Radurization Effect of Korean Mackerel

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(Received February 15, 1985)

To investigate potential application of radurization technique as an effective tool for controlling histamine accumulation, histamine contents were determined in fresh Korean mackerel meat samples irradiated at six dose levels of gamma radiation up to 1 *Mrad* during 35 day-storage at 0°, 5° and 10°C. Total bacterial counts, total volatile bases and trimethylamine contents were also estimated.

The minimum and maximum doses for Korean mackerel were determined to be 0.2 and 0.5 Mrad, respectively. Irradiation induced redening of fish muscle tissue and redening appeared to be dose dependent. At low doses, this redening contributed positively to the overall sensory qualities. At 0.5 and 1.0 *Mrad*, on the other hand, meat samples were excessively redened to the extent that red and white muscle layers were not readily distinguishable.

No appreciable histamine accumulation occured during entire 35 day-storage in fresh mackerel meats, whether irradiated or not, when they were kept at ice temperature. At 5°C, histamine started to accumulate slightly during late storage in the samples irradiated at low doses. Even in the unirradiated samples, the histamine accumulation did not exceed the level of 10.0 *mg* per 100*g* meat until the 28th day at 5°C. At 10°C, however, histamine accumulation in the unirradiated was phenomenal from very early storage and the values reached 827.5 *mg* per 100 *g* meat by the 35th day, while histamine accumulation in the irradiated was severely suppressed.

Irradiation invariably brought about a significant reduction of initial microflora, disrupting normal spoilage pattern. This was reflected in the suppression of not only histamine but also total volatile bases and trimethylamine accumulation during post-irradiation storage at refrigerated temperatures.

Introduction

There is no well documented case of histamine poisoning outbreak reported in Korea as yet from eating fresh fish and fishery products. However, the histamine poisoning related problems (Ferencik, *et al.*, 1961) are well known in many countries where raw fish consumption is very high. Although mackerel meats are not usually eaten raw in Korea, the danger of such problems can not be ruled out because people traditionally buy mackerel as fresh whole fish at market stores. It was only very recent that mackerel, often of second quality, were put into canned products for both domestic and oversea markets.

Although the literature abounds with information on the application of radurization techniques to almost all species of commercial fish (Nickerson and Goldblith, 1950) and shellfish (Miyauchi, *et al.*, 1964) including mackerel, no information is available on potential application of such techniques for controlling histamine poisoning related problems in fresh fish meats of species that are often incriminated for outbreak of histamine or histamine-like poisoning problems.

In this study, fresh Korean mackerel meats were exposed to six doses of gamma radiation below 1.0 Mrad and accumulation of histamine, total volatile bases (TVB) and trimethylamine (TMA) contents and total microbial counts were followed up for 35 day-storage at 0°, 5°, and 10°C.

Material and Methods

Storage studies on irradiated fresh mackerel meat samples

The mackerel (*Scomber japonicus*) of fresh quality was purchased at Fisheries Cooperative in Pusan, the major fish landing port in Korea. The fish were gutted and ten fishes each packed in polyethylene pouches were immediately frozen to −20°C. The frozen gutted fish were then transported to Seoul in a dry ice box and being stored in −40°C freezer until used. Only necessary numbers of the frozen fish pouches at a time were taken out of the freezer and the fish thawed overnight at refrigerated temperatures. The thawed fish were then immediately filleted and the fillets cut into small cubes (approximately 0.5×0.5×1.0 *inch* in dimensions). Approximately 80*g* of fish meats were packed in nylon pouches and heat sealed after pressing out as much of air as possible with fingers. The sample packages were then well iced in an insulated polyurethane box and transported to Korea Atomic Energy Research Institute in Seoul, where a shipboard irradiator of approximately 15,000 Ci of Co-60 source is available for general use.

The samples were irradiated at 0.1, 0.2, 0.25, 0.4, 0.5 and 1.0 *Mrad*. The unirradiated control was kept in ice while other samples were being irradiated. All samples, irradiated and unirradiated, were transported back to the processing laboratory for planned storage studies.

