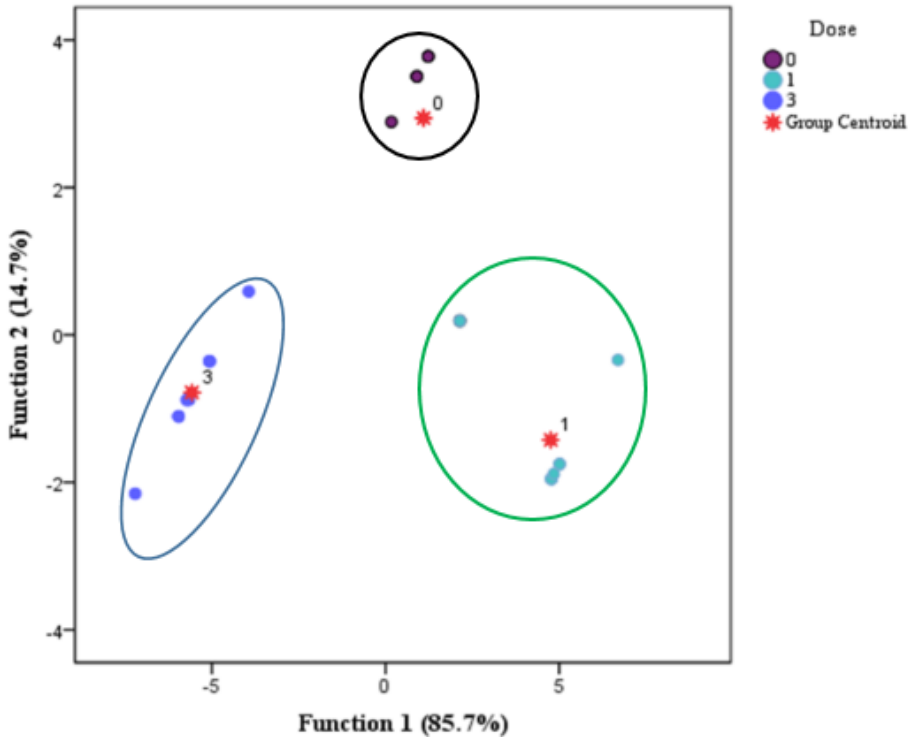

Figure 6. Canonical discriminant function analysis relationship between fatty acids and irradiation dose of tilapia fish muscles

Regarding irradiation dose, the LDA resulted in a discriminant model with two significant discriminant functions. The first function explained 85.7% of the variance and the second function explained 14.7% of the variance (Figure 6).

The first function separated samples irradiated with 1- kGy and 3-kGy doses (MCV: 0-kGy = 1.094; 1-kGy = 4.754; 3-kGy = -5.575), and was more strongly correlated with stearic acid (C 18:0), myristic acid (C 14:0), and oleic acid (C 18:1). The second function supported the separation of the 0-kGy and 1-kGy treatments (MCV: 0-kGy = 2.942; 1-kGy = -1.424; 3-kGy = -0.782) and was more correlated with stearic acid (C 18:0), palmitic acid (C 16:0), and oleic acid (C 18:1).

The model showed satisfactory classification performance, classifying 95.5% of the samples for the original groups and 81.8% for the cross-validation procedure (Table 6).

Table 6. Classification Results of DFA of Fatty Acid Composition in Tilapia with Irradiation Dose

		Predicted Group %			Total
	Dose	0	1	3	
Original group %	0	100	0	0	100
	1	12.5	87.5	0	100
	3	41.7	0	100	100

The results of the LDA verified the changes in the fatty acid profiles of gamma-irradiated Tilapia muscle stored on ice. The results suggested that predominant fatty acids, such as palmitic acid, palmitoleic acid, oleic acid, stearic acid, and myristic acid have adverse effects related to storage duration and also gamma irradiation.

4. Conclusion

The shelf-life of muscle tissue was extended to 70 days following 3-kGy irradiation, and 56 days following 1-kGy irradiation, by reducing the microbial load of fish muscle. Both the 3-kGy and 1-kGy dose eliminated coliform bacteria. Lipid oxidation and hydrolysis were extended throughout the storage period by the interaction of storage time and dose. Changes in fatty acid composition throughout the study showed that 1-kGy irradiation was the optimum dose for minimizing deterioration of quality. Therefore, the results indicate that a 1-kGy irradiation dose is the safest dose for the preservation of Tilapia stored in ice.

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