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Original Article

Isolation of Food-Borne Microorganisms from Atlantic Mackerel and Disinfection of the Raw Fish by Radiation, Low Temperature and Combination Treatments

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An investigation was undertaken for the isolation and identification of fish-borne microorganisms from mackerel fish (*Scomberomorus guttatus*) collected from Savar Bazar. Radiation, low temperature (-20°C) and combination treatments were then applied for the decontamination of associated organisms. The ranges of total viable bacterial count, total coliform count, total faecal coliform count and total staphylococcal count varied from 6.5 x 10^4 to 1.04 x 10^5, 2 x 10^2 to 4.0 x 10^2, 0 to 1 x 10^2 and 4.4 x 10^4 to 3.8 x 10^4 cfu/g respectively, while the total fungal count was nil. Sixty-four bacterial isolates were identified including S*taphylococcu*s (19%), *Micrococcus* (11%), *Enterobacter* (8%), *Klebsiella* (17%), *Bacillus* (19%), *Escherichia* (17%) and *Pseudomonas* (9%). To disinfect the microorganisms, the samples were irradiated at different doses (0-10.0 kGy) of gamma irradiation. Total coliform and total faecal coliform bacteria were inactivated below the detectable level at 2.5 kGy of irradiation. The number of total viable bacteria decreased by three logs when irradiated at 5.0 kGy and the number of *Staphylococcus* bacteria were eliminated completely after the irradiation dose of 5.0 kGy. During frozen storage, the total viable bacterial counts were gradually declined in all the samples. After six months of storage the bacterial counts were decreased about one log in all of the samples. It has been observed that combination treatments (irradiation and freezing) are more effective than the single treatment for eliminating the fish-borne bacteria.

Keywords: Mackerel fish, Microbial contamination, Irradiation, Low temperature

Introduction

Fish is a good source of protein and minerals such as calcium, phosphorous and iron, trace elements like iodine (in marine fishes), as well as vitamins A and D[1]. The high content of polyunsaturated fatty acids requirement probably helps lower cholesterol levels. Thus, from nutritional point of view, fish is important in the diet of the developing world[2]. Though fish is highly nutritious and tasty, it is very perishable and cannot be kept for long times for consumption. Thus, in question of preservation, spoilage of fish has drawn the attention of people and had put effort to know the reasons of spoilage. The deterioration is believed to cause mainly by the bacterial activity, which brings about very noticeable changes in the texture, flavour, odour and general appearance of the product. For this reason, we are concerned primarily with the deterioration of fish by microorganisms and microbial enzymes.

Mackerel fish (*Scomberomorus guttatus*, Bloch and Schneider) is a popular sea fish among the mackerel variety of fishes. It is found in around the Bay of Bengal and adjoining seas. In the Indian sub-continent it is called Surmai[3]. It is very cheap, tasty and easily available in the market, very popular and highly nutritious, used to make fish pickle and usually eaten as a condiment with rice, sought after food either cooked or as Sashimi, extremely high in vitamin B_{12}, very high in omega 3, very low in mercury and can be eaten twice a week according to EPA guidelines and in Scandinavia, canned mackerel in tomato sauce is commonly used as sandwich filling[3].

Microorganisms differ in their responses to freezing, some survive virtually unharmed, some resist freezing but are susceptible to damage during frozen storage. Gram-negative organisms such as *Escherichia, Pseudomonas, Alcaligenes, Vibrio* and *Salmonella* are more sensitive to freezing than Gram-positive organisms[4]. The bacteria from skin and gills of fish and shellfish are predominantly aerobic. The population is inevitably facultative in nature. Matches *et al*.[5] demonstrated that facultative anaerobic bacteria are predominating.

The spoilage of one fish has been demonstrated to cause mainly by bacterial activity. Spoilage bacteria grow almost entirely on the surface of fish. Some bacterial changes occur during spoilage

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of marine fish[6]. These are dependent not only on the strains of bacteria present, but also on the types of fish. Doyle[7] reported that spoilage is the result of whole series of complicated deteriorative changes brought about by chemical action. The course of spoilage in any instance is subjected to the influence of environmental factors, particularly temperature[8]. The quality of fresh food like fish continuously changes during storage.