At intervals of 0, 3, 7, 14, 21, 28 and 35 days during storage at 0°, 5° and 10°C, 2 sample pouches from each group were withdrawn and the changes of histamine contents, total bacterial counts, TVB and TMA contents were determined. Since histamine poisoning related problems are generally known to be associated with eating fish of inferior quality, the TVB and TMA contents were determined in order to relate the histamine levels to the freshness of each sample as indicated by the levels of two common spoilage indicating substances for fish.

Analytical methods

For estimating histamine contents, the cation exchange procedure of Kawabata *et al.* (1960) was employed. Detailed procedure was described previously (Lee *et al.*, 1985)

For total bacterial counts (Hartman and Huntsberger, 1961) sample meats were weighed into 4-oz. glass blending jars, presterilized and chilled, and homogenized with three volumes of 0.1% peptone water, also presterilized and chilled, at high speed for two min, using an Osterizer blender. Each homogenate was then made into a series of decimal dilutions and plates poured, allowed to solidify and incubated at 20°C. Tryptone glucose yeast extract agar (Scholz *et al.*, 1962) and 0.1% peptone water were used as medium and diluent, respectively. Colonies were counted on 5th day and results expressed as total viable cell number per *g* meat.

TVB and TMA nitrogens were estimated by microdiffusion method as detailed procedure was described previously (Lee *et al.*, 1985). Results were expressed as *mg* N per 100 *g* meat.

Results and Discussions

Minimum and maximum dose for mackerel meats

The mackerel meat samples were exposed to 0.1, 0.2, 0.25, 0.4, 0.5 and 1.0 *Mrad* and immediately after the irradiation treatments, the meat samples were presented to a group of six trained panels for establishing the minimum and maximum dose range.

The minimum dose is defined as an irradiation dose that shall retain the quality attributes characteristic of fresh sample and at above the dose the irradiation will impart undesirable changes in the products. The maximum dose on the other hand, is defined as the dose at above which the irradiated products become unacceptable due to adverse changes in sensory attributes as fresh products.

No adverse effect on sensory qualities of meat samples irradiated at doses up to 0.2 *Mrad* was noticed. The 0.2 *Mrad* irradiated had an almost neutral odor and rather hard in texture as compared to the unirradiated. At the same time they were distinctively brighter in color due to irradiation induced redening of tissue.

Irradiated samples at doses above 0.2 *Mrad*, on the other hand, imparted an irradiation odor which was clearly dose dependent. The samples irradiated at 0.4 *Mrad* were considered to be still acceptable by some panel as fresh products, whereas the 0.5 *Mrad* irradiated were unacceptable due to a strong irradiation induced odor.

Therefore, the dose of 0.2 *Mrad* was considered to be the minimum dose and of 0.5 *Mrad* to be the maximum permissible for mackerel meat samples (Sasayama and Amano, 1970 and Venugopal *et al.*, 1973).

Redening of meat due to irradiation at various dose levels

As a phenomena, one most apparent effect of exposing fresh mackerel meat samples to gamma radiation was redening of tissue. Initially the fresh meat samples had red and white muscle very distinctively zoned. Irradiation treatment caused the red muscle redder and brighter and the white muscle pinkish in color.

Thus, at dose above 0.4 Mrad, the redening of meat was intensive enough to make the meat more or less uniformly redish throughout regardless of the red or white muscle layers. This redening also appeared to be discipating upon storage, although, once redened, it remained noticeable during the practical storage period.

This redening appeared to have contributed positively to the overall sensory qualities of samples irradiated at low doses; thus the samples irradiated at 0.1, 0.2 and 0.25 Mrad were definitely better in appearance than the unirradiated control. On the other hand, the samples irradiated at 0.5 and 1 Mrad has an odd looking due to excessive redening of muscle together with the strong irradiation odor and they were not acceptable(Yamanaka and Amano, 1969). Exact mechanism of reddening of mackeral meat by irradiation has not been pursued, since this was beyond of our purpose of investigation. It shall be of interest to study the mechanism in the future.

Histamine accumulation in mackerel meats

The data on histamine contents in mackerel meats irradiated at various doses and changes during subsequent storage period at 3 temperature levels are presented in Table 1. There occured no appreciable accumulation of histamine contents in all meat samples, irradiated and unirradiated, during entire 35 day-storage at 0°C, although all meat samples passed into inedible stage long before the 35th day.

