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تأثیر توأمان پرتودهی گاما و انجماد بر کیفیت فیله ماهی کپور نقره‌ای (*Hypophthalmyctis moltrix*)

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چکیده: اثرات دزهای مختلف پرتو گاما (۰/۷۵، ۳ و ۵ کیلوگری) و انجماد بر کیفیت میکروبی و شیمیایی فیله ماهی کپور نقره‌ای مورد بررسی قرار-گرفت. جمعیت باکتری‌های مزوفیل هوازی در دزهای ۳ و ۵ کیلوگری نسبت به گروه شاهد و تیمار ۰/۷۵ کیلوگری بطور موثری کاهش یافت، جمعیت این باکتری‌ها در فیله‌های پرتو دیده مذکور ۳ ماه پس از نگهداری در دمای ۱۸- درجه سانتی‌گراد تقلیل پیدا کردند (P<0/05). پرتودهی با دزهای ۳ و ۵ کیلوگری به شکل معنی‌داری جمعیت باکتری‌های کلی‌فرم را کاهش‌داد (P<0/05) و پس از نگهداری در انجماد جمعیت آنها به صفر رسید. البته پرتودهی با دوز ۵ کیلو گری مقادیر TVN را نسبت به سایر تیمارها افزایش‌داد (P<0/05) و به ۲۷/۳ mg/100g رساند، و ۳ ماه پس از نگهداری در انجماد به ۳۱/۳ mg/100g افزایش‌یافت. PV نیز به تدریج با افزایش دز پرتودهی و پس از نگهداری در انجماد افزایش یافت و در تیمار ۵ کیلوگری به شکل معنی داری از سایر تیمارها بالاتر بود (P<0/05). بنابراین پرتودهی با دز ۳ کیلو گری و انجماد در کاهش باکتری‌های مزوفیل هوازی و کلی‌فرم‌ها در فیله ماهی کپور نقره‌ای موثر می‌باشند و بدون ایجاد تغییر معنی‌داری در کیفیت شیمیایی گوشت ماهی باعث طولانی شدن عمر ماندگاری فرآورده منجمد می‌شوند.

کلید واژه: نگهداری در انجماد، پرتودهی گاما، کیفیت میکروبی و شیمیایی، کپور نقره‌ای

Effect of different dose of gamma irradiation and freezing storage on microbial and chemical quality of silver carp (*Hypophthalmyctis moltrix*) fillet

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Abstract: The effect of different dose of gamma irradiation (0.75, 3 and 5 kGy) and freezing on microbial and chemical quality of silver carp fillet was assessed. Population of mesophilic aerobic bacteria were decreased significantly in 3 and 5 kGy groups than control and 0.75 kGy groups, also after 3 months of storage in -18 °C population of aerobic bacteria were decreased (P<0.05). Population of coliform bacteria decreased significantly by irradiation at 3 and 5 kGy (P<0.05), and reached to zero after freezing storage. However, Total volatile nitrogen (TVN) increased by 5 kGy irradiation than other treatments (P<0.05) and reached to 27.3 mg/100g, and increased to 31.3 mg/100g after freezing storage. Also, peroxide value (PV) increased gradually by irradiation dose increase and freezing storage, and it was significantly higher in 5 kGy group than other groups (P<0.05). So irradiation at 3 kGy and freezing storage is effective in reduction of mesophilic aerobic and coliform bacteria in silver carp fillet and enhances the shelf life of frozen product without significant changes in chemical quality of fish meat.

Keywords: freezing storage, gamma irradiation, microbial and chemical quality, Silver carp

Introduction

Fresh fish is an extremely perishable food as compared to other food products. Hygienic quality of fish and aquaculture products come down quickly because of the different sources of microbial contamination and ultimately will lead to corruption [1]. Silver carp (*Hypophthalmyctis moltrix*) belongs to the Cyprinidae family, and is one of the main fish species farmed in Iran. Along with high production of this species, there is an obvious need for development of new technologies and efficient fish preservation methods which permit shelf-life extension of these products. Besides traditional methods used to prolong the shelf-life of fish and fisheries products including rapid freezing and storage in ice [2], various methods including the use of organic acids, antimicrobial materials, modified atmosphere packaging and ionizing radiation have been suggested [3].