The use of low doses of radiation to destroy a sufficient number of microorganisms and enhance the storage life of goods is called radiation pasteurization[9]. Investigation on the fresh mackerel had shown the effectiveness of radiation for extending shelf life. John *et al*.[10] observed that low dose of irradiation reduced bacterial growth. Radiation treatment can be a suitable method for mackerel fish preservation for further removal of associated microorganisms. Still there is no enough report regarding preservation of mackerel fish by radiation and low temperature. Therefore, the aim of this study was quantitative and qualitative microbiological analysis of economically important marine fish sample *Scomberomorus guttatus* (Atlantic mackerel) and the effect of frozen storage, gamma irradiation and combination treatment (frozen storage and gamma irradiation) on the associated microorganisms.

Materials and Methods

Sample

Atlantic mackerel (*Scomberomorus guttatus*) was used for the studying of the microbial spoilage of the fish. Combinations of different parts of the fish such as muscle, skin, fin etc. were used as materials for the study. The fish samples were collected from Savar market. The samples were thawed by keeping at room temperature and then they were cut into different pieces and kept into the pre-sterilized polythene bags. The investigation was carried in the laboratory of Microbiology and Industrial Irradiation Division (MIID), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka.

Microbiological analysis

Total viable bacterial count (TVBC) was done by the standard plate count method following the method described by Sharp and Lyles[11]. Nutrient agar (pH 7.0-7.4) was used to determine TVBC as well as for isolation purposes. Plates were incubated at 37ºC for 24 h and the count was expressed as colony forming unit per gram (cfu/g). Total viable coliform count (TCC) was done in the same way using MacConkey agar medium at 37ºC. mFC agar medium and staphylococcal agar media were used for total faecal coliform count and total staphylococcal count respectively. With a view to identify some selected isolates various morphological characteristics, biochemical and carbohydrate fermentation tests were performed. All the bacterial isolates were identified according to the Bergey's Manual of Determinative Bacteriology[12] and Manual for the Identification of Medical Bacteria[13]. Potato dextrose agar was used for total fungal count. The plates were incubated at 28º C and counts were recorded after 5 days of incubation. The fungal isolates were identified following the procedures described by Gilman[14], Raper and Fennel[15] and Koneman *et al*.[16].

Gamma irradiation

The fish samples were subjected to different radiation doses such as 0 (control), 2.5, 5 7.5 and 10 kGy of ionizing radiation at a dose rate of 1.25 Mrad/h from a 50,000 curie Co^{60} source (Gamma beam, 650, AECL, Canada) situated at the Institute of Food and Radiation Biology of AERE.

Storage condition

All of the untreated control and irradiated samples were stored in low temperature (-20°C) in a deep freezer. The samples were then examined for microbiological qualities. Three replicas were studied for each of the sample. The bacteriological analyses were carried out before keeping the samples in the deep freeze and during storage once in a month up to six months.

Results and Discussion

The highest total viable bacterial count (TVBC) of the raw mackerel varied from 6.5×10^4 to 1.04×10^5 cfu/g (Table 1) and the average count of the five samples was 8.9×10^4 cfu/g. The total coliform count (TCC) of the samples varied from 2.0×10^2 to 4.0×10^2 cfu/g with an average 3.0×10^2 cfu/g. Total faecal coliform count (TFCC) of the samples varied from nil to 2×10^2 cfu/g and the average count of the five samples was 6.0×10^1 cfu/g. Total staphylococcal count (TSC) was also observed of the fish samples and the count varied from 3.8×10^4 to 4.4×10^4 cfu/g with an average count of 4.2×10^4 cfu/g. There was no fungal count in any of the samples (Table 1). The similar bacteriological status of raw fish was observed previously by Rashid *et al*.[17], Rahman *et al*.[18] and Khatun *et al*.[19].

Table 1. *Quantitative assessment microorganisms in raw Atlantic mackerel fish*

Sample	Count of viable microorganism (cfu/g)				
	TVBC	TCC	TFCC	TSC	TFC
1	1.04×10^5	3.0×10^2	Nil	4.4×10^4	0
2	8.9×10^4	4.0×10^2	2.0×10^2	3.8×10^4	0
3	6.5×10^4	3.0×10^2	1.0×10^2	4.0×10^4	0
4	8.9×10^4	2.0×10^2	0	3.9×10^4	0
5	9.8×10^4	3.0×10^2	0	3.9×10^4	0

TVBC = Total viable bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSC = Total staphylococcal count (TSC); TFC = Total fungal count.

