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Changes of Fatty Acid profile during Gamma Irradiation on of Rainbow Trout (*Oncorhynchus mykiss*) Fillet

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ABSTRACT: The present study was conducted to evaluate the effect of different doses of gamma rays (0, 0.75, 1.5, 2.25, 3, 3.75 and 4.5 kGy) on fatty acids composition of Rainbow trout fillet. Irradiation of fillets was performed by gamma rays from a Co60 source. The results showed that total saturated fatty acid concentrations increased significantly (p<0.001) with increasing irradiation dose , so that the control sample and fish muscles that irradiated with 4.5 kGy had the lowest and highest amounts of total saturated fatty acids (20.454 ± 0.011% and 19.228 ± 0.040% , respectively). The amount of total polyunsaturated fatty acids (PUFAs) in irradiated samples were significantly lower than control sample (p<0.001) and amounts of total monounsaturated (MUFAs) were significantly higher than control samples (p<0.001). The results were indicated that the highest content of MUFAs and PUFAs were in samples irradiated with 3.75kGy (37.783± 0.092 %) and control samples (37.677± 0.104 %), respectively .The lowest level of MUFAs and PUFAs were in control samples (36.596 ± 0.024%) and 4.5 kGy (36.459 ± 0.047%), respectively. Overall, results of this study determined that gamma irradiation changes the fatty acids profile especially polyunsaturated fatty acids in rainbow trout fillet significantly (P<0.001).

Keywords: Effect, fillet, fatty acid, irradiation, Rainbow trout, profile.

INTRODUCTION

Food irradiation is a means of food preservation that has been in development since the early part of the 20th century. Irradiation as a method of meat, poultry and fish preservation has excellent potential to improve meat safety and extend the shelf life (Fu e*t al.,* 2000; Chwla *et al.,* 2003; Jeevanandam *et al.,* 2001; Mahrour *et al.,* 2003; Chouliara *et al.,* 2004, 2005 Morehouse., 2002). It is capable of improving the safety of many foods, and extending their shelf life. There has been worldwide interest in using irradiation for preservation of various foods, including fishery products (Kreuzer, 1969; Kilgen, 2001). Irradiation of food up to an overall dose of 10 kGy is accepted in several countries for commercial food processing (Lacroix & Quattara, 2000). The quality of meat is influenced by its lipid content and fatty acid composition. Fish meat is more or less susceptible to oxidative deterioration, depending on the degree of lipid saturation. Polyunsaturated fatty acids (PUFAs) benefit human health, but also increase susceptibility to lipid oxidation. Oxidation leads to negative effects on quality parameters such as flavour, colour, texture and nutritive value (Buckley *et al.,* 1995). During recent decades, research on n-3 highly unsaturated fatty acid (n-3 HUFA) in marine foods has intensified due to their beneficial effects on human health. They have been shown to have curative and preventive effects on cardiovascular diseases, mortality and neurodevelopment in infants (Kinsella., 1986; Kinsella *et al.,* 1990; Hu *et al.,* 2001; Okada, & Morrissey, 2007).

Gamma rays can convert water molecules to ions and free radicals (OH , H, H3O, e-) Double bonds of carbon - carbon on unsaturated fatty acids and carbonyl groups of fatty acid and amino acids, are especially prone to attack of free radicals. These unsaturated fatty acids are the primary source for the oxidation of lipids. With increasing irradiation dose, the amount of unsaturated fatty acids decreased at the end time of exposure (Brewer, 2009).

A review of the scientific and technical literature revealed some information about the effect of irradiation and other processes on fatty acid composition in fish. Ozden (2005) investigated about changes in fatty acid composition of marinated fish during its shelf-life.

He found that total saturated fatty acid concentrations increased significantly in marinated fish during chilled storage. Haliloglu *et al* (2002) compare fatty acids profiles of muscle lipids of three trout species (*Salvelinus alpinus*, *Salmo trutta fario* and *Oncorhynchus mykiss*) fed the same commercial diet and reared under the same conditions. They found that Palmitic acid (16:0) in total saturated fatty acid (SFAs) and oleic acid (18:1 n-9) in monounsaturated fatty acids (MUFAs) were the most abundant FAs and significant differences were observed between fish species. Gamma irradiation at 50 kGy of vacuum-packed herring fillets at 0 1C did not affect the proportion of polyunsaturated fatty acids (Adam *et al.,* 1982). Recently, Kalyoncu *et al* (2010) were studied on the seasonal fluctuations of fatty acid compositions of rainbow trout and ω3/ ω6 fatty acids ratio of this fish species. Yilmaz *et al* (2007) were evaluated the effects of irradiation (0, 1, 3, 5 and 7 kGy) of ground beef on fatty and trans fatty acids. Mbarki *et al* (2009) reported that low-dose irradiation had no adverse effect on the nutritionally important polyunsaturated fatty acids (PUFAs) of Mediterranean horse mackerel.

