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RESEARCH PAPER

Effect of gamma rays on microbial quality of salmon fish during storage in refrigerator at 4 °C

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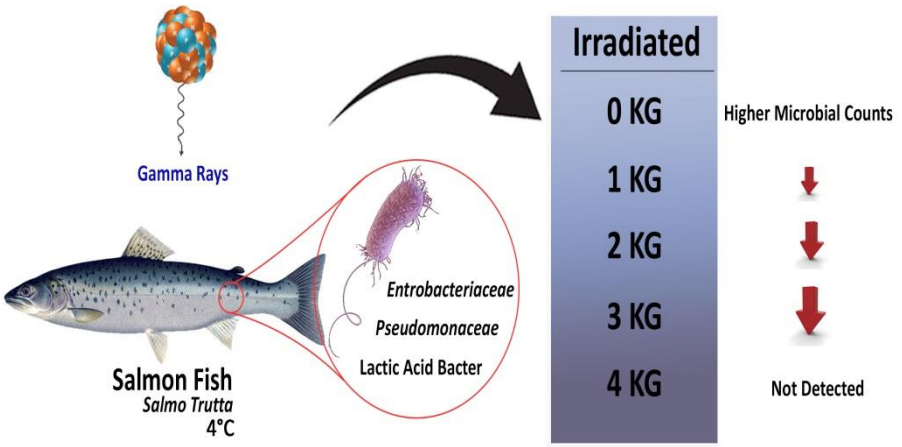
Highlights

- For the samples with 4 kg of irradiation no LAB and Entrobacteriaceae were detected
- With increasing the dose of irradiation, the microbial counts decreased (P> 0.05).
- The gamma rays had significant effects on the microbial quality of salmon fish during storage (4 °C).
- The effect is due to the charged particles generated by irradiation (to break DNA).

Graphical Abstract



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Abstract

Microbial quality, including total volatile counts (TVC), Entrobacteriaceae, Pseudomonaceae, and lactic acid bacteria (LAB) of salmon (*Salmo trutta*) irradiated at 0 (unirradiated), 2, 3, and 4 kg during 21 days of storage in a refrigerator (4 ± 1°C), was examined periodically (every 4 days). Unirradiated samples had higher bacterial counts than the others, and as the irradiation dose increased, the bacterial count decreased (P >0.05), as no LAB and Entrobacteriaceae were detected in the samples with 4 kg irradiation. The results showed that gamma irradiation inhibited bacterial growth in rainbow trout during storage at refrigeration temperatures. According to the results, the main effect of irradiation on microorganisms is due to the charged particles generated by irradiation, which are capable of breaking deoxyribonucleic acid (DNA). In the presence of water, cell damage during irradiation is due to both direct damage to cell DNA and indirect damage due to the reactivity of the radioactive products with cell components.

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1. Introduction

Fish and fish products are perishable foods. Spoilage of fish muscle results from biological reactions such as the oxidation of lipids, the action of endogenous enzymes, and the metabolic activities of microorganisms (Toughani et al., 2020). Microbial contamination reduces the shelf-life of foods and increases the risk of foodborne illness. Bacteria are one of the major organisms contributing to the rapid deterioration of fish quality and the risk of foodborne illness. When the fish dies, bacteria present on the surface and in the guts multiply rapidly and invade the flesh, which provides an ideal medium for growth and multiplication (Khan and Mustafa, 2012). Therefore, most perishable food products are stored at low temperature in order to extend their shelf-life. However, these steps do not eliminate undesirable microorganisms from these products. Alternative preservation techniques such as pulsed light, high pressure, pulsed electric and magnetic fields, natural antimicrobial ingredients and irradiation are being used or investigated for their application to food products (Quintavalla and Vicini, 2002; Holley and Patel, 2005).

The irradiation process is one of the few technologies which address both quality and safety due to its ability to control spoilage and foodborne pathogenic microorganisms by exposition to ionizing energy and also is the only alternative to heat processing for food preservation that has a lethal effect on micro-organisms. Nowadays, more than 60 countries have regulations allowing food irradiation of at least one product and total quantity of irradiated foods in the world was reported to be about 405,000 tons, while total amount of irradiated meat and seafood was about 32,000 tons in 2005 (Özogul et al., 2010; Badr, 2012). Salmon is a high quality product with considerable nutritional and economic importance. The main objective of this study was to investigate the effects of different doses of gamma irradiation on bacterial quality of fresh rainbow trout fillets during cold storage.

