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THE EFFECT OF SINGLE AND DOUBLE DOSES OF GAMMA RADIATION ON PRE-RIGOR MORTIS FILLETED SAITHE

by

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1. INTRODUCTION

Among the fish species especially suitable for treatment with low doses with ionizing radiation (radurization) prior to further processing are those with a low fat content.

Due to the difficulties of getting fresh raw material, most of the studies in this field have been performed either on deepfrozen fish, or on fish caught some days previously.

The possibility of giving the first irradiation on board the fish vessel just after catching the fish when the bacterial load is low and giving a further treatment on shore some days later, has now been recognized (Slavin et al 1966). More interest is therefore focused both on the irradiation of pre-rigor mortis fish and also on the effect of splitting the single dose. The latter effect has especially been studied by Liston et al (1969) a.o., who observed an additional reducing effect on the microflora of cod when comparing a double dose with a corresponding single dose.

In a previous paper the effect of low doses of ionizing radiation on pre-rigor cod was reported (Jebsen et al 1972). In order to get a better understanding of the effects introduced by low doses of ionizing radiation on pre-rigor fish these studies were continued. Saithe was selected as the next fish species to be studied. Besides measuring the spoilage bacterial flora in radiated and unirradiated samples and some of the more important criteria for freshness, the effect of double doses in comparison with single doses was also investigated.

2. MATERIALS AND METHODS

Sampling and Packaging

Saithe (*Gadus virens*), caught on the western coast of Norway, was brought to the laboratory alive. Immediately after killing, the fish was filleted and skinned under aseptic conditions at low temperature (+4 °C). The fillets were cut into pieces of about 50 g and packed in samples of half a kilo in laminated plastic bags ("Tropyten 80") with a low permeability to N_2 and CO_2. The samples were stored at 0° C.

Irradiation

The samples were irradiated in a cobalt-60 source at the Institute of Atomic Energy, Kjeller, Norway, at doses of 50, 100 and 150 krad. Two series of samples irradiated with 50 krad were re-irradiated after 7 days with 50 and 100 krad, respectively.

Analytical Procedure

Dimethylamine (DMA) was determined from a proteinfree extract made by homogenizing 100 grams of muscles in 200 ml of water, adjusting the pH to 5.2. Colloidal $Fe(OH)_3$ (15 ml) was added to the homogenate and the suspension was further diluted to 500 ml, heated to 70 °C and filtered. To 25 ml of the filtrate 225 ml H_2O and 0.5 g MgO was added, and the resulting solution was distilled for 45 min. The DMA determination in the distillate was carried out according to the method of Dyer and Mounsey (1945).

The content of formaldehyde (FA) was measured colorimetrically using the reaction with chromotropic acid. The method is described by Bremanis (1950) and has been modified by Antonacopoulos (1960). For the calibration curve hexamethylentetramin (Merck No. 4343, p.a.) was used as a source of FA, to which the same amount (3 g) of FA free fish muscle was added.

The analytical methods applied for the determination of total bacterial count, proteolytic activity, trimethylamine (TMA), ammonia, and volatile acid are described in detail in a previous paper (Jebsen et al 1972). The trimethylamineoxide (TMAO) was reduced by $TiCl_3$ (Ronold et al., 1947) and determined as TMA.

3. RESULTS

The total bacterial count is shown in Fig. 1. Initially the bacterial count in non-irradiated saithe fillets was about $5 \cdot 10^4$/g fish muscle, increasing to more than 10^6/g during the following 10 days storage at 0 °C. Irradiations with as low doses as 50 krad kept the bacterial count below 10^6/g for 30 days. It is seen from Fig. 1 that double doses, especially 50 + 100 krad, had a greater reducing (decimating) effect on the bacterial count than the corresponding single doses.

