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MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC EVALUATION OF FRESH FISH AND ITS PRODUCTS IRRADIATED BY GAMMA RAYS

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ABSTRACT

The present study evaluated the microbiological, chemical, and organoleptic aspects of irradiated fresh fish and its products to extend their shelf life. Fresh fish and its products were irradiated at three doses (1.5, 3.0, and 4.5 kGy) used for preservation to study the effects of irradiation on their microbiological properties, fatty acid composition, and organoleptic properties. Irradiated fresh bolti fish, smoked herring, and smoked mackerel were evaluated microbiologically, chemically, and organoleptically. Radiation treatment not only reduced the counts of aerobic bacteria, fecal streptococci, molds, and yeasts but also destroyed all the *Staphylococcus aureus* cells, improving the hygienic quality of the fresh and smoked fish samples. Irradiation increased the peroxide, acid, and thiobarbituric acid values, but they remained within acceptable levels. No new fatty acids or other artifacts due to irradiation were observed. Irradiation of 4.5 kGy greatly reduced the organoleptic quality scores of fresh bolti fish, indicating that the optimum radiation dose for this fish was 3.0 kGy. Smoked herring and mackerel could be irradiated with up to 4.5 kGy without adverse effects on their organoleptic properties.

Keywords: microbiological; chemical; organoleptic; fish; irradiated

INTRODUCTION

Currently, irradiated food (up to 10 kGy) has become acceptable and is commercially available in many countries worldwide **(Lacroix and Ouattara, 2000; Demartini et al., 2019)**.

Radiation sterilization, which is analogous to the processing techniques used for other meats (beef, poultry, and seafood), is also a new and promising method applicable to the processing of precooked meats (enzyme inactivation). Radiation sterilized foods can be stored for a long time (years) without refrigeration **(Duliu, Ferdes, and Ferdes, 2004; Sedeh et al., 2007; Fallah et al., 2008; Kakatkar et al., 2017)**. Many studies of potential techniques for the commercial radiation sterilization of seafood have been carried out because radiation extends the shelf life of the product and improves the hygienic quality of the seafood **(Chouliara et al., 2004; Brennan, 2005; Özkan et al., 2007)**.

The lipids in the fish muscle are different from those of other animal tissues due to their high contents of polyunsaturated fatty acids **(Al-Kahtani et al., 1996; Javanmard et al., 2006; Özden and Erkan, 2010)**.

The high polyunsaturated fat content can be considered the main reason for the oxidative rancidity of fish, which adversely affects its flavor. Irradiation has been reported to increase 2-thiobarbituric acid-reactive substances (TBARS) in aerobically packaged raw poultry meat **(Du et al., 2000; Turgis et al., 2008)**.

Irradiation has shown considerable promise in the microbial decontamination of fresh fish and fish products, extending their shelf lives and improving their hygienic quality **(Farkas, 2006; Mbarki et al., 2009)**. However, the acceptability of irradiated fish products is dependent on the chemical composition of the fish, particularly the lipids. Many investigators have found that irradiation in the presence of oxygen can accelerate lipid oxidation and induce oxidative rancidity; hence, lipid quality and fatty acid composition can be altered **(Byun et al., 2008; Mbarki et al., 2009)**. The progressive use of gamma radiation in the field of fish and fish product preservation makes it important to evaluate radiation processes and their effects on the qualities of these products **(Badr, 2012)**.

Bolti (*Tilapia nilotica*) is one of the most important and preferred freshwater fish. Smoked fish products have received more attention due to their pleasant taste. The most important types of smoked fish are herring and mackerel **(WHO, 2000; Nickelson et al., 2001)**.

Fresh and smoked fish products can be contaminated with many pathogenic microorganisms that affect their shelf life **(Moini et al., 2009; Rostamzad et al., 2010)**.

The main aim of the present study was to evaluate the effects of gamma irradiation on the microbiological, chemical, and organoleptic qualities of fish (fresh bolti fish as well as smoked herring and smoked mackerel).

Scientific hypothesis

H1: Radiation is considered a safe method of preserving food from microbial deterioration and consequently prolonging its marketing period.