2. Materials and methods

2.1. Sample preparation

60 salmon fish weighing from 250 to 300 g and the same age and were purchased from the Karaj fish farm transferred to the cold chain. The selection of fish was randomly taken from fish of almost equal size. After washing, the fish were placed in fresh ice containers (3: 1 ratio of ice and fish) in jelly boxes and then transferred to the aquaculture processing laboratory of the University of Tehran. In order to vacuum, the samples were placed in vacuum-vacuumed plastics after vacuuming and packed in vacuo vacuum conditions and then transferred to the Atomic Energy Organization of Iran for irradiation (Fig. 1).

Fig. 1. Fish preparation and fillet sample.

2.2. Irradiation

All groups were exposed to 220 gasses. After irradiation, the specimens were transferred to the laboratory and stored in a refrigerator at 4 °C for 21 days. During the maintenance period, samples for each treatment at

random intervals of 1, 5, 9, 13, 17, and 21 days were selected randomly and microbial tests (including total bacterial load measurements, pseudomonads count , The number of bacteria producing lactic acid, as well as the number of bacteria associated with secondary contamination, namely enterobacteriacea) were performed on them.

2.3. Microbial analysis

According to the standard method for preparing the specimen for microbial tests (2006, Hoosber), using a sterile knife and pencil, each fillet was sampled at 25 g, and then 225 ml of sterilized cold physiological serum (approximate temperature of 4 °C) and completely homogeneous (for 2 min). The specimens were then kept at room temperature for 30 minutes until resuscitation was performed. After preparation of the samples and to prepare different dilutions, the samples were removed using a sampler of 1 ml and transferred to the test tube containing 9 ml of sterilized cold physiological serum and then fully homogenized, To a dilution of 1/0. In this experiment, in order to measure total bacterial load, Pseudomonas, the bacteria producing lactic acid, *Vantrobactyrosa* were used from plate count agar, pseudomonas agar peptone, deMan Rogosa Sharpe agar, iron agar, respectively. Analysis of bacteria producing lactic acidIn the case of lactic acid producing bacteria, after the preparation of the plates, they were used for incubation at 30 °C for 48 to 72 days, and then the number of colonies formed was counted. Enterobacteriaceae analysis For examination of Enterobacteriaceae bacteria, the plates were placed at 37 °C for 24 hours, then large colonies were counted with purple halo (Fig. 2).

2.4. Statistical analysis

All data were normalized by Kolmogorov-Smirnov test. After realization of two main conditions, the parametric tests of variance analysis (homogeneity of variance and normalization of data (1999 Zar), two way ANOVA) were used to compare the variance between treatments and to check whether there is any difference in meaning All treatments (0.5 and 0.1% confidence level) were used by SPSS software using statistical software. Charts were also drawn using Excel software. In this experiment, 60 fishes, 4 experimental treatments A completely randomized design template was tested with 3 replications.

Fig. 2. Sterile environment for preparing the culture medium.

3. Results and Discussion

The total volatile counts per gram of irradiated and unirradiated rainbow trout during the storage time are presented in Fig. 3a. The unirradiated samples had an initial count of 2.8 log CFU microorganisms per gram. In samples irradiated to 2, 3 and 4 kGy, the initial bacterial count was reduced to 1.11, 0.5, and 0.1 log CFU organisms per gram, respectively.

