Effect of Gamma irradiation and frozen storage on microbial quality of Rainbow trout (*Oncorhynchus mykiss*) fillet

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Received: January 2010 Accepted: Agust 2010

Abstract

The effect of gamma radiation (1, 3 and 5 kGy) on microbial quality of farmed rainbow trout (*Oncorhynchus mykiss*) fillets which were stored under frozen conditions (-20° C) was studied by measuring microbiological changes in 5 months. Gamma irradiation and increasing of frozen storage time had significant effects ($P<0.05$) on the reduction of microorganism's population. The total count showed that all samples maintained acceptable microbiological quality until the end of the fifth month of frozen storage. The lowest microbial load at the end of the fifth month of frozen storage was related to irradiated samples at 3 kGy (2 Log CFU/g). Yeasts and molds were below the detection levels in both irradiated samples at 1 and 5 kGy until the end of the third month and in irradiated samples at 3 kGy throughout the frozen storage. The population of yeasts and molds increased in irradiated samples at 1 and 5 kGy in the fourth and fifth month of frozen storage. Growth of coliform bacteria and *Salmonella* wasn't observed in control and irradiated samples due to good hygienic quality of fish breeding, fishing, handling, filleting and packaging and also effect of freezing on elimination and inactivation of mesophilic microorganisms.

Keywords: Gamma irradiation, Frozen Storage, Rainbow trout (*Oncorhynchus mykiss*), Microbiological analysis

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[DOR: 20.1001.1.15622916.2011.10.1.7.6]

Introduction

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 (taste, aroma, white flesh) resulting in a high-quality product and nutritional value (FAO, $2010_{a,b}$; Iranian Fisheries Organization, IFO, 2009). The major problem of distribution of seafood or fishery products is their susceptibility to spoilage, mainly due to contamination of spoilage and pathogenic microorganisms (Özden and Erkan, 2010). Fish spoilage occurs following growth and activity of special microorganisms and lipid oxidation which cause off-odor and off-taste by production of some metabolites changing sensory characteristics and customer acceptability (Moini et al., 2009; Rostamzad et al., 2010). Therefore 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). Besides traditional methods such as ice storage, rapid chilling, freezing, smoking and heating (Farkas, 1990, 1999; Himelblooom et al., 1994), 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 fisheries products. The irradiation of food products 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). Cobalt-60 (^{60}Co) produces electromagnetic γ-rays which have too much energy. During radiation, DNA molecules undergo swelling and 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 they die (Lacroix and Ouattara, 2000). Therefore food irradiation provides safety and extends the shelf life of fisheries products because of its high effectiveness in inactivating pathogenic and spoilage microorganisms without deteriorating product quality (Özden and Erkan, 2010). The alteration in microbial population and composition as a result of irradiation depends on the dose of irradiation, storage temperature, packaging conditions and fish species (Özden et al., 2007). Freezing controls growth of microorganisms and biochemical changes in fish as a preservation method for long time storage (Motalebi et al., 2010). When irradiation is used in combination with freezing, the irradiation doses can be reduced through synergistic action without affecting the product quality (Lacroix and Ouattara, 2000). A review of the scientific and technical literature revealed some information about the effects of irradiation on microbiological characteristics of irradiated food (Lamuka et al., 1992; Dogbevi et al., 1999; Thayer and Boyd,

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2000; Ouattara et al., 2001, 2002; Badr, 2004; Chouliara et al., 2004; Javanmard et al., 2006; Özden et al., 2007; Sedeh et al., 2007; Fallah et al., 2008; Turgis et al., 2008 ; Ahmed et al., 2009; Moini et al., 2009).

The aim of this study was to determine the effect of gamma irradiation process in low-dose (1, 3 and 5 kGy) and frozen storage on microbial quality of rainbow trout fillets.