At 5°C, the histamine contents in the unirradiated started to increase appreciably during the late storage period, reaching 81.9 mg per 100 g meat by the 35th day., No appreciable increase in the histamine contents was noted in all irradiated samples stored at 5°C

The histamine accumulation in the unirradiated samples during 10°C storage was phenomenal; the contents started to increase very rapidly during very early storage, exceeding 72.8 mg per 100 g meat by the 3rd day and reaching 827.5 mg per 100 g meat by the 35th day. In the irradiated samples stored at 10°C on the other hand, the histamine accumulation was severely suppressed, thus the contents never reaching a level beyond 22.7 mg per 100 g meat during entire 35 day-storage. The histamine accumulation reached rather significant levels in the samples irradiated at doses below 0.25 Mrad during the late storage period at 10°C

Table 1. Changes of histamine contents in meats during post-irradiation storage

(*mg*/100*g* meat)

Storage temperature	Storage (days)	Irradiated at (*Mrad*) 0	0.1	0.2	0.25	0.4	0.5	1.0
0°C	0	2.5	1.2	1.7	2.4	1.2	1.4	1.4
	3	3.3	1.3	2.6	2.1	1.4	2.4	1.3
	7	3.9	2.1	2.5	3.2	1.2	2.1	2.3
	14	3.7	2.3	2.4	3.6	1.5	2.8	2.7
	21	5.3	2.5	2.7	4.1	1.5	2.5	2.7
	28	6.4	2.4	2.6	4.4	1.5	3.2	2.5
	35	7.5	3.1	4.2	4.4	2.1	2.9	2.6
5°C	0	2.1	1.3	1.7	2.3	1.2	1.4	1.4
	3	2.5	1.1	2.5	3.0	1.8	2.0	1.5
	7	2.6	2.3	2.1	2.2	1.6	1.9	2.2
	14	8.0	2.7	2.9	2.8	2.2	2.5	2.1
	21	8.5	2.4	4.7	3.6	2.1	3.6	2.3
	28	10.4	3.4	4.8	3.7	2.8	3.9	2.2
	35	81.9	4.8	5.6	3.7	1.8	4.2	2.7
10°C	0	2.4	1.9	1.6	1.8	1.8	1.2	1.3
	3	72.8	2.4	1.5	3.1	3.1	2.5	1.3
	7	89.9	3.2	2.3	6.4	3.2	2.9	2.6
	14	386.1	7.3	5.1	5.6	5.7	3.2	2.6
	21	588.5	10.2	14.2	22.7	5.5	4.0	2.7
	28	643.7	13.7	14.6	20.0	5.5	5.2	3.0
	35	827.5	14.6	16.4	19.1	5.7	6.8	3.6

and this is well beyond the practical storage period of fresh fish products.

It is important to note that a dose of 0.1 Mrad appears to be sufficient in bringing about a severe suppression of histamine accumulation during the practical storage of the products under refrigerated temperature conditions.

Microbiological changes in mackerel meats

One of the immediate effects of irradiating mackerel meats was, as with other marine products, reduction in number of total bacterial population. The total bacterial counts of the irradiated lagged behind those of the unirradiated throughout the storage period at 0°, 5° and 10°C (Table 2). At 0°C, the gap of the counts between the irradiated and the unirradiated remained more or less unchanged throughout the storage period, while at 5° and 10°C, the gap started to become narrow with progress of storage, approaching the level of the unirradiated by the 21st day. This was more apparent in the samples irradiated at the doses of 0.25 Mrad and less.

It is interesting to note that, although histamine accumulation in the unirradiated samples was more or less directly relatable to total bacterial numbers, irradiation treatment brought about disruption of normal spoilage pattern in the irradiated samples thereby suppressing severely histamine accumulation even through the late period. By this time the total bacterial numbers exceeded 10^9 cells per g meat, the count level sufficient to accumulate more than 72 mg histamine per 100 g meat in the unirradiated samples stored at 10°C, but in fact the histamine contents at this bacterial cell density level in the irradiated samples ranged only from 2.2 to 20.0 mg per 100 g meat. It may be that the histamine producing microorganisms in normal microflora of mackerel belong to groups that are highly radiosensitive and therefore they are more or less selectively removed by gamma radiation at doses as low as 0.1 Mrad (Zobell and Conn, 1940).

