Effect of Gamma Irradiation on Fatty Acid Composition of Rainbow Trout (*Oncorhynchus mykiss*) Fillets

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Abstract

The present study was conducted to evaluate the effect of low-dose gamma irradiation (0, 1, 3 and 5 kGy) on fatty acid composition of Rainbow trout (*Oncorhynchus mykiss*) fillet. Among all of the fatty acids, oleic acid (C18:1) (with mean 33.50±3.02 g/100 g fatty acids) and myristoleic acid (C14:1) (with mean 0.41±0.26 g/100 g fatty acids) were the most predominant and the lowest fatty acids in all irradiated and non-irradiated samples, respectively. Statistical analysis showed that there were no significant differences (P>0.05) in level of all fatty acids, saturated fatty acids (SFA), unsaturated fatty acids (USFA), mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) between Rainbow trout fillet control and irradiated in 1, 3 and 5 kGy. Therefore irradiation process and different doses of irradiation in this study (1, 3 and 5 kGy) had no significant effect (P>0.05) on fatty acid composition.

Keywords: Gamma irradiation, *Oncorhynchus mykiss*, Fatty acid composition

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Introduction

Fish is an extremely perishable food as compared to other food sources (Chouliara *et al.*, 2004). The Rainbow trout (*Oncorhynchus mykiss*) belongs to the Salmonidae, and is one of the main fish species farmed in Iran. The demand for Rainbow trout in Iran and other country markets has increased significantly over the past decade and this could be due to its desirable characteristics (aroma, taste, white flesh) resulting in a high-quality product (Figure 1) (Food and Agriculture Organization, FAO, $2010_{a,b}$; Iranian Fisheries Organization, IFO, 2009). Along with such a demand there is an obvious need for development of new technologies and efficient fish preservation methods which permit shelf-life extension of these products (Chouliara et al., 2004).

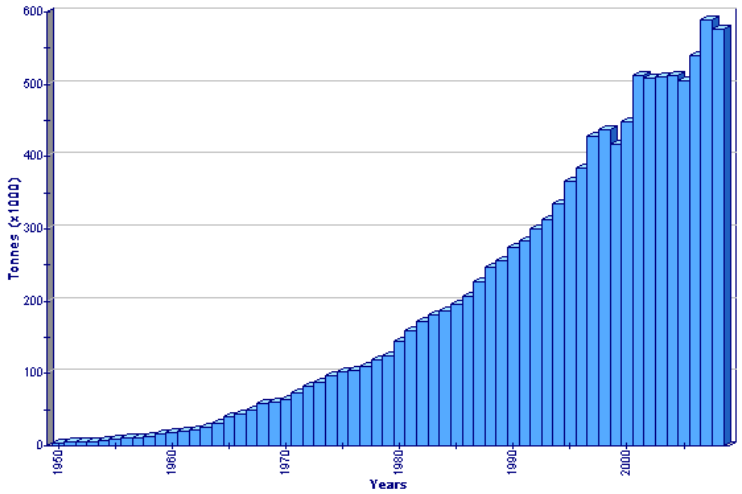

Figure 1: Global aquaculture production of *Oncorhynchus mykiss* (FAO, 2010_a)

Besides traditional methods such as rapid chilling, ice storage, freezing, smoking and heating (Himelblooom et al., 1994; Farkas, 1990; 1999), various methods involving the use of organic acids, antimicrobials (Al-Dagal and Bazarra, 1999; Gelman et al., 2001), antioxidants (Haghparast et al., 2010), edible coating (Motalebi et al., 2010), modified atmosphere packaging (Masniyom et al., 2002) and ionizing radiation (Savvaidis et al., 2002; Chouliara et al., 2004; Erkan and Özden, 2007) have been proposed to extend the shelf-life of fish and fishery products.