The objective of this study was to evaluate the effect of different doses of gamma rays on fatty acid composition in *O. mykiss* muscle samples.

MATERIALS AND METHODS

A. Fish sample

Cultured fresh Rainbow trout (*Onchorhynchus mykiss*) brought from Ranghinkaman farm in Guilan province in Iran during September 2010. The average weight and length of the fish were 390±53 gr and 33±2.2 cm, respectively. Then, fish were eviscerated, washed and packaged in polyethylene pouches and were packed in polystyrene boxes with ice (ice to fish ratio was 2:1) samples were transported to irradiation process within 18 hours.

B. Irradiation

Samples were irradiated at the Iranian Atomic Energy Organization- Tehran, Iran, using a Gamma cell facility with 60 Cobalt radiation source. The applied doses in this study were 0, 0.75, 1.5, 2.25, 3, 3.75 and 4.5 kGy. Exposure time showed in table 1. (Dose rate: 4.6 Gy/Sec, Transit dose: 20.0 Gy, Activity: 19823.7 Ci). The absorbed dose was monitored by an alanine transfer dosimeter. Dosimetry of irradiation process was showed that samples were irradiated with deviation of ± 20%.

Fish samples were maintained at 2±1 °C during irradiation by using sealed ice covering the samples. Internal temperature of the facility was 20±1 °C. For simulation of condition, non-irradiated fish muscle samples (control) were kept in polystyrene boxes in room temperature (20-22°C) until the end of irradiation process. Irradiation process and all analyses were performed using three samples (pouches) per treatment.

Table 1: Exposure time of irradiation of rainbow trout muscle.

Irradiation dose(kGy)	Exposure time	
	min	sec
0.75	2	20
1.5	5	41
2.25	8	35
3	11	22
3.75	13	42
4.5	17	6

C. Lipid extraction and fatty acid compositions

Total lipid was extracted according to the method of Weilmeier and Regenstein (2004). Fatty acid composition was determined after methylation, by gas chromatography (Agilent 6890, Italy) using a BPX-Column (70120 m, 250µm i.d, 0.2 µm film thickness'). The chromatographic conditions were as follows- injection volume: 0.5 ml; injection temperature: 250 ºC; detector and detector temperature: FID-280ºC; column temperature, 120 1C for 2 min, programmed at 5 1C/min up to 220 1C for 8 min. Fatty acids were identified by comparison of their retention time with those of authentic standard (Sigma Cod No.: 189-19) and their contents were calculated on a weight percentage basis(ISO 5508, ISIRI 4091)(Christie, 1989). All chemical (Methanol, n-Hexane, Potassium hydroxide pellets) brands was MEREK.

D. Statistical analysis

The complete experiment was performed in triplicate. Significant differences between the samples were calculated by Spss 13.00 by One way analysis of variance (ANOVA) .Comparison of means were based on Post Hoc multiple test (Duncan’s test). Level of significance was set at $P<0.001$.

RESULTS

Fatty acid composition in non-irradiated and irradiated rainbow trout muscle samples with 0.75, 1.5, 2.25, 3, 3.75 and 4.5 kGy are shown in Table 2. Content of total saturated fatty acids (SFAs) in the non irradiated muscle of rainbow trout was 19.228%. The highest and lowest content of SFAs between treatments were in samples irradiated with 4.5 kGy (20.454%) and 0.75 kGy (19.043%), respectively. The predominant saturated fatty acid in all treatments was Palmitic acid (C16:2).

Table 2. Variations in total fatty acid composition of irradiated fillets of *O. mykiss* * (% of total FA).