Food irradiation is considered as a kind of cold process (no heat) for food storage that is widely investigated during the past half century [4]. Some benefits of this method are extending the shelf life of Rooty products, disinfecting spices, fruits and cereals, reducing microorganisms that cause to corruption, delay in fruit ripening, improving sensory characteristics and reducing pathogenic factors of food materials specially animal originated raw foods[4]. Moreover, in this method, foods are affected by radiation after packaging as a final process, so it eliminates the potential contamination before consuming until foods are packed[5]. Rays used in food irradiation include ultraviolet (UV), beta ray, gamma ray, X-ray and microwaves. Gamma ray is considered as one of the cheapest forms of Food irradiation because its radiation source comes from nuclear fission byproducts. Furthermore, gamma ray, unlike beta ray and UV has high penetration ability. This radiation type belongs to electromagnetic waves, which is irradiated from evoked core of elements such as cobalt 60 and cesium 137 that are important in food preservation [6]. Food irradiation with a higher dose than 10 kGy is an effective, economical and safe method for food storage both in animal foods and in plant foods that has not nutritional, toxicology or microbial problems [7]. Irradiation with low dose in the range 1 to 3 kGy as Radurization was used to extend shelf-life of fisheries products [8].

Moreover, temperature is a main factor in food preservation, and it was shown combination of antimicrobial effects of irradiation and freezing storage on pathogens in poultry and fish carcasses. So the food irradiation in combination with proper cooling increases the shelf-life of fisheries products [9].

In this study the effect of gamma irradiation and post irradiation in freezing storage (-18 °C) as a combination of two shelf life extension methods was examined. Another aim of this study was to determine the lower dose of radiation without negative effects from irradiation to achieve a standard dose of irradiation on fish trade in Iran. In this study, microbial and chemical characteristics of fish meat and the possibility of using irradiation

to control pathogenic bacteria in the food and shelf life extension of fish meat in freezing temperatures was assessed.

Materials and Methods:

Fish samples

To examine the effect of gamma irradiation and post storage in freezing on the chemical and microbial properties of fish meat 30 aqua-cultured fresh silver carp (*Hypophthalmyctis moltrix*) from a fish farm were prepared. The average length and weight of the fish was 53±3.1 cm and 1835±42 g, respectively. After receiving the fish, each was divided into 12 equal parts and then was packed into the special bags in the vicinity of ice. Samples were divided into four groups, one group wasn't exposed to radiation (control) and three other groups were irradiated by 0.75, 3 kGy of gamma rays.

Irradiation

Samples were irradiated at the Nuclear Research Center of Agriculture, Medicine and Industry-NRCAM (Karaj - Iran) of Nuclear Energy Organization of Iran, using a 60 Cobalt radiation source (Gamma cell PX-30-ISSIE, Dose rate: 0.23 Gy/Sec, Russia). The applied doses in this study were 0.75, 3 and 5.0 kGy. During irradiation, the samples were kept out of the ice and at first, one side of samples were irradiated and then all directions of those were irradiated through 180 degrees rotation. The absorbed dose was monitored by copper sulfate-iron dosimeter. Fish samples were maintained at 4±1 ºC during irradiation by using sealed ice covering for the samples. Non-irradiated (control) fish samples were kept in polystyrene boxes with sealed ice at the ambient temperature for 24 h.

Storage conditions

After irradiation, the non-irradiated and irradiated fishes were transported to the laboratory of food Health, Faculty of Veterinary Medicine, Tehran University in packed ice via insulated polystyrene boxes, within 1.5 h. Then these samples were kept at -18 °C in a freezer. The storage of the fish lasted 3 months, and then samples were taken for chemical and microbiological analyses.

Microbiological analyses

Counting the Mesophilic aerobic and coliform bacteria was done on the day of receiving the samples and in the third month of their storage and experimental methods for each group of bacteria was based on APHA [10]. 25g of fish were cut and separated by a sterile knife in sterilized condition for counting the mesophilic aerobic bacteria. Samples (25 g) were mixed with 225ml of peptone water (Merck) diluents in a Stomacher for 2 minutes. 0.1 ml of prepared dilution was plated in two plates containing Nutrient Agar (Merck), 35 º C, 1-2 days for mesophilic aerobic bacteria and 1 ml of prepared dilution was plated on Violet Red Bile Agar (VRBA) (Merck), 37 º C, 2 days for coliform bacteria. Results are expressed as a logarithm of colony forming units (log CFU) per gram of sample.

Chemical Analysis

Determination of Total Volatile Nitrogen (TVN):

To calculate total volatile nitrogen (TVN), 10 g of fish meat together with 2 grams of magnesium oxide and 300 ml distilled water were distilled inside the Kjldal balloon. After distillation, 25 ml of boric acid reagent (2%) was added to distillate. Then, the distillate was titrated with H_2SO_4 0.01 N[11].