The irradiation of foods is a physical treatment involving direct exposure to electron or electromagnetic rays, for their long time preservation and improvement of quality and safety (Mahindru, 2005). ^{60}Co (Cobalt-60) produces electromagnetic γ-rays which are similar to light but with much higher energy. During irradiation treatment, DNA molecules undergo break alongside the chain, preventing them from functioning normally. As a result, the parasites and microorganisms that have been affected are no longer capable of reproducing themselves and so they die (Lacroix and Ouattara, 2000).

Fishery products are also comparatively rich in unsaturated and essential lipids. Poly unsaturated fatty acids (PUFA) were reported to have beneficial effects on human health and also

are susceptible to peroxidation damage (Haghparast et al., 2010). Therefore, stability of these components needs to be considered for the standardization of the radiation process (Erkan and Özden, 2007). Ionizing radiation causes the radiolysis of water which is present to a great extent in fish. This generates free radicals such as OH^-, H^+ and hydrated electron, all of which react with the food constituents. The most susceptible site for free radical attack in a lipid molecule is adjacent to the double bonds. The most affected lipids during irradiation are thus the polyunsaturated fatty acids that bear two or more double bonds (Brewer, 2009). A review of the scientific and technical literature revealed some information about the effects of irradiation on fatty acids and fatty oxidation products of irradiated food (Katta et al., 1991; Ghadi and Venugopal, 1991; Monica et al., 2002; Yılmaz and Gecgel, 2007; Erkan and Özden, 2007; Chen et al., 2007; Hong et al., 2010).

The aim of this study was to determine the effects of irradiation process in low-dose and different doses of gamma irradiation (1, 3 and 5 kGy) on fatty acid composition of Rainbow trout fillets.

Material and methods

A total of 3.5 kg freshwater Rainbow trout (*Oncorhynchus mykiss*) (with the average weight of 300-500 grams) was obtained from a local aquaculture farm located at Saravan-Foman road, in the north of Iran. The fish were killed and then transported to the laboratory at the National Fish Processing Technology Research Center at Anzali port in Iran. For greater precision in determining the fatty acid composition, three fish were randomly selected. After passing into rigor mortis, the fish were washed with potable water, skinned, beheaded, gutted and then filleted by a sterile scalpel and washed again. Each fish was divided into four fillets, each fillet weighing approximately 70-80 grams. Each fillet was separately placed in a plastic film bag and was marked (control 1 and 1 kGy; control 3 and 3 kGy; control 5 and 5 kGy) (Moini et al., 2009). The fillets were divided into three lots (4 fillets in each lot): each lot included irradiated samples and their controls (0 kGy). Packed samples were delivered to the radiation plant in insulated polystyrene boxes with ice/fillets weight ratio to 2:1 to keep at 0-4° C. The ice was placed in plastic film bags.

Gamma irradiation was carried out in a ^{60}Co source irradiator (Gamma cell Px-30, dose rate 0.23 Gy sec^{-1}, Atomic Energy Organization of Iran, Karaj Nuclear Research Center for Agriculture and Medicine, Karaj, Iran). The applied dose levels were 0 (control), 1, 3, and 5 kGy (Moini et al., 2009). During irradiation the packaged fish were next to the sealed ice covering. The dose rate was established using alanine transfer dosimeter.

After irradiation, fillets were transported to the laboratory at the National Fish Processing Technology Research Center at Anzali port in Iran in insulated polystyrene boxes with ice/fillets weight ratio to 2:1 to keep at 0-4° C. In the laboratory, fillets were exposed to rapid freezing in a spiral freezer. Fillet depth temperature reached to -20° C within 25

minutes. Then frozen fillets were kept in a cold storage at -20° C.

The edible parts of each fillet (with skin) were homogenized and 5g of the homogenized sample was mixed well with 10g cleaned sea sand and 20g anhydrous sodium sulfate, and then percolated overnight 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, the remaining fat residue was weighed (Association of Official Analytical Chemists, AOAC, 1990).