H2: Gamma irradiation we will be using for the fresh fish and smoked improving the hygienic quality and increasing its shelf life without adverse effects on its organoleptic properties.

MATERIAL AND METHODOLOGY

Three types of fish, fresh bolti (*Tilapia nilotica*), smoked herring, and smoked mackerel, were used in this experiment. Fresh bolti fish samples were obtained from the Dammam fish market in the eastern province of Saudi Arabia. This market is one of the main sources of fish in the kingdom, while smoked herring and smoked mackerel were obtained from the market in Riyadh, Saudi Arabia. The fish samples were wrapped with non-perforated plasticized polyvinyl chloride (PVC) film, which is a stretch film with high transparency, 12-micron thickness, self-clinging, and the following rates of permeability: CO_2 >100 $cm^3/m^2/24$ hr; O_2 1800 $cm^3/m^2/24$ hr; and water vapor 700 $g/m^2/24$ hr.

Irradiation process

Fish samples in bags were irradiated for 30 Minutes. The samples temperature was when irradiation at 2 – 3 °C ±1 °C were exposed to 1.5, 3.0, and 4.5 kGy from cobalt 60 in a Gammacell 220 at King Abdul Aziz City for Science and Technology (KACST) in Riyadh (Model Gammacell 220 from MDS; Nordion Initial Canada Activity source (Co-60) was 24.000). All fish samples were stored at 3° C ±1 °C (90 – 92% RH).

Microbiological tests

The total aerobic bacterial counts were enumerated on agar (Merck, Darmstadt, Germany) plates as described by **Maturin and Peeler (2001)**. The quantification of fecal streptococci was done through the MPN technique using the Dextrose Azide Broth **(APHA, 1998)**, while *Staphylococcus aureus* was counted on Baird-Parker medium as described by **Tallent et al. (2001)**. The total molds and yeasts were counted on malt extract agar plates **(Tournas et al., 2001)**.

Lipid extraction

The parts of fish were homogenized and 5 g of the homogenized sample was mixed well with 10 g cleaned sea sand and 20 g anhydrous sodium sulfate, and then percolated for 6 hours with a hexane-acetone mixture (2:1) in a glass column with a Teflon stopcock. After evaporation of the solvent from the percolate (600 mL) under vacuum (all chemicals were from Fischer, USA), the fish lipids extracted were weighed **(AOAC, 1990)**.

Lipid quality attributes

The peroxide value (as equivalents/kg lipid) and the acid value were determined as described by **AOAC (1990)**. The thiobarbituric acid value was determined according to performed as described by **Lynch and Frei (1993**).

Fatty acid profiles

The fatty acid composition was determined by a gas-liquid chromatography apparatus according to the method by **AOAC (1990)**.

Organoleptic tests

The appearance, odor, texture, and taste of the fish samples were served to a taste panel of 10 members using a hedonic scale of 1 to 9 and the dishes were rated as 9 for excellent, 6 for good and below 4 as poor or unacceptable. **(WHO, 2000)**.

Statistical Analysis

The obtained data are reported as the mean ±*SD* and were statistically analyzed using SPSS program version 22.0 (SPSS, 2018, SPSS Inc., Chicago, Illinois, USA). Significant differences were evaluated by Duncan's multiple range test (DMRT), with differences considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The results of the microbiological tests are shown in Table 1. A comparison of the fresh bolti fish and smoked fish (herring and mackerel) indicated that most microbial counts were higher for fresh bolti fish and lowest for smoked mackerel. The use of salt and the smoking process might have affected the microbial counts. Irradiation caused a substantial reduction in all the microorganisms evaluated, and the reduction percentage was proportional to the irradiation dose. The lowest irradiation dose used (1.5 kGy) decreased the total aerobic bacterial counts of the fresh bolti fish by 7.5×10^3, while it decreased the total aerobic bacterial counts of smoked fish (herring and mackerel) by 4.4×10^3 and 2.0×10^3, respectively.