Determination of Peroxide Value (PV):

One gram of homogenized fish meat were weighed, blended with 1g of KI powder and 20 ml solvent solution (acetic acid and chloroform) in clean and dry test tubes. Then, it was put in boiling water for 30 seconds. The content of the tube immediately was transferred to an Erlenmeyer flask containing 20 ml of potassium iodide (5%). Then, tube was washed to Erlenmeyer flask with a 25 ml of distilled water for twice and then it was titrated with sodium hyposulfite 0.002 N and the starch glue were used as a reagent. And Peroxide value was expressed as mEq peroxide/kg meat [12].

2-5- Statistical analysis:

All analyses were run in triplicate (three different packaged samples). Statistical analysis of the data was done by standard methods. Data were subjected to analysis of variance (ANOVA) to find out the level of significance between different treatments. Tukey procedure was used to test the differences between averages at the 5% significance level [13].

Results and Discussion:

Effect of irradiation and freezing on total count of mesophilic aerobic bacteria and coliform bacteria of fish meat was investigated. The total mesophilic aerobic count for silver carp fillets is given in Figures 1 and 2. The results indicated that the population of mesophilic aerobic bacteria decreased in irradiated groups at 3 and 5 kGy than the 0.75 kGy and control group. The 3 and 5 kGy irradiated silver carp samples had a lower mesophilic aerobic bacteria counts, reached 6 and 5 log CFU/g respectively, while non-irradiated group was 6.5 log CFU/g that was close to the value of 7 log CFU/g, which is considered the upper acceptability limit for fresh water and marine species by ICMSF[14]. Jeevanandam *et al.* [15] reported that initial total viable count of non-irradiated and irradiated (1 and 2 kGy) sea bream was 4.64, 3.36 and 2.75 log cfu/g reaching the counts of 8 log cfu/g at day 20 in non-irradiated samples, and at day 30 for irradiated group samples. Also Savvaidis *et al.* [17] reported counts of 7 log cfu/g for vacuum packed trout after 9, 14 and 24 days for non-irradiated and irradiated samples at 0, 0.5 and 2 kGy, respectively.

In addition to irradiation, the number of mesophilic aerobic bacteria was reduced effectively after freezing storage at -18 °C ($P \leq 0.05$), but this reduction was significant only in 5 kGy treatment (Fig. 1). It was reported that bacteria at the exponential state that

undergo abrupt temperature fall from 37 to 0˚C lose viability and at −20˚C most cells will have lethal or sub-lethal injury. However, the temperature effect on gram negative bacteria such as coliform bacteria, *Escherichia coli* is more pronounced [16]. Our results are also in agreement with this report on the preservation using freezing storage and freezing was effective in reduction of the mesophilic aerobic bacteria.

At initial time after exposure to radiation, coliform bacteria had the highest count in the non-irradiated and 0.75 kGy irradiated groups and reached to 6.8 and 6.5 log CFU/g respectively (Fig. 2). It is clear that irradiation at both 3 and 5 kGy significantly reduced ($P<0.05$) population of these bacteria in the silver carp fillet and decreased it to 5 and 3.5 log CFU/g respectively (Fig. 2). After third month of freezing storage at -18°C population of coliform bacteria in fish fillet reached zero in 3 and 5 kGy groups, but in the control and 0.75 kGy groups decreased to 6.2 and 6 log CFU/g respectively (Fig. 2). According to the microbiological results, both irradiation at the doses of 3 and 5 kGy and freezing storage were more effective than using one of them alone in reducing the total number of mesophilic aerobic bacteria and coliform bacteria in the silver carp fillet.