The total lipids (2-2.5g) were saponified for 3h at 100° C in alcoholic KOH. From the saponification mixture, the non-saponifiable material and the fatty acids were extracted with diethyl ether, directly and after acidification with H_2SO_4, respectively. After evaporation of diethyl ether under vacuum, the residues were weighed. The free fatty acids were converted into their methyl esters with diazomethane and analyzed on a 10% Silar-10C column (on 100-120 mesh Gas-Chrom Q II; 2m×2mm ss) with a Varian 1400 gas chromatograph equipped with FID. The injector and the detector temperatures were 250° C and 260° C, respectively. The following temperature program was used: initial oven temperature, 175° C for 12 min, rising to 215° C at 6° C/min and a final hold time of 35-45 min. The gas flow rates were as follows: nitrogen 11 ml/min, hydrogen 20 ml/min and air 300 ml/min. The standard fatty acid methyl esters (Applied Science and Sigma) were used for the identification of peaks. The areas of the peaks were measured and the relative amounts of the fatty acids were calculated by Waters 730 data module (AOAC, 1990).

All experiments for each dose and its control were carried out in one replicate. Comparisons were carried out in the groups which included some fatty acids. The fatty acids which were in one compared group (e.g. group of mono unsaturated fatty acids) were considered as replicates in each comparison. All data were subjected to one-way analysis of variance and Duncan's multiple range test ($P<0.05$) to evaluate the effect of irradiation and different applied doses in this study on fatty acid composition. SPSS version 18.0 was used for statistical analysis.

Results

Fatty acid composition of Rainbow trout fillets (irradiated and their controls) are shown in table 1. Among all of the fatty acids, oleic acid (C18:1) (with mean 33.50±3.02 g/100 g fatty acids) was the most predominant fatty acid in all irradiated and non-irradiated samples. The lowest level of fatty acids was related to myristoleic acid (C14:1) and then linolenic acid (C18:3) (with mean respectively 0.41±0.26 and 0.66±0.58 g/100 g fatty acids) in all irradiated and control samples. Among the saturated fatty acids, the highest level was related to palmitic acid (C16:0) (with mean 21.04±2.32 g/100 g fatty acids) in all irradiated and control samples. The mean content of highly unsaturated fatty acids (HUFA), docosa hexaenoic acid (DHA) and ecosa pentaenoic acid (EPA) were respectively 2.27±2.11 and 0.69±0.32 g/100 g fatty acids, in all irradiated and control samples.

Table 1: Fatty acid composition (g /100g fatty acids) of Rainbow trout fillets irradiated (1, 3 and 5 kGy) and their controls.

Sample	Fatty Acid [a]															
	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:5 EPA	C22:6 DHA	Other Fatty Acids	ΣSFA	ΣUSFA	ΣMUFA	ΣPUFA
Control1 (0kGy)	1.84	0.42	18.91	3.01	5.24	29.43	32.26	0.34	3.38	0.72	2.43	2.02	29.37	68.61	32.86	35.75
1 kGy	1.86	0.54	22.08	3.25	5.05	33.70	27.25	0.20	2.79	0.43	0.81	2.04	31.78	66.18	37.49	28.69
Control3 (0kGy)	2.08	0.64	24.25	3.95	5.93	30.13	23.21	0.16	2.25	0.26	0.41	6.73	34.51	58.76	34.72	24.04
3 kGy	2.89	0.68	22.46	4.96	4.60	36.30	19.78	1.42	1.52	1.19	0.43	3.77	31.47	64.76	41.94	22.82
Control5 (0kGy)	2.45	0.11	20.45	4.68	4.24	35.77	20.31	1.40	1.42	0.87	4.21	4.07	28.56	67.35	40.56	26.79
5 kGy	1.78	0.07	18.09	4.36	4.24	35.67	21.40	0.47	1.52	0.71	5.36	6.33	25.63	68.04	40.1	27.94

[a] SFA (saturated fatty acid), USFA (unsaturated fatty acid), MUFA (mono unsaturated fatty acid), PUFA (poly unsaturated fatty acid)