The higher reduction in the total aerobic bacterial count for fresh bolti fish might be due to the direct effect of the radiation as well as indirect effects resulting from water radiolysis, which is greater in fresh fish than in smoked fish. The highest irradiation dose used, i.e., 4.5 kGy, reduced the total aerobic bacterial counts by approximately 99.9% (<100).

Considering that Staphylococcus aureus is the main food poisoning microorganism, irradiation, even at the lowest dose, destroyed almost all the cells of this dangerous microorganism (<100). It could be concluded that irradiation doses used were sufficient to substantially reduce the counts of all the microbial species investigated and improve the hygienic quality of both fresh and smoked fish, rendering these products safe for human consumption. Many other investigators reported substantial reductions in the microbial counts on fish and fish products as a result of irradiation **(Noomhorm et al., 2003; Duliu, Ferdes and Ferdes, 2004; Özkan et al., 2007; Demartini et al., 2019)**.

It has been reported that irradiation doses of 1, 3, and 5 kGy significantly reduced the total viable counts of microorganisms on rainbow trout fillets **(Moini et al., 2009; Kakatkar et al., 2017)**.

Mendes et al. (2005) and **Nickelson et al. (2001)** found that the mesophilic bacterial counts of irradiated shrimp, crab, and fish were lower than those of nonirradiated samples during storage at 4 °C.

Table 1 Microbiological quality of fresh and smoked fish and before and after irradiation.

Microbiological quality (Fresh bolti fish)	Fresh bolti fish			
	0 kGy	1.5 kGy	3 kGy	4.5 kGy
Total aerobic bacteria	8.2×10^5	7.5×10^3	5.0×10^2	1.1×10^2
Fecal streptococci	3.0×10^3	3.2×10^2	<100	<100
Staphylococcus aureus	3.3×10^2	<100	<100	<100
Yeasts	8.0×10^3	6.1×10^2	1.0×10^2	<10
Molds	0.7×10^2	3.3×10	<10	<10

Microbiological quality (Smoked herring and Smoked mackerel)	Smoked herring				Smoked mackerel			
	0.0 kGy	1.5 kGy	3 kGy	4.5 kGy	1.5 kGy	0.0 kGy	3 kGy	4.5 kGy
Total aerobic bacteria	6.2×10^4	4.4×10^3	2.0×10^2	7.1×10	2.0×10^3	3.0×10^4	4.1×10^2	6.0×10
Fecal streptococci	1.9×10^2	1.1×10	<100	<100	1.1×10	7.0×10^2	<100	<100
Staphylococcus aureus	7.2×10^2	<100	<100	<100	<100	2.2×10^2	<100	<100
Yeasts	7.4×10^3	5.6×10^2	5.0×10	<10	8.0×10	7.1×10^2	2.1×10	<10
Molds	1.5×10^3	7.3×10	<10	<10	<10	<10	<10	<10

Table 2 Effect of irradiation on the peroxide values, acid values and thiobarbituric acid values (TBA) of fish products.

Parameters	Fresh bolti fish				Smoked herring				Smoked mackerel			
	0 kGy	1.5 kGy	3 kGy	4.5 kGy	0.0 kGy	1.5 kGy	3 kGy	4.5 kGy	1.5 kGy	0.0 kGy	3 kGy	4.5 kGy
Peroxide value	5.7 ±0.26	7.9 ±0.13	9.8 ±0.22	14.3 ±0.05	7.5 ±0.55	9.2 ±0.33	11.1 ±0.58	15.9 ±0.10	8.3 ±0.70	9.9 ±0.90	12.5 ±0.60	13.7 ±0.22
Acid value	0.8 ±0.05	0.96 ±0.04	1.2 ±0.03	1.87 ±0.03	1.15 ±0.23	1.52 ±0.21	2 ±0.22	2.55 ±0.19	1.37 ±0.18	1.7 ±0.13	2.11 ±0.21	2.7 ±0.15
TBA	0.178 ±0.04	0.211 ±0.07	0.25 ±0.08	0.592 ±0.04	0.279 ±0.46	0.372 ±0.23	0.446 ±0.33	0.635 ±0.23	0.353 ±0.04	0.415 ±0.04	0.481 ±0.03	0.722 ±0.06

Note: Results are presented as mean ±*SD* (n = 3). Values are significantly differen (p <0.05).