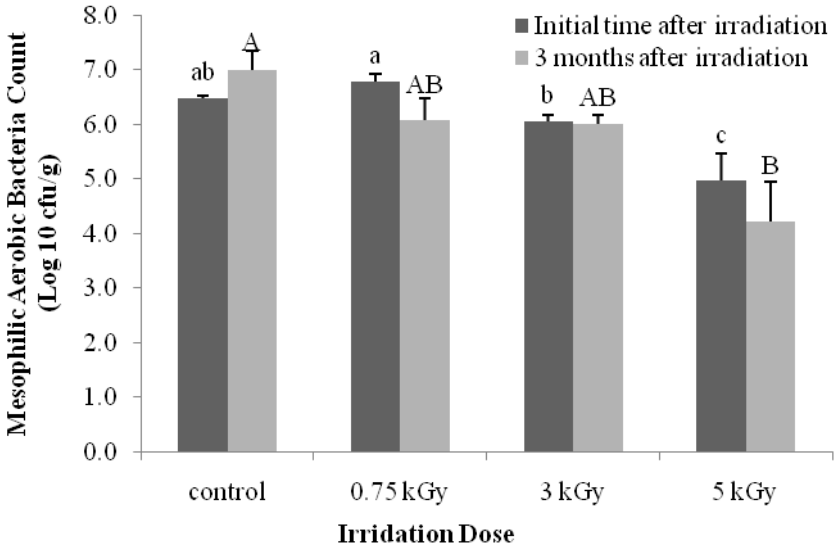

Fig. 1. The effects of irradiation on the population of mesophilic-aerobic bacteria at initial time after irradiation and 3 months after freezing storage (-18°C) in Silver carp fillet. Different letters shows significantly differences between treatments ($P<0.05$).

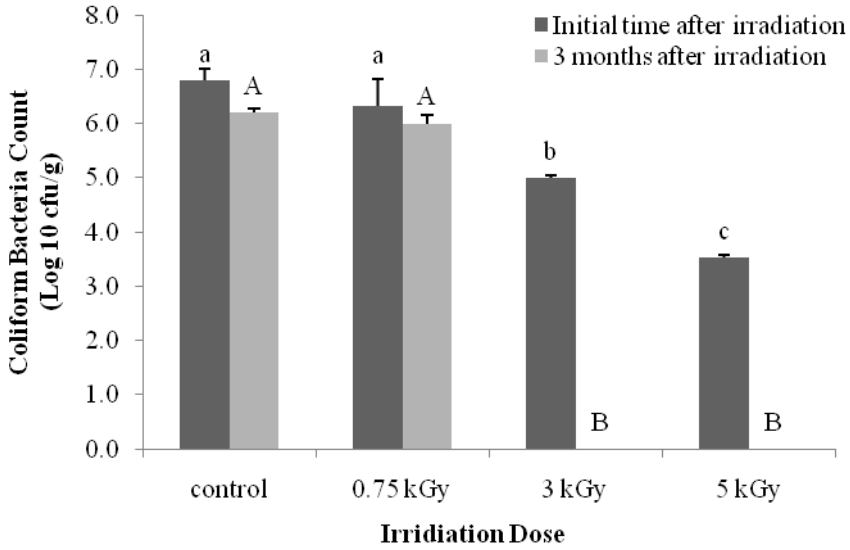

Fig. 2. Effects of irradiation on the population of coliform bacteria at initial time after irradiation and 3 months after freezing storage (-18°C) in Silver carp fillet. Different letters shows significantly differences between treatments (P<0.05).

TVN values were gained after irradiation and freezing storage in silver carp fillet are shown in Fig. 3. As Fig. 3 shows, the initial values of TVN, in the control and 0.75 kGy groups, were less than 20 mg/100g, and 3 months after freezing storage, with slightly increase, it reached to 20.6 and 21.7 mg/100g respectively. The incensement in TVN value in the irradiated fillets at the dose of 3 kGy was slightly higher after irradiation and freezing storage and reached to 23.6 and 26.6 mg/100g, while in 5 kGy irradiated group increased to 27.3 and 31.3 mg/100g and reached to more than 100 mg/100g that indicates severe corruption in the frozen fish[16].

Peroxide value (PV) is a widely used index for lipid oxidation assay. Fig. 4 shows the PV increased gradually from the lowest value in the control group (0.57 mEq/kg) to the values of 0.68 and 0.71 mEq/kg in the 0.75 and 3 kGy groups respectively and reached to highest value in the 5 kGy group (0.76 mEq/kg)(P <0.05). Three months after storing fish fillets in -18°C, PV increased in all treatments, but it was significantly higher in 5 kGy group than other groups (P <0.05) and its value reached to 0.66, 0.74, 0.75 and 0.84 mEq/kg in control, 0.75, 3 and 5 kGy group respectively (Fig. 4).