Table 2: Statistical analysis of comparisons of fatty acids of Rainbow trout fillets irradiated (1, 3 and 5 kGy) and their controls

Fatty Acids [a]	Treatment	Statistical Parameter [b]			
		Mean	SD	n	P-value
TFA	**Control 1 (0 kGy)**	8.90	12.04	11	1.00
	1 kGy	8.90	12.41		
	Control 3 (0 kGy)	8.47	11.42	11	0.95
	3 kGy	8.74	11.96		
	Control 5 (0 kGy)	8.71	11.58	11	0.96
	5 kGy	8.51	11.54		
SFA	**Control 1 (0 kGy)**	29.37	7.83	4	0.92
	1 kGy	31.78	9.51		
	Control 3 (0 kGy)	34.51	10.56	4	0.91
	3 kGy	31.47	9.80		
	Control 5 (0 kGy)	28.56	8.94	4	0.90
	5 kGy	25.63	7.88		
USFA	**Control 1 (0 kGy)**	68.61	14.43	7	0.96
	1 kGy	66.18	14.51		
	Control 3 (0 kGy)	58.76	12.71	7	0.90
	3 kGy	64.76	13.77		
	Control 5 (0 kGy)	67.35	13.45	7	0.95
	5 kGy	68.04	13.64		
MUFA	**Control 1 (0 kGy)**	32.86	16.05	3	0.91
	1 kGy	37.49	18.41		
	Control 3 (0 kGy)	34.72	16.15	3	0.87
	3 kGy	41.94	19.44		
	Control 5 (0 kGy)	40.56	19.40	3	0.99
	5 kGy	40.10	19.43		
PUFA	**Control 1 (0 kGy)**	35.75	15.57	4	0.86
	1 kGy	28.69	13.38		
	Control 3 (0 kGy)	24.04	11.46	4	0.96
	3 kGy	22.82	9.39		
	Control 5 (0 kGy)	26.79	9.19	4	0.96
	5 kGy	27.94	9.87		

[a] TFA (total fatty acids), SFA (saturated fatty acids), USFA (unsaturated fatty acids), MUFA (mono unsaturated fatty acids), PUFA (poly unsaturated fatty acids)

[b] Mean (unit: g /100g fatty acids), SD (standard deviation), n (number of compared fatty acids); The comparison with $P>0.05$ is not significantly different.

Discussion

According to table 2 statistical analysis showed that there is no significant difference (P>0.05) between levels of fatty acids in irradiated (1, 3 and 5 kGy) Rainbow trout fillets and their controls. Also the difference of saturated fatty acid (SFA) contents (myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and arachidic acid (C20:0)), difference of mono unsaturated fatty acid (MUFA) contents (myristoleic acid, palmitoleic acid and oleic acid), difference of poly unsaturated fatty acids (PUFA) contents (linoleic acid (C18:2), linolenic acid, EPA (C20:5) and DHA (C22:6)) which are also essential fatty acids, in irradiated sample (1, 3 and 5 kGy) and their controls were not statistically significant (P>0.05). Irradiation at cold temperature and also subsequent frozen storing reduced free radicals production and therefore changes in fatty acid composition were not significant. Researchers have studied the effects of different methods of fish preservation on fatty acid composition of fish. Özden (2005) studied fatty acid composition changes in marinated fish during the marinating process and cold storage. Voldrich et al. (1991) examined the effect of smoking and marination on fatty acids in mackerel meat. Yang et al. (1981) investigated fatty acid changes caused by salting in gray fish. Oladapa et al. (1984) studied changes in the fatty acid composition of traditionally processed (smoked, solar-dried) freshwater fish species.