The initial TVC content of fish sample indicated that the quality of fish was good. Sallam, 2007 and Hamzeh and Rezaei, 2012, reported that the initial number of bacteria less than 4 log CFU per gram of sample refers to good quality of fish. The initial microbial load of freshwater fishes is related to water condition, temperature and handling condition (Sallam, 2007).

pseudomonaceae was not detected immediately after irradiation (Fig. 3b). Generally, pseudomonaceae increased during the storage time but it was greater in unirradiated samples ($P< 0.05$) and irradiaton with 3 and 4 kg reduced pseudomonaceae counts as there was not significant differences between 3 and 4 kg if irradiation. *Enterobacteriaceae*, a hygiene indicator, were also found to be members of the microbial populations associated with the spoilage of fresh rainbow trout during refrigerated storage. This finding is in agreement with the results reported for different fish species, including salmon (Sallam, 2007), Mediterranean octopus (Atrea et al., 2009), and rainbow trout (Mexis et al., 2009; Pezeshk et al., 2013), in which *Enterobacteriaceae*were part of the microflora found at the end of the shelf life of products under refrigerated storage. The initial amount of *Entrobacteriaceae* in rainbow trout was 0.89 and 0.1 log cfu/g for control and irradiated samples with 2 kg, respectively and were not detectable for irradiated to 3 and 4 kg (Fig. 3c). The *Enterobacteriaceae* of the controls increased significantly during the storage and decrease after 13 and 9 day for 2 and 3 kg of irradiation, and was not detectable for 4 kg of irradiation samples during the storage time.

The LAB contents of samples were not detectable immediately after irradiation with different doses (Fig. 3d). The initial content was 0.99 log CFU per gram of unirradiated samples (as control) and were appeared in 5 and 9 days for 2 and 3 kg of irradiation, respectively. Toward the end of storage time the LAB contents increased significantly for 0 (unirradiated), 2 and 3 kg and it was not detectable for samples with 4 kg of irradiation. The increase for unirradiated samples was greater than the others ($P<0.05$) (Grecz et al., 1983).

Riebroy et al., 2007 investigated the effects of irradiation at different doses (0, 2 and 6 kilogray (kgy) on the microbiological quality of Som-fug, a Thai fermented fish mince (Riebroy et al., 2007). They reported that Lactic acid bacteria (LAB) and TVC, yeast and mold counts in samples irradiated at 6 kgy were not detectable throughout the storage of 30 days at 4 °C, whereas no growth was found in the sample irradiated at 2 kgy within the first 10 days.

Abu-Tarboush et al., 1996 exposured the Tilapia (*Tilapia nilotica* × *T. aurea*) and Spanish mackerel (*Scomberomoruscommerson*) to gamma irradiation doses of 1.5, 3.0, 4.5, 6.0, and 10.0 kg. They reported that Doses of 3.0 and 4.5 kgy extended the sensory acceptability (appearance, odor, texture, taste) and the microbial quality (total count and coliforms) by 8 days compared to the unirradiated controls (Abu-Tarboush et al., 1996).

Hocaoğlu et al., 2012 investigated the effects of different doses of gamma irradiation 1, 3 and 5 KGy on shrimp at two different temperatures (+4 and -18). They reported that high dose of irradiation (5 kgy) might enhace lipid oxidation, although inhibited the growth of microorganisms (aerobic mesophilic bacteria, *Staphylococcus aureus*, *Escherichia coli* and coliform count) at both temperatures (Hocaoğlu et al., 2012).

Generally, bacterial counts of the samples increased during the storage, with a marked decrease at day 17 for TVC. This result suggested that bacteria in rainbow trout might be inhibited or killed by lactic acid produced with sufficient exposure time. Additionally, bacteriocin from LAB might function as a bacteriocidal agent, leading to a lowered count (Riebroy et al., 2007).

The results showed that gamma irradiation inhibit bacterial growth in rainbow trout during storage. Gamma irradiation has been considered as an interesting method of preservation to extend the shelf life of chilled, stored fish and also to reduce, qualitatively and quantitatively, the microbial population in fish and fishery products (Riebroy et al., 2007; Abu-Tarboush et al., 1996). Ionisation irradiation affects microorganisms, such as

bacteria, yeasts, and moulds, by causing lesions in the genetic material of the cell, effectively preventing it from carrying out the biological processes necessary for its continued existence (Rahman, 2007).