Dose / Fatty acid	0	0.75	1.5	2.25	3	3.75	4.5
c12:0, Lauric acid ***	0.020 ± 0.001^{b}	0.013 ±0.003^{ab}	0.011 ±0.002^{a}	0.019 ± 0.005^{b}	0.022 ± 0.003b^{c}	0.030 ± 0.002^{c}	0.029 ±0.000^{c}
c14:0 , Myristic acid	0.972 ± 0.003^{d}	0.860 ±0.026^{b}	0.878 ±0.016^{bc}	0.872 ± 0.008^{b}	0.918 ± 0.012^{c}	0.870 ± 0.004^{b}	0.707 ± 0.006^{a}
c15:0 , Pentadecanoic acid	0.181 ± 0.002^{c}	0.176 ±0.005$^{a\ bc}$	0.167 ±0.003^{a}	0.170 ± 0.002$^{a\ bc}$	0.169 ± 0.001$^{a\ b}$	0.172 ± 0.003$^{a\ bc}$	0.181 ± 0.005^{bc}
c16:0 , Palmitic acid	12.245± 0.013^{a}	12.303 ±0.045^{a}	12.836±0.553$^{a\ b}$	12.270±0.047^{a}	12.839± 0.105$^{a\ b}$	12.931± 0.341$^{a\ b}$	13.388± 0.431^{b}
c17:0 , Heptadecanoik acid	.0830 ± 0.003^{a}	0.083 ±0.006^{a}	0.071 ±0.006^{a}	0.082 ± 0.008^{a}	0.078 ± 0.003^{a}	0.310 ± 0.006^{a}	0.080 ± 0.003^{b}
c18:0 , Stearic acid	4.471 ± 0.014^{c}	4.256 ±0.040^{a}	4.636 ±0.121^{e}	4.545 ± 0.035^{d}	4.325 ± 0.022^{b}	4.251 ± 0.039^{a}	4.489 ± 0.014$^{c\ d}$
c20:0 , Arachidic acid	0.321 ± 0.002^{c}	0.307 ±0.006^{b}	0.322 ±0.003^{c}	0.308 ± 0.006^{b}	0.290 ±0.004^{a}	0.291 ± 0.003^{a}	0.333 ± 0.003^{c}
c22:0 , Behenic acid	0.823 ± 0.003^{b}	0.943 ±0.003^{e}	0.863 ±0.036^{d}	1.155 ±0.115^{f}	0.780 ± 0.023^{a}	0.840± 0.025^{c}	1.158 ± 0.094^{f}
c24:0 , Lignoceric acid	0.111 ± 0.002^{ab}	0.102 ±0.002^{ab}	0.112 ±0.003^{c}	0.103 ± 0.006^{ab}	0.098 ± 0.004^{b}	0.080 ± 0.004^{a}	0.109 ± 0.036^{ab}
total saturated	**19.228**	**19.043**	**19.897**	**19.525**	**19.520**	**19.777**	**20.454**
c14:1 , Myristoleic acid	0.059 ± 0.002^{ab}	0.040 ± 0.005^{c}	0.060 ± 0.000^{ab}	0.069 ±0.004^{a}	0.059 ± 0.004^{ab}	0.060 ± 0.003^{ab}	0.058 ± 0.003^{b}
c15:1 ,	0.039 ± 0.007^{a}	0.041 ± 0.003^{a}	0.042 ± 0.003^{a}	0.039 ± 0.001^{a}	0.040 ± 0.001^{a}	0.041 ± 0.002^{a}	0.039 ± 0.002^{a}
c16:1 , Palmitoleic acid	2.093 ± 0.005^{c}	2.030 ± 0.036^{c}	2.408 ± 0.011^{ab}	2.525 ± 0.005^{a}	2.498 ± 0.012^{a}	2.320 ±0.073^{b}	2.343 ± 0.061^{b}
c17:1 , Heptaenoic acid	0.249 ± 0.006^{ab}	0.241 ± 0.004^{b}	0.248 ± 0.008^{ab}	0.262 ± 0.003^{a}	0.262 ± 0.003^{a}	0.250 ± 0.002^{ab}	0.250 ± 0.004^{ab}
t.c18:1, trans -Oleic acid - ω9	0.142 ± 0.003^{a}	0.171 ± 0.007^{b}	0.137 ± 0.006^{a}	0. 131 ± 0.001^{a}	0.140 ± 0.002^{a}	0.140 ± 0.002^{a}	0.130 ± 0.001^{a}
c.c18:1 , *cis*-Oleic acid - ω9	32.121 ± 0.213^{a}	32.148 ± 0.451^{a}	32.340± 0.017^{ab}	32.177 ± 0.449^{a}	32.189 ± 0.142^{a}	32.813± 0.105^{b}	32.150 ± 0.442^{a}