Table 3 Relative percentages of fatty acids in nonirradiated and irradiated fish products.

Fatty acids	Carb No.	Fresh Bolti Fish				Smoked Herring				Smoked Mackerel			
		0.0 kGy	1.5 kGy	3.0 kGy	4.5 kGy	0.0 kGy	1.5 kGy	3.0 kGy	4.5 kGy	0.0 kGy	1.5 kGy	3.0 kGy	4.5 kGy
Lauric	12.0	00.12	00.12	00.34	00.18	00.31	00.38	00.34	00.44	00.20	00.14	00.24	00.16
Myristic	14.0	06.56	09.41	09.64	11.17	09.81	09.70	09.61	09.91	08.00	10.01	08.52	09.75
Pentadecanoic	15.0	01.70	00.92	00.92	00.79	00.82	00.21	00.65	00.65	01.11	01.33	01.60	01.12
Palmitic	16.0	28.65	28.22	27.70	27.79	00.21	00.15	00.87	01.50	00.24	00.21	00.20	00.24
Palmitoleic	16.1	18.01	18.10	18.00	17.75	19.13	18.57	17.26	14.40	19.63	18.00	16.20	15.20
Heptadecanoic	17.0	03.55	5.75	05.33	05.85	00.50	00.12	00.75	00.95	07.61	08.75	07.70	08.90
Heptadecanoic	17.1	0.3.68	3.00	04.12	04.28	00.83	00.12	00.19	01.34	02.35	01.26	01.31	00.72
Stearic	18.0	0.6.25	6.00	06.29	08.45	00.92	00.57	00.95	00.97	00.52	00.67	00.22	00.71
Oleic	18.1	21.71	20.01	19.70	16.93	03.20	03.11	03.62	03.66	06.35	06.66	05.14	05.26
Linoleic	18.2	05.16	4.51	03.89	02.32	18.43	18.70	18.03	19.00	20.51	17.29	17.29	24.00
Linolenic	18.3	00.55	00.55	00.68	00.55	19.01	25.00	18.27	22.14	08.61	08.22	18.60	17.10
Arachidic	20.0	0.1.34	1.20	01.07	01.10	02.00	02.03	02.52	02.82	02.74	03.13	02.71	02.92
Gadoleic	20.1	01.00	00.95	01.18	01.30	00.92	01.66	04.00	09.06	05.31	04.73	04.78	08.32
Eicosadienoic	20.2	00.26	00.28	00.28	00.23	23.38	21.70	23.0	13.22	14.69	18.82	15.90	05.27
Eleostearic	20.3	00.24	00.24	00.30	00.37	00.70	-	-	-	00.27	00.50	00.22	00.31
Arachidonic	20.4	01.12	01.10	00.50	00.85	00.31	-	-	-	01.90	00.20	-	-
T. sat *		48.17 ±1.23	51.42 ±2.62	51.29 ±2.05	55.33 ±2.75	14.57 ±2.05	13.16 ±1.65	15.69 ±0.65	17.24 ±0.58	20.42 ±1.23	24.24 ±2.62	21.19 ±0.63	23.80 ±0.45
T. Unsat **		51.73 ±2.75	48.78 ±2.27	48.95 ±1.10	44.58 ±0.50	85.41 ±1.37	88.86 ±2.27	84.37 ±2.03	82.82 ±2.62	79.62 ±2.52	75.68 ±1.37	78.90 ±1.57	62.21 ±0.57

Note: Results are presented as mean ±*SD* (n = 3). Values are significantly differen (p <0.05).

Table 4 Organoleptic evaluation of nonirradiated and irradiated fresh and smoked fish.