For observation of irradiation effect on microorganisms, five replicas of the samples were irradiated at 0, 2.5, 5.0, 7.5 and 10.0 kGy of irradiation doses and the residual microbial counts were analyzed. It was observed that in the non-irradiated samples the average of total viable bacterial count (TVBC) was 8.9×10^4 cfu/g and, after the irradiation, the count was decreased to 3.5×10^3 cfu/

g and nil at radiation doses of 2.5 and 5.0 kGy respectively (Figure 1). Similar finding was also found in a previous study[20] who reported that TVBC was reduced by two logs in all the samples at 2.5 kGy of irradiation doses. Ito *et al.*[21] also reported the same result. Total coliform count (TCC) was 3.0 x 10^2 cfu/g in the non-irradiated samples and after the irradiation at a dose of 2.5 kGy the count was reduced to nil in all the samples. In case of total faecal coliform (TFC), the count in non-irradiated samples was 6.0 x 10^1 cfu/g and after the irradiation at a dose of 2.5 kGy the count was decreased to nil. Irradiation effect was also observed in the total staphylococcal count (TSC). In the non-irradiated samples total staphylococcal count (TSC) was 4.0 x 10^4 cfu/g and the count was reduced to 3.0 x10^2 cfu/g at a irradiation dose of 2.5 kGy. After the irradiation at a dose of 5.0 kGy, no count was observed. Similar results have been reported by Rashid[20] and Ito *et al*.[21].

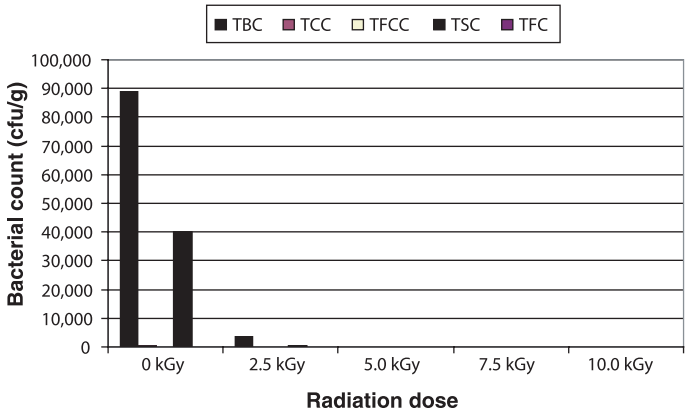

Figure 1. *Effect of radiation on the survivability microorganisms associated with Atlantic mackerel fish. TVBC = Total viable bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSC = Total staphylococcal count (TSC); TFC = Total fungal count.*

The effect low temperature on the survivability of bacteria in fish was studied and the results are shown in Figure 2. After six month of storage the average of the total viable bacterial count (TVBC) reduced from an initial count of 8.9 x 10^4 to 5.7 x 10^3 cfu/g. It was a common trend that the microbial count decreases gradually during storage at sub-freezing temperature. Like TVBC, total coliform count (TCC) and total faecal coliform count (TFCC) were also found to decrease gradually in all the samples during storage. No TFCC was found after storage for four months. The total staphylococcal count (TSC) was also decreased to about one log after six month of storage in all the samples. These results are in agreement with the results of several investigators[22-25].

The declination in the rate of bacteria with time indicated their gradual adaptation to storage temperature. Slow freezing is more detrimental than quick freezing, because of the formation of large ice crystal that disrupts cell membranes as well as brings out solute of the cell[26]. Thus, freezing causes the death of the bacterial cell. Microorganisms differ in their responses to freezing[27]. Some

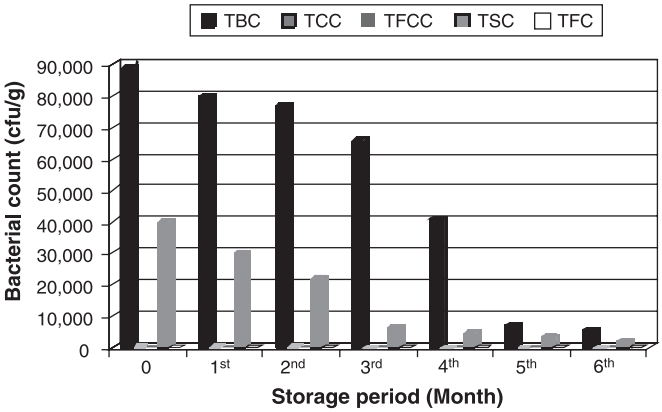

Figure 2. *Effect of storage at low temperature (-20°C) on the survivability microorganisms associated with Atlantic mackerel fish. TVBC = Total viable bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSC = Total staphylococcal count (TSC); TFC = Total fungal count.*

survive virtually unharmed, some resist freezing but are susceptible to damage during frozen storage or thawing, others are sensitive to freezing, storage and thawing and others are inactivated by freezing under nearly all conditions. Most spores and vegetative cells survive virtually unchanged. Most other non-spore forming organisms are sensitive to one or more steps of the freezing process[27].