c20:1 , *cis*-11-Eicosaenoic acid - ω 9	1.381 ± 0.009^{e}	1.470 ± 0.010^{d}	1.576 ± 0.005^{b}	1.625 ± 0.014^{a}	1.529 ± 0.006^{c}	1.560 ± 0.004^{b}	1.615 ± 0.015^{a}
c22:1 , Erucic acid - ω 9	0.402 ± 0.003^{d}	0.474 ± 0.008^{c}	0.570 ± 0.013^{a}	0.565 ± 0.005^{a}	0.508 ± 0.008^{b}	0.489 ± 0.009^{b}	0.538 ± 0.013^{a}
c24:1 , Nervonic acid - ω 9	0.110 ± 0 .005^{ab}	0.158 ± 0.003^{b}	0.109 ± 0.002^{ab}	0.133 ± 0.003^{a}	0.101 ± 0.003^{ab}	0.110 ± 0.002^{ab}	0.139 ± 0.004^{ab}
total monounsaturated	**36.596**	**37.773**	**37.490**	**37.526**	**37.326**	**37.783**	**37.262**
t.c18:2 , trans-Linoleadic acid - **ω 6**	0.218 ± 0.003^{c}	0.169 ± 0.010^{a}	0.189 ± 0.002^{b}	0.180 ± 0.004^{bc}	0.180 ± 0.005^{bc}	0.176 ± 0.005^{bc}	0.250 ± 0.005^{d}
c.c18:2 , *cis*-Linoleadic acid - ω 6	24.166 ± 0.306^{ab}	24.447 ± 0.586^{a}	24.547 ± 0.172^{a}	24.296 ± 0.004^{a}	24.103 ± 0.025^{ab}	24.105 ± 0.031^{ab}	23.830 ± 0.295^{b}
gamma.c18:3 , γ-Linolenic acid - **ω 6**	0.573 ± 0.015^{ab}	0.564 ± 0.018^{ab}	0.595 ± 0.007^{a}	0.579 ± 0.002^{ab}	0.605 ± 0.005^{a}	0.547 ± 0.006^{b}	0.535 ± 0.005^{b}
alpha.c18:3 , α- Linolenic acid **ω 3**	3.033 ± 0.015^{d}	3.352 ± 0.008^{b}	3.334 ± 0.013^{b}	3.380 ±0.005^{b}	3.507 ± 0.040^{a}	3.226 ±0.007^{c}	3.318 ± 0.020^{b}
C 20:3 , *cis*-8,11,14-Eicosatrienoic acid **ω 6**	0.010 ± 0.010	0.010±0.01	0.010±0.00	0.020 ± 0.010	0.010 ± 0.000	0.010±0.00	0.010 ± 0.01 0
C 20:3 , *cis*-11,14,17-Eicosatrienoic acid **ω 3**	1.170 ± 0.050	1.180 ± 0.08	1.160 ± 0.07	1.150 ± 0.010	1.200 ± 0.010	1.200 ± 0.05	1.100 ± 0.010
C 20:4 ,Arachidonic acid **ω 6**	1.935 ± 0.015	2.010±0.31	1.970±0.10	2.080 ± 0.010	2.010 ± 0.030	1.890±0.04	1.971 ± 0.021
c20:5*cis*-5,8,11,14,14-Eicosapentaenoic **ω3 (EPA)**	3.392 ± 0.008^{b}	3.507 ± 0.033^{a}	3.435 ± 0.053^{ab}	3.440 ± 0.002^{ab}	3.427 ± 0.006^{ab}	3.450 ± 0.017^{ab}	3.373 ± 0.012^{b}
c22:4 *cis*-7,10,13,16-Docosatetraenoic acid **ω6**	0.395 ± 0.005^{b}	0.453 ± 0.015^{a}	0.448 ± 0.008^{a}	0.450 ± 0.004^{a}	0.388 ± 0.008^{b}	0.470 ± 0.006^{a}	0.338 ± 0.008^{c}
c22:6 *cis*-4,7,10,13,16,19-Docosahexaenoic **ω3 (DHA)**	1.785 ± 0.005	1.733 ± 0.067	1.798 ± 0.053	1.702 ± 0.023	1.722 ± 0.055	1.800 ± 0.050	1.734 ± 0.005
total polyunsaturated	**37.677**	**37.425**	**37.468**	**37.277**	**37.152**	**36.874**	**36.459**
not detected	**6.499**	**5.145**	**5.336**	**5.672**	**6.002**	**5.566**	**5.825**