Materials and methods

A total of 10 kg freshwater rainbow trout (*Oncorhynchus mykiss*) with an average weight of 300-500 grams were obtained from a local aquaculture farm located at Saravan-Foman road, in the north of Iran. The fish were then transferred to the laboratory at the National Fish Processing Technology Research Center at Anzali port in Iran. After passing into rigor mortis, the fish were washed with tap water, skinned, beheaded, gutted and then filleted by a sterile scalpel and washed again. Each fish was divided into four fillets (about 70-80 g each). Each fillet was separately placed in a plastic film bag. The fillets were divided into four lots (20 fillets in each lot): 0 kGy (control) and irradiated samples (1, 3 and 5 kGy) (Moini et al., 2009). Packed samples were delivered to the radiation plant in insulated polystyrene boxes with ice/fillets weight ratio to 2:1. The ice was placed in plastic film bags. Gamma irradiation was carried out in the Nuclear Research Center for Agriculture and Medicine, Karaj, Iran. Fish samples were gamma irradiated using a ^{60}Co source irradiator (Gamma cell Px-30, dose rate 0.23 Gy sec^{-1}). The applied dose levels were 0 (control), 1, 3, and 5 kGy (Moini et al., 2009). During irradiation the packed fish were next to sealed ice covering. The dose rate was established using alanine transfer dosimeter. After irradiation, irradiated and non-irradiated 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. In the laboratory, fillets were exposed to rapid freezing in a spiral freezer (Koppens SVR C400/17-50, UK). Fillet depth reached to -20° C within 25 minutes. Then frozen fillets were kept in a cold storage at -20° C for 5 months. Rainbow trout fillets were analyzed for microbiological quality at the first day of frozen storage as 1-month sampling intervals for 5 months. The first day of the first interval was registered as day zero. For the microbiological analysis 10 g of rainbow trout fillet was removed with a sterile scalpel and minced under aseptic conditions. Then it was homogenized for 2 minutes with 90 ml of 0.1% (w/v) sterile peptone water (Merck, Germany) using a lab-blender 400 stomacher (Seward medical, UK). Subsequent dilutions were prepared by mixing a 1-ml sample with 9 ml of sterile peptone water. All analyses were carried out in duplicate. For determination of total bacterial count, 1 ml of appropriate dilutions were poured-plated with melted plate count agar (PCA) (Merck, Germany) and then were incubated at 35-37° C for 48 h. For the numeration of total coliforms, 1 ml of appropriate dilutions were poured-plated with melted violet red bile agar (VRBA); plates were incubated at 37°C for 48 h. Total yeasts and molds were enumerated on potato dextrose agar (Merck, Germany)

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after incubation at 25° C for 3–5 days. For detection of *Salmonella* spp., 10 g of the sample was homogenized with 90 ml lactose broth (Merck, Germany) and incubated at 35° C for pre-enrichment. Selective enrichment was performed in tetrathionate broth (Merck, Germany) at 43° C for 24 h and selenite cystine broth (Merck, Germany) at 35° C for 24 h followed by plating on *Salmonella-Shigella* (SS) agar (Merck, Germany) and brilliant-green phenol-red lactose sucrose (BG) agar (Merck, Germany) incubated at 35° C for 24 h. Suspected colonies developed on each plate served to biochemical and serological analysis (American Public Health Association, APHA, 1992).All data from microbial analysis were subjected to factorial analysis of variance (ANOVA) and Duncan's multiple range test (P<0.05) to evaluate the effect of irradiation and different applied doses in this study and frozen storage time on microbiological characteristics of rainbow trout fillets. Differences between means were considered significant when P<0.05. SPSS version 18.0 was used for statistical analysis.

Results

The values of total count, yeasts and molds count, coliforms count and *Salmonella* detection of non-irradiated (control) and irradiated (1, 3 and 5 kGy) rainbow trout fillets during frozen storage (-20° C) are shown in Table 1.Initial total bacterial counts of the control samples were 4.38 Log CFU/g, whereas the counts in samples irradiated at 1 kGy were 3.45 Log CFU and in irradiated samples at 3 and 5 kGy were not detectable at day 0 of frozen storage. Microbial load of irradiated samples at 5 kGy until the end of the first month and in the irradiated samples at 3 kGy until the end of the fourth month were below detection level.

Table 1: Microbial flora count (Log CFU/g) in non-irradiated and irradiated (1, 3 and 5 kGy) of rainbow trout fillets during frozen storage (-20 °C)

Microbial Flora	Radiation Dose (kGy)	Storage Time (Month)					
		0	1	2	3	4	5
Total Count	0	4.38±1.53 ^{aw}	3.65±0.06 ^{abw}	3.30±0 ^{aw}	3.65±0.06 ^{aw}	3.30±0 ^{aw}	3.45±0.21 ^{bw}
	1	3.45±0.21 ^{ax}	3.23±0.33 ^{abx}	2.00±0 ^{ax}	2.45±0.21 ^{ax}	2.00±0 ^{ax}	2.69±0.12 ^{bx}
	3	ND ^{ay}	2.00±0 ^{aby}	ND ^{ay}	ND ^{ay}	ND ^{ay}	2.00±0 ^{by}
	5	ND ^{az}	ND ^{abz}	2.00±0 ^{az}	1.00±1.41 ^{az}	2.00±0 ^{az}	2.47±0 ^{bz}
Yeasts and Molds	0	ND	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	1.00±0	1.00±0
	3	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	1.00±0	1.15±0.21
Coliforms	0	ND	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND
Salmonella	0	ND	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND
	5	ND	ND	ND	ND	ND	ND