Parameters	Fresh bolti fish				Smoked herring				Smoked mackerel			
	0 kGy	1.5 kGy	3 kGy	4.5 kGy	0.0 kGy	1.5 kGy	3 kGy	4.5 kGy	1.5 kGy	0.0 kGy	3 kGy	4.5 kGy
Appearance	8.5 ±0.01	8.4 ±0.06	8.5 ±0.04	8.3 ±0.04	8.6 ±0.01	8.6 ±0.04	8.7 ±0.03	8.2 ±0.03	8.9 ±0.01	8.7 ±0.04	8.7 ±0.04	8.5 ±0.03
Odor	8.2 ±0.04	8 ±0.07	8 ±0.07	7.3 ±0.11	8.5 ±0.02	8.3 ±0.04	8 ±0.07	8 ±0.09	8.7 ±0.01	8.4 ±0.02	8.4 ±0.02	8 ±0.04
Texture	8.7 ±0.01	8.6 ±0.04	8.3 ±0.05	8.3 ±0.11	8.3 ±0.04	8.5 ±0.05	8.2 ±0.07	8 ±0.11	8.3 ±0.05	8 ±0.04	8 ±0.04	7.8 ±0.07
Taste	8.4 ±0.03	8.2 ±0.07	8 ±0.03	8 ±0.12	8.5 ±0.02	8.5 ±0.04	8 ±0.05	8 ±0.05	8.8 ±0.01	8.5 ±0.04	8.3 ±0.05	8.2 ±0.05

Results are presented as mean ±SD (n = 3). Values are significantly differen ($p<0.05$) .

The effects of irradiation on the fish lipid quality attributes are presented in Table 2. Generally, irradiation increased the peroxide value, acid value, and thiobarbituric acid value (TBA) of both fresh and smoked fish, but the levels were still indicative of acceptable quality.

Irradiated fresh bolti fish and smoked fish (herring and mackerel) showed significantly higher peroxide values (14.3, 15.9 and 13.9 meqO_2.kg^{-1} at 4.5 kGy) than the control samples (5.7, 7.5 and 8.3 meqO_2.kg^{-1}), respectively, and the peroxide values were positively correlated with the dose used. The results of this study are in agreement with the findings of other studies that have reported an increase in oxidation activity and lipid peroxidation as a result of radiation treatment of fish and fish products **(Ahn et al., 2000; Byun et al., 2008; Rostamzad et al., 2010).** In contrast, **Javanmard et al. (2006)** reported no significant ($p>0.05$) differences in the peroxide value between irradiated and control chicken meat after irradiation.

TBA values revealed that irradiation caused an increase in lipid oxidation in fish samples. The rate of the increase was correlated with the irradiation dose, and the highest increase was observed in the thiobarbituric acid values (0.592, 0.635, and 0.722 mg MDA.kg^{-1} at 4.5 kGy), indicating that the peroxides and hydroperoxides degraded into low-molecular-weight compounds. Similarly, some researchers have shown increases in TBA values during irradiation of various fish and fish products **(Byun et al., 2008; Turgis et al., 2008)**. In contrast, **Chun et al. (2010)** reported no significant differences in the TBARS values with both increasing irradiation doses and increasing storage periods in chicken breasts.

Comparing fresh bolti, smoked herring and smoked mackerel revealed that the greatest increase in lipid quality attributes occurred in fresh bolti fish, and the smallest increase occurred in smoked mackerel fish.

The fatty acid compositions of fresh bolti fish and smoked fish (herring and mackerel) are shown in Table 3. The compositions of the irradiated and nonirradiated samples were qualitatively similar since no new fatty acids or other artifacts were generated by irradiation. Among all the fatty acids, palmitoleic acid (C16:1) (17.75, 14.40, and 15.20% at 4.5 kGy and 18.01, 19.13, and 19.63% in the control, respectively) was the most abundant fatty acid in all the irradiated and nonirradiated samples. The least abundant fatty acid was lauric acid (C12:0) (0.18, 0.44, and 00.16% at 4.5 kGy and 0.12, 0.31, and 0.20% in the control, respectively) in all the irradiated and nonirradiated samples. The relative percentage of total polyunsaturated fatty acids for all fats in the fish samples decreased slightly with increasing radiation dose (44.58, 82.82, 62.21% at 4.5 kGy and 51.73, 85.41, 79.62% for the control), suggesting the potential for the oxidation of unsaturated compounds by irradiation.