Table 2. Changes of total bacterial counts in mackerel meats during post-irradiation storage (No. cells/*g* meat)

Storage temperature	Storage (days)	Irradiated at (*Mrad*)						
		0	0.1	0.2	0.25	0.4	0.5	1.0
0°C	0	3.8×10^{6}	8.0×10^{4}	8.0×10^{4}	1.8×10^{4}	2.6×10^{2}	1.2×10^{2}	N.C.
	3	1.4×10^{6}	1.2×10^{3}	1.3×10^{5}	3.0×10^{4}	1.8×10^{2}	3.4×10^{2}	N.C.
	7	1.6×10^{6}	1.2×10^{4}	3.2×10^{4}	1.2×10^{4}	2.6×10^{3}	1.4×10^{3}	N.C.
	14	4.0×10^{7}	6.0×10^{5}	1.5×10^{6}	4.6×10^{4}	6.0×10^{4}	4.0×10^{3}	N.C.
	21	3.0×10^{8}	2.3×10^{4}	8.0×10^{5}	4.6×10^{5}	1.2×10^{5}	8.0×10^{4}	2.5×10^{2}
	28	8.1×10^{9}	4.4×10^{6}	9.3×10^{8}	7.7×10^{6}	3.2×10^{5}	1.5×10^{5}	4.6×10^{3}
	35	5.4×10^{10}	4.9×10^{7}	1.1×10^{8}	6.2×10^{7}	1.7×10^{7}	4.0×10^{7}	3.8×10^{4}
5°C	0	3.6×10^{6}	8.5×10^{4}	8.0×10^{4}	1.8×10^{4}	2.6×10^{3}	1.0×10^{3}	N.C.
	3	1.4×10^{7}	6.0×10^{4}	6.6×10^{4}	6.4×10^{4}	1.2×10^{4}	4.0×10^{3}	N.C.
	7	8.1×10^{9}	4.5×10^{6}	1.8×10^{6}	6.4×10^{4}	1.0×10^{5}	9.0×10^{4}	2.0×10^{2}
	14	1.6×10^{11}	7.2×10^{9}	3.0×10^{9}	3.4×10^{6}	2.0×10^{5}	1.3×10^{5}	1.0×10^{6}
	21	2.9×10^{11}	8.0×10^{10}	1.7×10^{10}	1.2×10^{9}	8.8×10^{9}	6.9×10^{8}	8.0×10^{7}
	28	8.4×10^{10}	1.3×10^{9}	6.6×10^{9}	1.6×10^{9}	6.0×10^{9}	4.0×10^{9}	3.0×10^{8}
	35	1.7×10^{11}	4.3×10^{10}	1.4×10^{10}	7.0×10^{9}	1.0×10^{8}	6.0×10^{9}	3.0×10^{7}
10°C	0	7.2×10^{5}	4.8×10^{3}	7.8×10^{3}	5.4×10^{3}	8.0×10^{2}	2.0×10^{2}	N.C.
	3	9.8×10^{9}	5.6×10^{5}	8.7×10^{4}	7.6×10^{4}	4.0×10^{3}	3.6×10^{3}	N.C.
	7	6.2×10^{9}	1.3×10^{8}	4.6×10^{7}	1.2×10^{6}	6.0×10^{5}	3.2×10^{5}	3.3×10^{3}
	14	3.2×10^{10}	8.0×10^{8}	1.3×10^{9}	8.6×10^{7}	1.1×10^{6}	5.8×10^{5}	1.8×10^{5}
	21	4.0×10^{10}	3.0×10^{10}	8.6×10^{9}	9.8×10^{8}	6.0×10^{8}	1.4×10^{7}	2.8×10^{7}
	28	1.2×10^{11}	5.0×10^{10}	7.5×10^{9}	7.8×10^{9}	7.8×10^{9}	2.4×10^{9}	4.8×10^{9}
	35	1.5×10^{11}	5.5×10^{10}	8.6×10^{10}	7.4×10^{9}	6.5×10^{8}	1.8×10^{9}	2.8×10^{9}

N.C.: Count less than 32 cells/*g* meat

Relationship between histamine level and freshness

Accumulation of TVB and TMA contents in the meat samples during 35 day-storage at 0°, 5° and 10°C is shown in Table 3 and 4, respectively. The effect of irradiation treatment was well reflected in the rate of TMA accumulation. In the unirradiated, TVB contents increased from initial values ranging from 16.0 to 24.0 mgN per 100 g meat to the level exceeding 69.6 mgN per 100g meat by the 7th day of storage at 10°C, reaching the value of 289.5 mgN per 100 g meat by the 35th day. At 0° and 5°C, the rate of TVB accumulation was much less with much reduced terminal values.