ND = not detected; $^{a-b}$ Means within a row, which are not followed by a common superscript letter(s) are significantly different (P<0.05); $^{w-z}$ Means within a column, which are not preceded by a common superscript letter(s) are significantly different (P<0.05).

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The lowest microbial load at the end of the fifth month of frozen storage was related to irradiated samples at 3 kGy (2 Log CFU/g). Yeasts and molds were not detected in irradiated samples at 3 kGy throughout the frozen storage time and in irradiated samples at 1 and 5 kGy until the end of the third month of frozen storage.

The population of yeasts and molds increased in irradiated samples at 1 and 5 kGy in the fourth and fifth month of frozen storage.

Coliform and *Salmonella* bacteria were not detected in all irradiated and control samples throughout the storage.

Discussion

Although it is widely accepted that the initial microbial load of freshwater fish varies depending on water conditions and temperature, most of the available literature on different freshwater species (Tilapia, Striped bass, Rainbow trout, Silver perch and Sea bream) reports bacterial counts of 2 to 7 Log CFU/g (Moini et al., 2009). The initial counts and the counts in all the time during the frozen storage indicated to an acceptable fish quality, considering the proposed upper acceptability limit for total bacterial counts of 2×10^7 CFU/g for fresh and frozen fish (ISIRI, 1999).

The results of total microbial count showed that microbial load of irradiated samples (at 1, 3 and 5 kGy) were significantly ($P<0.05$) lower than controls throughout the storage period. This finding confirms the significant effect ($P<0.05$) of irradiation on the reduction of microbial count. Food spoilage microorganisms are generally very susceptible to irradiation; a 90% reduction of most vegetative cells can be accomplished with 1–1.5 kGy (Brewer, 2009). In irradiated samples the highest and lowest microbial counts were related to 1 and 3 kGy, respectively. Because the highest radiation dose in this study (5 kGy) might induce lipid oxidation. These reaction metabolites made a good media for microbial growth. In this study increasing frozen storage time caused a significant ($P<0.05$) reduction effect on microbial count. Freezing is known to reduce viable cell counts by 1-2 Log units, with extended storage causing additional, time dependent reductions (Yammamoto and Harris, 2001).

Moini et al. (2009) reported that irradiation at 1, 3 and 5 kGy had a significant reduction effect on the total viable count of rainbow trout fillets. Ahmed et al. (2009) in evaluating the efficiency of gamma radiation (3, 5 and 8 kGy) in combination with low temperature (-20° C) storage of degutted fresh *Pampus chinensis*, reported that total bacterial count (TBC) was affected by the radiation. In their study, initial bacterial load of control was maximum (1.3×10^4 CFU/g) followed by 3 kGy irradiated fishes (2×10^2 CFU/g) and at 5 and 8 kGy the samples were completely sterilized resulting in no bacterial growth. TBC values in their investigation suggest that the irradiated samples remain acceptable after 90 days at -20°C. Sedeh et al. (2007) reported that irradiation (0.5, 1, 2 and 3 kGy) and storage at low temperature had a significant reduction effect on microbial loads of bovine meat. They reported that the combined effect of irradiation and frozen storage was more effective than each treatment alone on decreasing total bacteria counts. Javanmard et al. (2006)

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reported that irradiation (0.75, 3 and 5 kGy) and freezing storage (-18°C) had a significant reduction effect on microbial loads of chicken meat. The combination of frozen storage plus irradiation resulted in greater overall reductions of microbial loads, extending shelf life of chicken meat for commercial application and critical conditions. Jørgensen and Hansen, (1965) reported that the irradiation of vacuum-packed gutted trout at 2 kGy a total viable aerobic count of 10^6 CFU/g was not reached within 4 weeks in ice storage. At doses of 1 and 0.5 kGy this count was reached after 26 and 23 days, respectively. Non-irradiated fish which were spoiled in the third week of ice storage reached a count of 10^6 CFU/g after 15 days.