Fresh bolti fish, which are considered lean fish, have a lower percentage of unsaturated fatty acids than the other tested fish, suggesting that the higher susceptibility of the fat in bolti fish to oxidation by irradiation may be due to the high content of monounsaturated fatty acids that are easily oxidized.

There were no significant differences ($p>0.05$) in the levels of all fatty acids, saturated fatty acids, or unsaturated fatty acids between the control and irradiated fish samples at 1.5, 3.0, and 4.5 kGy. Therefore, the irradiation process had no significant effect ($p>0.05$) on the fatty acid composition.

Javan and Motallebi (2015) reported an increase in fatty acid oxidation with increasing dose of gamma irradiation in their study of the effects of different doses of gamma radiation (0, 0.75, 1.5, 2.25, 3, 3.75, and 4.5 kGy) on the fatty acid composition of rainbow trout fillets.

Oraei et al. (2011) also reported that different irradiation processes and doses of radiation (1, 3, and 5 kGy) had no significant effects ($p>0.05$) on the fatty acid composition of rainbow trout fillets.

Al-Kahtani et al. (1996) reported the influence of irradiation on the chemical components of tilapia and Spanish mackerel, and radiation doses of 1.5 – 10 kGy caused a decrease in some fatty acids. **Erkan and Özden (2007)** reported that the total fatty acid contents in the muscle of nonirradiated sea bream were lower than in sea bream irradiated with 2.5 kGy and higher than in sea bream irradiated with 5 kGy.

Mbarki et al. (2009) reported that low-dose irradiation had no adverse effect on the nutritionally important polyunsaturated fatty acids in Mediterranean horse mackerel.

Özden and Erkan (2010) reported that the total saturated and total monounsaturated fatty acids in irradiated sea bass increased at 2.5 and 5 kGy, and the total polyunsaturated fatty acid contents in irradiated samples were higher than that in nonirradiated samples.

The organoleptic scores (for appearance, odor, texture, and taste) for fresh fish and smoked fish are shown in Table 4). Smoked mackerel earned the highest scores, while fresh bolti fish earned the lowest scores. Irradiation reduced the organoleptic scores of all the tested fish samples, but the reduction was not significant. The reduction was proportional to the radiation dose, and the largest reduction

was observed for fresh bolti fish, especially in odor and taste. The average appearance scores for the nonirradiated samples were 8.5, 8.6, and 8.9. For the irradiated samples, the scores were 8.3, 8.2, and 8.5 at 4.5 kGy. Both the irradiated and nonirradiated samples were in acceptable conditions. Considering the organoleptic evaluation, the optimum radiation dose for fresh bolti fish is less than or equal to 3.0 kGy. Smoked herring and smoked mackerel can be irradiated up to 4.5 kGy without adversely affecting their chemical and organoleptic properties.

Our results agreed with the results of **Prakash et al. (2014) and Badr (2012)**, who reported that irradiated and nonirradiated dry fish were in acceptable condition. **WHO (2000)** and **Ahmed et al. (2009)** showed that radiation doses up to 5 kGy had a significant effect on the visual qualities, decay rate, color, and texture of minimally processed foods. **Alam, Ahmed and Shahin (2009)** showed good correlations between bacterial populations and the overall acceptability scores with the shelf life of hilsa.

CONCLUSION

The irradiation of food enhances the safety and the hygienic qualities of fresh and smoked fish products because of its high efficacy for inactivating pathogenic and spoilage microorganisms without deteriorating the quality of the product.

According to all the obtained data, gamma irradiation, especially 4.5 kGy, can be applied for microbial control and improving the safety of smoked fish, and increasing its shelf life without adverse effects on its organoleptic properties. Also, the current study showed that irradiation of 4.5 kGy greatly reduced the organoleptic quality scores of fresh bolti fish, indicating that the optimum radiation dose of this fish is 3.0 kGy.

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Conflict of Interest:

The authors declare no conflict of interest.

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