Also researches on meat irradiation and its effect on lipids have been done in the last decades. Researches done on chicken by Rady et al. (1988) showed no significant difference in total saturated and unsaturated fatty acids between irradiated (1, 3, 6 kGy) and non-irradiated frozen chicken muscle. Maxwell and Rady, (1989) also reported a steady increase in oleic acid in the polar fractions with increasing doses of gamma irradiation. However, Hafez et al. (1985) did not find changes in the fatty acids (C16:0, C18:1 and C18:2) of soybeans at different radiation doses (1, 5, 10, 20, 40, 60, 80 and 100 kGy). Katta et al. (1991) found significant decrease in the amount of palmitic acid and increase in oleic acid as irradiation dose level increased (0.5-3 kGy) in chicken meat. These authors determined that the levels of other fatty acids notably polyunsaturated fatty acid (linoleic and arachidonic acid) did not change.

Gamma irradiation at 50 kGy of vacuum-packed herring fillets at 0° C did not affect the proportion of polyunsaturated fatty acids (Adam et al., 1982). Hau and Liew, (1993) examined the effect of irradiation at 10 kGy on the linoleic and linolenic acid contents of grass prawns. Irradiation caused a 16% decrease in linoleic acid content, whereas linolenic acid was not affected significantly. Armstrong et al. (1994) reported no changes in fatty acid compositions of two species of Australian marine fish irradiated at doses of up to 6 kGy. The influence of irradiation on chemical components of tilapia and Spanish mackerel has been reported (Al- Kahtani et al., 1996). Irradiation of tilapia at 1.5–10 kGy caused a decrease in some fatty acids (C14:0, C16:0 and C16:1). In the case of Spanish

mackerel, C16:0 and C16:1 fatty acids decreased when irradiated at 1.5–10 kGy.

Yılmaz and Gecgel, (2007) have reported that irradiated (1, 3, 5 and 7 kGy) ground beef samples had higher concentrations of total *trans* fatty acids than the control samples. Irradiated ground beef samples with 7 kGy had the highest total *trans* fatty acids, total monounsaturated and total unsaturated fatty acids than the other samples. Results showed an increase in *trans* fatty acids related to the increase on irradiation dose in ground beef and irradiation dose changed fatty acids composition especially *trans* fatty acids in ground beef.Erkan and Özden, (2007) reported that the contents of total SFA in the muscle of non-irradiated sea bream was respectively lower than in 2.5 kGy irradiated sea bream and higher than in 5 kGy irradiated sea bream. There was significant difference in the content of total MUFA between 2.5 kGy and 5kGy irradiated sea bream and no significant difference was determined in the content of MUFA between non-irradiated and irradiated fish. The content of PUFA in the muscle of 5 kGy irradiated sea bream was significantly lower than in non-irradiated and 2.5 kGy irradiated sea bream. Özden and Erkan, (2010) reported that total saturated and total monounsaturated fatty acid contents were 27.97% and 24.72% for non-irradiated sea bass, respectively. The amounts of these two fatty acids in irradiated samples increased to 28.18 and 25.75% for 2.5 kGy and 29.08 and 28.54% for 5 kGy. Significant difference was found in the content of total MUFA between 2.5 kGy (25.75%) and 5 kGy (28.54%) irradiated sea bass and between non-irradiated and irradiated fish. Total polyunsaturated fatty acid content for irradiated samples was higher than non-irradiated samples. Chen et al. (2007) reported that total SFA and MUFA of beef lipid increased with irradiation (1.13, 2.09 and 3.17 kGy), ratios of MUFA to SFA did not change. Whilst, total PUFA reduced with irradiation, which resulted in PUFA to SFA ratio decrease. Alfaia et al. (2007) reported that no significant differences were observed for fatty acid composition between non-irradiated (control) and irradiated (7 kGy) meat samples. Brito et al. (2002) reported that the total *trans* fatty acids in non-irradiated ground beef is smaller than the irradiated one.

Therefore irradiation process and different doses of irradiation in this study (1, 3 and 5 kGy) had no significant effects (P>0.05) on fatty acid composition of Rainbow trout fillets.

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