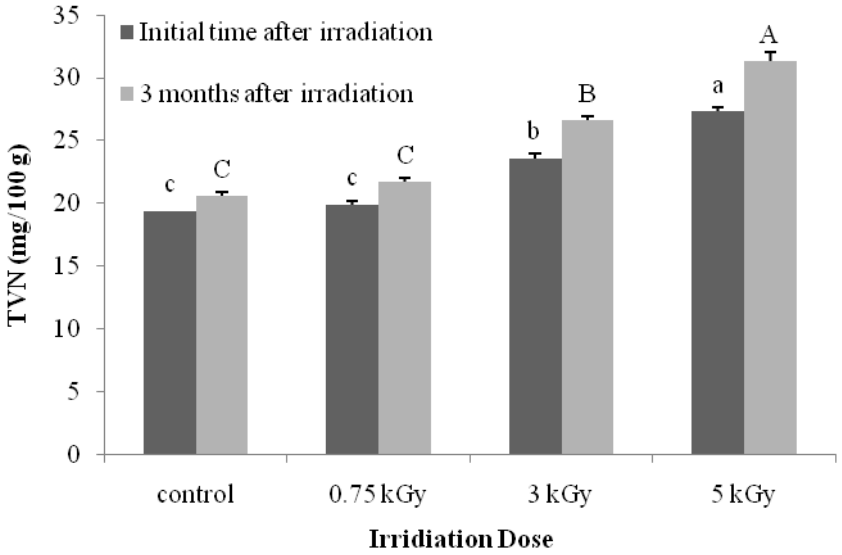

Fig. 3. Effects of irradiation on TVN value at initial time after irradiation and 3 months after freezing storage (-18°C) in Silver carp fillet. Different letters shows significantly differences between treatments (P<0.05).

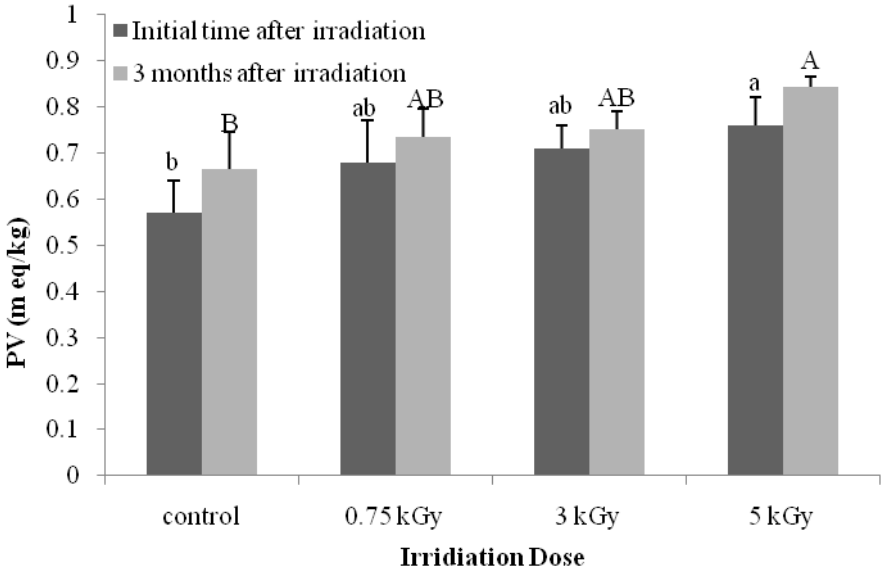

Fig. 4. Effects of irradiation on peroxide value (PV) at initial time after irradiation and 3 months after freezing (-18°C) storage in Silver carp fillet. Different letters shows significantly differences between treatments (P<0.05).

The shelf life extension of fish is detected by microbial, chemical and sensory parameters. TVN and PV values, as indexes of corruption in the present study, gradually increased with gamma-ray irradiation (P <0.05), but only in 5 kGy group TVN significantly

increased and reached to the border of corruption and also PV was significantly higher in 5 kGy group than other irradiated and control groups ($P < 0.05$).

Freezing also increased TVN and Peroxide values and it reports that six-month freezing storage of deboned silver carp increases TVN and PV during the period of storage [16].

Conclusion

Shelf life extension by using low doses of gamma irradiation results necessarily changes in micro flora and reduction in the average of gram-negative bacteria which are causing corruption [17, 18]. This is also true about irradiated fish in freezing condition. Irradiation doses at 1 to 3 kGy are suggested to expand shelf life of freshwater fish [16, 19, 20]. Our results shows that irradiation at the dose of 3 kGy and freezing storage are very effective together as compared to using one of them alone in reducing the total number of mesophilic aerobic bacteria and coliform bacteria to extend shelf life on the silver carp fillet. So Gama irradiation reduces bacterial count in fish meat and therefore prolongs shelf life of frozen products without causing significant changes in chemical quality of fish meat. Gama irradiation in the production of fish and fishery products in Iran is not currently used. It is hoped that additional data on the promising results obtained by the combined use of low dose irradiation and freezing for fish preservation will lead to the use of irradiation as a food preservation technology in the future.

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