According to my results, yeasts and molds were not detected in irradiated samples at 3 kGy throughout the frozen storage time and in irradiated samples at 1 and 5 kGy until the end of the third month of frozen storage. It has been stated that yeasts and molds are sensitive to the irradiation process because of their large genomic structure (Fallah et al., 2010). Because of some metabolite production in lipid oxidation and bacterial growth reactions in 5 and 1 kGy, the population of yeasts and molds increased in the fourth and fifth months of frozen storage. Ahmed et al. (2009) reported that in evaluating the efficiency of gamma radiation (3, 5 and 8 kGy) in combination with low temperature (-20° C) storage of degutted fresh *Pampus chinensis*, the total mold count (TMC) increased with the increase of storage period. So that TMC values were 3.1×10^5, 5.3×10^3, 3.8×10^4 and 3.5×10^4 CFU/g in control, 3, 5 and 8 kGy treated samples respectively at the end of 90 days. Fallah et al. (2008) reported that the irradiation dose of 1.5 kGy reduced the initial counts of yeasts and molds by 2 Log units, while at 3 kGy yeasts and molds were below the detection levels during 6 days of storage. Badr (2004) reported that irradiation of rabbit meat at 1.5 and 3 kGy significantly reduced the counts of yeasts and molds by 84% and 94%, respectively. H_2S-producing bacteria such as *Salmonella* are generally predominant in spoiled fish flora (Moini et al., 2009). Because of good hygienic quality of production, fishing, handling, filleting, washing and packaging, coliform bacteria and *Salmonella* were not detected in irradiated and control samples. Radiation sensitivity of non-sporeforming pathogenic bacteria such as *Salmonella* in meat and fishery products is well documented (Badr, 2004; Fallah et al., 2008; Moini et al., 2009). Like other gram negative bacteria, *Salmonella* and coliforms have a very low resistance to radiation. Therefore elimination of these bacteria by radiation could be beneficial to the preservation of fish products in view of the major role that these species play in the spoilage of fish (Moini et al., 2009). In addition, rainbow trout fillets were exposed to quick-freezing and then stored at -20° C. About 90% of bacteria are present in fish die at the time of freezing. These bacteria such as *Salmonella* spp. are related to the mesophilic bacteria group. Psychrophilic bacteria that survive in cold conditions are inactive until fish are frozen due to absence of free water for their growth and activation (Johnstone et al., 1994). Moini et al. (2009) have reported that the H_2S-producing bacteria in the control rainbow trout samples reached a maximum count of 4.89 Log CFU/g on

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day 35 and were not observed at dose levels of 1, 3 and 5 kGy for 7, 21 and 42 days, respectively. Fallah at al. (2008) have reported that no coliforms were detected in irradiated (1.5 and 3 kGy) camel meat during refrigerated storage at 3±1° C. Sedeh et al. (2007) reported that the optimum dose of gamma radiation in order to decrease coliforms and specially for elimination of *Salmonella* of red meat was obtained at 3 kGy. With an increase in irradiation, the number of coliforms decreased. therefore irradiation significantly reduced them. Also irradiation and frozen storage was more effective than each treatment alone at decreasing coliform counts. Javanmard et al. (2006) reported that at the first day of frozen storage *Salmonella Typhimurium* was found in one non-irradiated chicken meat. However at irradiated samples (0.75, 3 and 5 kGy) no *Salmonella* was observed. Irradiation and frozen storage was reported more effective than each treatment alone at decreasing total and coliform counts. Badr (2004) reported that irradiation at 1.5 kGy was not enough for complete elimination of *Salmonella* of rabbit meat, while at 3 kGy *Salmonella* was not detected.

According to all obtained data from microbial analysis, low-dose gamma irradiation (especially 3 kGy) can be applied for microbial control and the safety of rainbow trout and shelf life extension in frozen state. Gamma irradiation at 3 kGy was more effective than irradiation at 1 and 5 kGy in eliminating microorganisms of rainbow trout fillets. In addition, the current study showed the synergistic effect of two preservation methods, food irradiation and freezing in low temperature on extending the shelf-life of rainbow trout fillet by reducing the microorganism's load.

Acknowledgments

This study was supported by Iranian Fisheries Research Organization. We wish to thank National Fish Processing Technology Research Center at Anzali port in Iran for performing the experiments and Atomic Energy Organization of Iran, Karaj Nuclear Research Center for Agriculture and Medicine for performing the irradiation.

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