For observation of combination effect (irradiation and storage) on microorganisms the fish samples were irradiated at 0, 2.5, 5.0, 7.5 and 10.0 kGy of irradiation doses and kept at -20°C for six months. The microbial counts were observed monthly. It has been found that total viable bacteria were gradually decreased in all the samples and no count was observed after six months of storage in case of combination treatment. Total staphylococcal count was also gradually decreased and the count was nil after six months of storage. Total coliform and total faecal coliform bacteria were nil at the initial period of storage at the radiation level of 2.5 kGy, *i.e.*, no count was observed during storage period of six months in the irradiated samples (Table 2). The bacteria survived after irradiation also gradually decreased during frozen storage and after six months of storage it was decreased about three to four log more in all the samples. Similar results have been reported by other investigators[20,23,28].

On the basis of agar colony morphology different bacterial isolates were selected from different media for identification. A total of 64 isolates recovered from non-irradiated and irradiated fish samples were identified including *Staphylococcus aureus, Micrococcus varians, Enterobacter cloacae, Klebsiella ozaenae, Bacillus subtilis, Escherichia coli, Bacillus megaterium, Klebsiella edwardsii, Pseudomonas aerugenosa* and *Micrococcus radiodurans*. The bacteria isolated after six months of storage at -20°C were *S. aureus, M. varians, B. subtilis, E. coli, B. megaterium, K. edwardsii, P. aerugenosa* and *M. radiodurans*. So, it was found that *E. cloacae, K. ozaenae, B. subtilis* and

Table 2. *Effect of combination treatments on the survivability microorganisms associated with Atlantic mackerel fish*

Storage	Count of viable microorganism (cfu/g)				
	TVBC	TCC	TFCC	TSC	TFC
Non-irradiated (Control)					
Before storage	8.9×10^4	3.0×10^2	6.0×10^1	4.0×10^4	0
After 1 month	8.0×10^4	2.5×10^2	5.0×10^1	3.0×10^4	0
After 2 months	7.7×10^4	2.0×10^2	3.0×10^1	2.2×10^4	0
After 3 months	6.6×10^4	1.0×10^2	3.0×10^1	6.5×10^3	0
After 4 months	4.1×10^4	1.0×10^1	1.5×10^1	4.5×10^3	0
After 5 months	7.2×10^3	6.6×10^1	0	3.3×10^3	0
After 6 months	5.7×10^3	3.0×10^1	0	2.0×10^3	0
Irradiated at 2.5 kGy					
Before storage	3.5×10^3	0	0	3.0×10^2	0
After 1 month	3.0×10^3	0	0	2.5×10^2	0
After 2 months	7.5×10^2	0	0	2.0×10^2	0
After 3 months	4.5×10^2	0	0	1.0×10^2	0
After 4 months	3.3×10^1	0	0	0	0
After 5 months	2.5×10^1	0	0	0	0
After 6 months	0	0	0	0	0
Irradiated at or above 5.0 kGy					
Before storage	0	0	0	0	0
After 1 month	0	0	0	0	0
After 2 months	0	0	0	0	0
After 3 months	0	0	0	0	0
After 4 months	0	0	0	0	0
After 5 months	0	0	0	0	0
After 6 months	0	0	0	0	0

TVBC = Total viable bacterial count; TCC = Total coliform count; TFCC = Total faecal coliform count; TSC = Total staphylococcal count (TSC); TFC = Total fungal count.

M. radiodurans were eliminated after six months of storage. Among the 64 isolates 12 (19%) were *Staphylococcus*, 7 (11%) *Micrococcus*, 5 (8%) *Enterobacter*, 11 (17%) *Klebsiella*, 12 (19%) *Bacillus*, 11 (17%) *Escherichia* and 6 (9%) *Pseudomonas*. Bacteria associated with stored fish muscle and their great variation in the percentage has been reported by Anwar *et al*.[22].

It can be concluded that irradiation at frozen condition is useful to improve the keeping quality and lower the risk of food-borne illness caused by microorganisms.

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