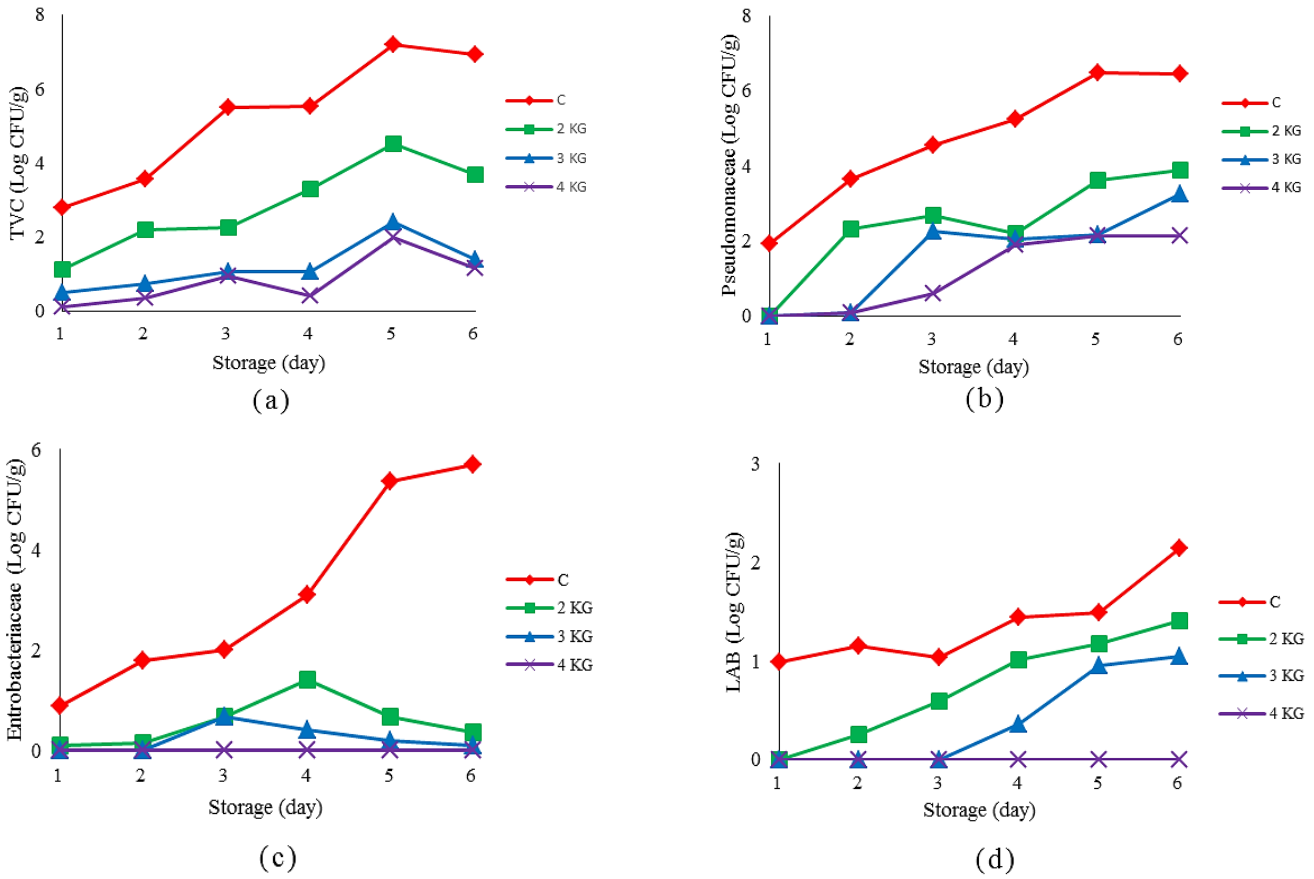

Fig. 3. Changes in total viable counts (TVC) (a), Pseudomonaceae (b), entrobacteriaceae (c), and lactic acid bacteria (d), of rainbow trout (*Oncorhynchus mykiss*) irradiated with 1, 3, and 5 kg.

4. Conclusion

According to the results, the major effect of irradiation on microorganisms is due to the charged particles generated by irradiation, which are able to break deoxyribonucleic acid (DNA). In the presence of water, cell injury during irradiation is due to both, direct damage to cell DNA and indirect damage through reactivity of the radiolytic products with cell components.

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