In the irradiated samples, the TVB accumulation was severely suppressed and in the samples irradiated at high doses the contents remained virtually unchanged during entire storage period. The TMA accumulation followed a similar pattern for the TVB except that the initial values were much lower (Table 4).

This pronounced suppressin of TVB and TMA accumulation in the irradiated samples indicated that, as it was with histamine accumulation, micro-organisms capable of producing TVB and TMA are removed more or less selectively by irradiation. This phenomenon of TVB and TMA suppression during post-irradiation storage of marine products has been well established (Pelory *et al.*, 1967; Kumta *et al.*, 1970). Interrelating the data on the accumulation of histamine, TVB and TMA, it is evident that histamine accumulation goes hand in hand with that of TVB and TMA. This confirms the well known fact that histamine poisoning related problems are invariably associated with eating certain species of fish of inferior quality. This point was well demonstrated by Takagi *et al.* (1969) who related histamine accumulation to the freshness of 21 species of white and dark fleshed fish.

Table 3. Changes of total volatile bases in mackerel meats during post-irradiation storage

(*mg* N/100 *g* meat)

Storage temperature	Storage (days)	Irradiated at (*Mrad*)						
		0	0.1	0.2	0.25	0.4	0.5	1.0
0°C	0	16.0	20.9	20.6	16.5	21.4	19.3	16.7
	3	21.6	20.5	16.9	20.3	20.5	20.9	19.6
	7	19.6	21.5	20.3	18.3	19.6	14.7	21.0
	14	22.6	21.9	21.6	17.9	21.9	22.6	21.0
	21	29.6	28.9	26.3	35.6	33.6	25.6	24.7
	28	36.6	27.6	26.6	23.3	33.1	33.3	28.9
	35	50.0	30.8	30.3	25.3	33.6	28.0	29.0
5°C	0	24.0	21.0	20.7	16.5	21.4	19.3	21.6
	3	26.1	23.8	21.0	20.3	25.6	19.6	25.2
	7	28.0	28.0	24.0	27.3	29.4	23.3	27.0
	14	65.0	27.5	31.0	28.6	23.3	27.6	28.0
	21	75.3	73.7	55.3	36.0	35.9	40.6	34.5
	28	93.2	95.3	113.2	73.3	42.9	33.3	31.7
	35	168.5	145.2	125.2	102.2	50.3	35.6	34.5
10°C	0	16.9	17.4	17.2	18.2	16.8	17.7	16.8
	3	33.6	20.5	18.6	20.9	22.4	21.4	20.6
	7	69.6	24.7	24.9	24.2	23.8	25.6	21.4
	14	98.6	45.8	28.4	30.1	24.7	33.1	24.1
	21	107.2	98.4	68.2	59.8	28.4	30.2	25.8
	28	268.5	103.5	101.2	117.0	45.2	43.4	35.4
	35	289.5	166.9	118.9	151.0	71.3	65.6	50.7

Table 4. Changes of trimethylamine in horse mackerel meats during post-irradiation storage

(*mg*N/100 *g* meat)