From Table 1 the proteolytic activity can be read. In non-irradiated samples the proteolytic activity increased rapidly during the first two weeks, then the activity levelled out. In irradiated fillets the proteolytic

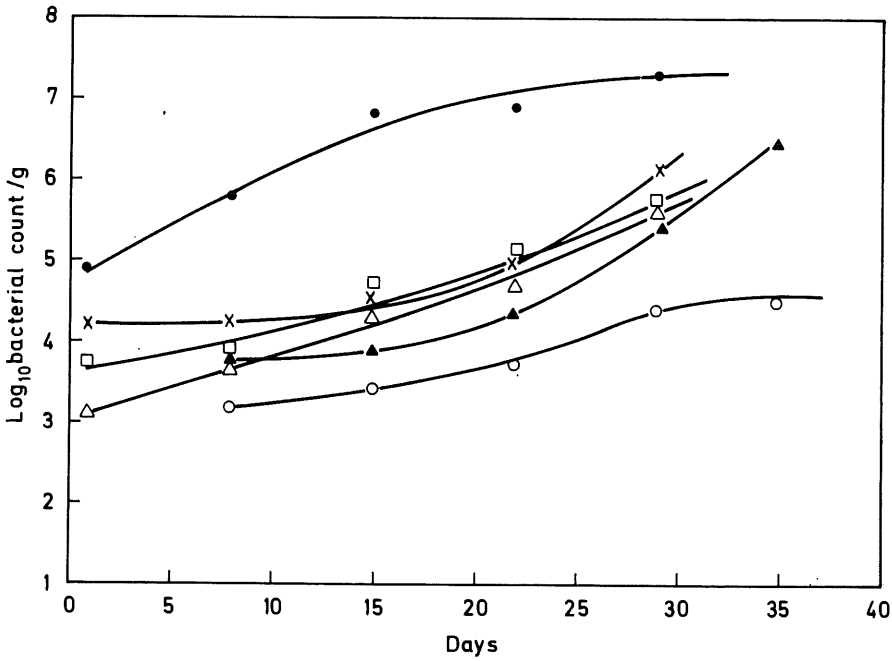

Fig. 1. Total bacterial count in non-irradiated (●), 50 krad (x), 100 krad (□), 150 krad (△), 50 + 50 krad (▲) and 50 + 100 krad (○) irradiated saithe fillets.

activity was low during at least three weeks of storage at 0 °C. During this period there seemed to be no differences between the proteolytic activities of samples which had received different radiation doses.

After three weeks the proteolytic activity increased more rapidly in the fillets receiving 50 + 50 krad than in the other irradiated samples (The series receiving only 50 krad was at this time disrupted.) Fig. 2 illustrates the relation between the total bacterial count and the proteolytic activity for the following doses, 0, 150 and 50 + 100 krad.

The quantity of volatile acids formed during storage is shown in

Table 1. Proteolytic activity in non-irradiated and irradiated saithe. Storage temperature 0° C.

Storage (days)	Non-irradiated	Irradiation dose (krad) 50	100	150	50 + 50	50 + 100
1	35	53	43	28	—	—
8	80	54	58	40	35	49
15	180	93	47	59	62	63
22	189	—	65	88	57	68
29	184	—	89	102	147	67
36	190	—	—	—	154	91