* Average of three lots analyzed. **Values reported are means± S.D. *** abcd Values for each sample with different superscript letters in the same rows are significantly different at $p < 0.001$.

The content of this fatty acid had a significant differences between treatments($p < 0.001$) and the lowest and the highest amount of this fatty acid were in control sample (12.245 ± 0.013 %) and in samples irradiated with 4.5 kGy (13.338 ± 0.431%), respectively. There was a significant differences ($p<0.001$) in the content of total monounsaturated fatty acids (MUFAs) between treatments. The lowest and highest content of MUFAs in muscle of rainbow trout were in control samples (36.596 %) and those irradiated with 3.75 kGy (37.783 %), respectively. The most dominant MUFAs in muscle of rainbow trout were cis-oleic acid ($C_{18:1\ \omega 9}$) and cis-11-Eicosaenoic acid($C_{20:1\ \omega 9}$). The difference these fatty acids between treatments were statistically significant ($p<0.001$).

The quantity of polyunsaturated fatty acids(PUFAs) in control samples and samples irradiated with 0.75, 1.5, 2.25, 3, 3.75 and 4.5 kGy were 38.425, 38.468, 37.484, 37.277, 37.152, 37.152, 36.677, 36.459%, respectively.

The dominant polyunsaturated fatty acids in control sample were cis-Linoleadic acid $C_{18:2\ \omega 6}$ (25.447± 0.580 %), -Linolenic acid $C_{18:3\ \omega 3}$ (3.352± 0.008%) and cis-5, 8, 11, 14, 14-Eicosapentaenoic acid $C_{20:5\ \omega 3}$ (3.507 ± 0.003%).

At the end of irradiation process, the content of cis-5,8,11,14,14-Eicosapentaenoic acid $C_{20:5\ \omega 3}$ irradiated samples with 0.75, 1.5, 2.25, 3, 3.75 and 4.5 kGy, were 3.435±0.053 %, 3.450 ± 0.017%, 3.440 ±0.002 %, 3.427±0.006, 3.392± 0.053 % and 3.373 ± 0.012 %, respectively. These values for cis- 4, 7, 10, 13, 16, 19-Docosahexaenoic acid $C_{22:6\ \omega 3}$ were 1.733± 0.067%, 1.798±0.053%, 1.800±0.050%, 1.702± 0.023%, 1.722±0.055 %, 1.785 ± 0.005% and 1.734± 0.005 %, respectively.

The differences between the values of the former fatty acid was significant ($p<0.001$) but this difference for the latter fatty acid was not significant ($p<0.001$).

DISCUSSION

Present findings showed an increase in saturated fatty acids related to the increasing of irradiation dose in rainbow trout trout. Also irradiation was changed fatty acids composition especially MUFAs and PUFAs fatty acids in fish muscle as the content of PUFAs was 37.677% in control sample and declined to 36.459% in samples irradiated with 4.5 kGy.

In our study, amount of Palmitic acid (SFA) significantly increased during increasing irradiation dose and Oleic acid content was not significantly changed. However, Katta *et al.,* (1991) found significant decrease in amount of Palmitic acid and increase in Oleic acid as irradiation dose level increased (0.5 to 3 kGy) in chicken meat.

Similar results for other fish species have also been reported (Rahman *et al.,* 1995). 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. Armstrong *et al.,* (1994) reported no changes in fatty acid compositions of two species of Australian marine fish irradiated at doses up to 6.0 kGy. This finding was contradicted with our results.

Yilmaz & Geçgel (2007) showed that concentration of total trans fatty acids in irradiated ground beef samples had higher than the control samples and irradiated ground beef samples with 7 kGy had the highest total trans fatty acids. Hau & Liew (1993) reported that irradiation at 10 kGy caused a 16% decrease in the linoleic contents of grass prawns. Whereas Linolenic acid was not affected significantly. Ozden & Erkan (2010) reported that total saturated fatty acid contents were increased in irradiated fish samples. These results were similar to our finding about SFAs. These study results generally agreed that irradiation of sea foods and meats had marginal effects on the lipids, including essential fatty acids .In this study.

Maxwell & 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 amount of Palmitic acid and increase in oleic acid as irradiation dose level increased (0.5–3 kGy) in chicken meat. These authors determined levels of other fatty acids notably polyunsaturated fatty acid (linoleic and arachidonic acid) did not change.

Al- Kahtani *et al.,* 1996 reported Influence of irradiation on chemical components of tilapia and Spanish mackerel whereas Irradiation of tilapia at 1.5–10 kGy caused a decrease in some fatty acids (C14:0, C16:0 and 16:1) and In Spanish mackerel, C16:0 and C16:1 fatty acids decreased when irradiated at 1.5–10 kGy.

CONCLUSION

The effects of irradiation on oxidation process of lipids are well known. The results of our study showed that the different doses of irradiation especially high doses (3-4.5 kGy) affect fatty acid composition in rainbow trout fillet. It is stated that there was relationship between irradiation dose and lipid oxidation whereas with increasing of irradiation dose, lipid oxidation will urge.

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