Storage temperature	Storage (days)	Idrraiated at (*Mrad*)						
		0	0.1	0.2	0.25	0.4	0.5	1.0
0°C	0	4.1	4.7	4.3	4.0	4.6	4.3	4.7
	3	3.3	5.6	4.7	5.3	4.2	5.3	5.1
	7	4.7	5.1	4.7	4.0	4.6	4.1	4.2
	14	5.3	6.0	4.3	4.7	4.5	4.0	5.5
	21	7.7	5.6	4.7	5.7	5.6	3.0	5.5
	28	13.3	6.5	6.7	6.7	6.1	6.7	6.0
	35	16.7	6.5	5.7	5.3	6.4	6.3	6.5
5°C	0	4.8	4.7	3.8	4.8	4.6	4.3	4.7
	3	6.3	4.7	4.0	4.7	4.7	4.7	5.1
	7	6.3	4.8	4.7	4.3	7.0	4.3	4.1
	14	14.7	8.8	7.8	6.7	5.7	6.9	5.1
	21	16.3	14.5	8.9	8.7	8.9	6.7	6.8
	28	23.3	21.9	19.9	13.3	11.7	10.0	7.9
	35	25.3	23.3	21.6	15.9	13.6	8.7	9.8
10°C	0	4.1	4.5	3.7	4.3	4.7	4.3	4.1
	3	10.3	4.7	5.1	4.7	4.2	4.0	4.2
	7	12.6	6.1	5.6	4.2	4.8	5.0	4.5
	14	21.4	13.1	6.5	5.1	6.8	5.7	5.1
	21	24.4	19.1	9.6	8.9	7.4	6.2	5.2
	28	123.5	17.7	14.9	13.9	8.4	10.7	9.7
	35	138.9	30.8	29.8	31.2	19.1	15.9	11.3

Conclusions

The optimum dose for Korean mackerel appears to be 0.2 Mrad and the maximum dose at 0.5 Mrad. The seemingly positive contribution to the sensory qualities of fresh by low dose radiation induced redening should be further investigated with a practical view to establish its technological significance to fresh fish trade.

It appears that, when fresh of first quality are kept at ice temperature, the danger of histamine poisoning related problems is completely nonexistent, whether the fishes are irradiated or not. The practical application of radurization techniques, therefore, should be sought mainly with the products to be stored at 5°C and above within refrigerated temperature range. Further studies should be made microorganims of isolated from both irradiated and unirradiated mackerel undergoing spoilage in order to establish the effectiveness and safety of radurization techniques as a tool for controlling histamine related problems in certain species of poisoning fresh fish.

Acknowledgements

This work was supported in part by a research grant of International Atomic Energy Agency (IAEA), and in part by a research grant of Cheongju College of Education

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한국산 고등어의 방사선 조사효과

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(1985년 2월 15일 수리)

생선 부패에 의한 히스타민 발생을 저지하는 방법으로 방사선 조사 기술의 응용 가능성을 살펴보았다. 한국산 고등어를 선량 0.1∼1.0 *Mrad* Co-60 방사선으로 조사한 후 0, 5, 10°C에 각각 저장하였다. 저장기간 중 히스타민, 총 휘발산, 트리메틸아민 양의 변화와 총 미생물 수를 측정한 결과 다음과 같은 사실을 알았다.

1. 히스타민 함량의 변화는 0°C 이하에서 저장하였을 시 방사선 조사에 관계없이 심각하게 변화하지 않았으나 5°C에서 저장하였을 시는 저선량 조사의 경우에도 저장 후기에 어육 100 *g*당 10 *mg*정도로 약간 증가하였다. 10°C 저장시에는 저장 초기에 이미 히스타민이 축적되어 저장 35일째는 어육 100 *g*당 827.5 *mg*의 높은 양에 달 하였으나 방사선에 조사 (0.4 *Mrad*) 의하여 어육 100 *g* 당 5.7 *mg* 정도로 현저하게 떨어졌다.
2. 방사선 처리는 초기의 미생물을 확실하게 사멸시키므로서 냉장 온도의 조건에서 히스타민 뿐 아니라 총 휘발산, 트리메틸아민의 생성을 억제하였다.
3. 방사선 조사 효과의 최저치는 0.2 *Mrad* 이었고 최고치는 0.5 *Mrad* 이었다. 0.5 *Mrad* 이상에서는 미생물의 억제 효과, 히스타민, 총 휘발산, 트리메틸아민의 생성을 현저하게 저해하여 방사선 효과가 컸으나 관능적인 면에서 좋지 못한 결과를 초래하였다. 반면 0.5∼1.0 *Mrad*의 선량조사 시에는 신선 고등어의 붉은 부분과 흰 부분이 구별되지 않을 정도로 고등어 근육이 붉어졌다.