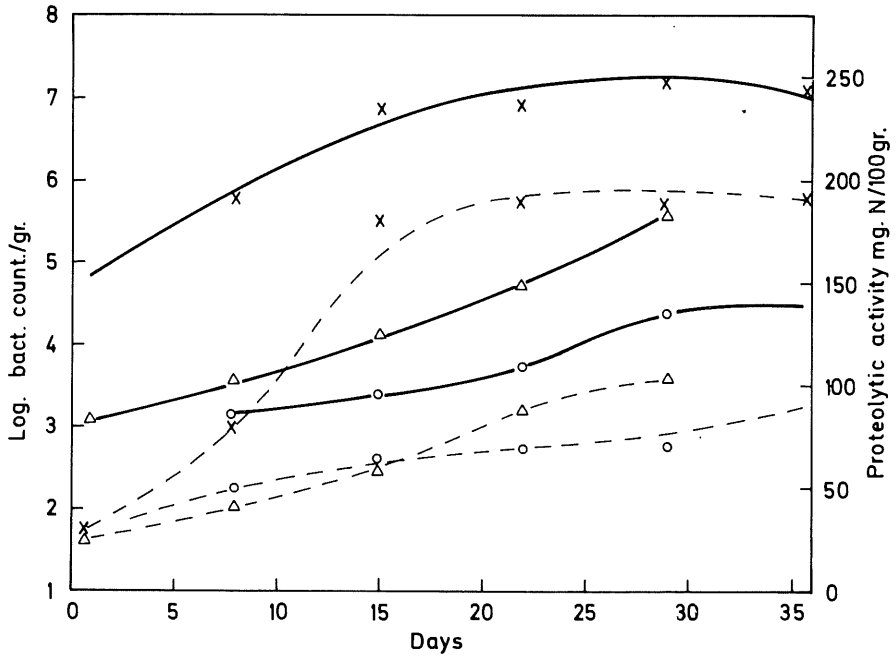

Fig. 2. Total bacterial count (—) and proteolytic activity (— — —) of non-irradiated (x), 150 krad (△) and 50 + 100 krad (o) irradiated saithe fillets.

Table 2. In non-irradiated fillets the concentrations of volatile acids increased rapidly during the second week of storage, from 12 mg/100 g on the 8th day to 172 mg/100 g on the 15th day. In fillets receiving 100, 150 and 50 + 100 krad the concentration of volatile acids was rather low throughout the experimental period, whilst the double dose 50 + 50 krad had only a temporary retarding effect on the formation of volatile acids. This sample took approximately four weeks to reach the same level which non-irradiated samples attained in two weeks.

Table 2. Volatile acid formation in non-irradiated and irradiated saithe during storage at 0 °C (mg acetic acid/100 g fish muscle).

Storage (days)	Non-irradiated	Irradiation dose (krad)				
		50	100	150	50 + 50	50 + 100
1	1	0	0	0		
8	12	6	6	3	1	0
15	172	35	6	3	11	9
22	244	—	11		26	11
29	201	—	15		135	6
36	265	—	—	—	128	16

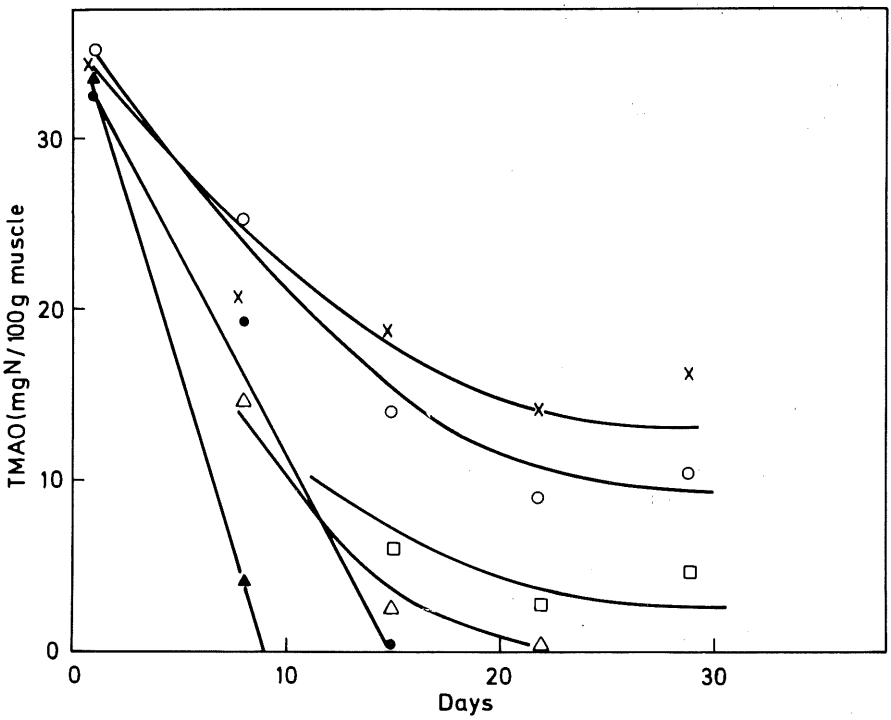

Fig. 3. Trimethylamineoxide concentration in non-irradiated (▲), 50 krad (●), 100 krad (o), 150 krad (x), 50 + 50 krad (△) and 50 + 100 krad (□).

Fig. 3 shows the initial trimethylamineoxide (TMAO) and the disappearance of TMAO during storage at 0 °C. It should here be noted that although the slope of the curves may be a little uncertain due to few measurements, they nevertheless give a good idea of the retarding effect which the ionizing radiation has on the TMAO reduction. Initially, the TMAO concentration was on average 33 mg/100 g. This value decreased in non-irradiated samples to near 0 during the first week of stor-

Table 3. Trimethylamine concentration (mg N/100 G fish muscle) in non-irradiated and irradiated saithe during storage at 0 °C

Storage (days)	Non-irradiated	Irradiation dose (krad)				
		50	100	150	50+50	50 + 100
1	2	0	0	0	—	—
8	9	0	0	0	0	1
15	31	5	6	0	5	0
22	40	—	0	0	9	0
29	38	—	8	0	23	0
36	37	—	—	—	21	3

Table 4. Dimethylamine (mg N/100 g fish muscle) in non-irradiated and irradiated saithe stored at 0 °C

Storage (days)	Non-irradiated	Irradiation dose in krad 50	100	150	50 + 50	50 + 100
1	2.3	3.8	2.1	2.4	—	—
8	4.4	8.7	9.3	13.7	5.4	4.6
15	4.7	16.0	17.3	18.4	17.0	30.2
22	7.2	—	16.8	16.5	18.3	27.0
29	5.8	—	17.9	18.3	14.7	29.8
36	5.1	—	—	—	14.6	31.6

age. In irradiated fillets the degradation of TMAO is much slower. Here the single dose of 150 krad seems to have a greater retarding effect on the degradation than the splitted dose of 50 + 100 krad.

The results of the trimethylamine (TMA) determination are shown in Table 3. The TMA concentration in non-irradiated fillets increased during storage especially during the second week, and was significantly higher than the TMA concentration in many of the irradiated samples.

After 29 days storage the TMA content in fillets irradiated with 100 krad was of the same order as that found in non-irradiated fillets after 8 days storage. In fillets receiving a single dose of 150 krad no detectable amount of TMA could be measured during the storage period. Splitting a 150 krad dose into 50 and 100 krad had nearly the same effect on TMA as the single dose, a split 100 krad dose, however, had less effect on the TMA formation than the corresponding single dose.

The dimethylamine (DMA) formation was low in non-irradiated fillets throughout the storage period (Table 4). In irradiated samples a significant increase was observed in the course of the second week of storage. A splitting of a 100 krad dose in 50 + 50 krad did not influence the DMA concentration significantly compared to the corresponding single dose. On the other hand a considerably higher DMA concentration was measured when a 150 krad dose was split into 50 + 100 krad.

Table 5. Formaldehyde (mg/100 g fish muscle) in non-irradiated and irradiated saithe stored at 0 °C

Storage (days)	Non-irradiated	Irradiation dose (krad) 50	100	150	50 + 50	50+ 100
1	3.7	3.8	3.3	5.5	—	—
8	4.9	11.8	10.6	15.3	8.4	18.8
15	2.8	21.4	20.2	16.8	9.8	28.1
22	2.6	—	25.5	17.5	18.7	23.6
29	3.1	—	22.0	24.0	14.3	31.2
36	1.2	—	—	—	11.2	31.4

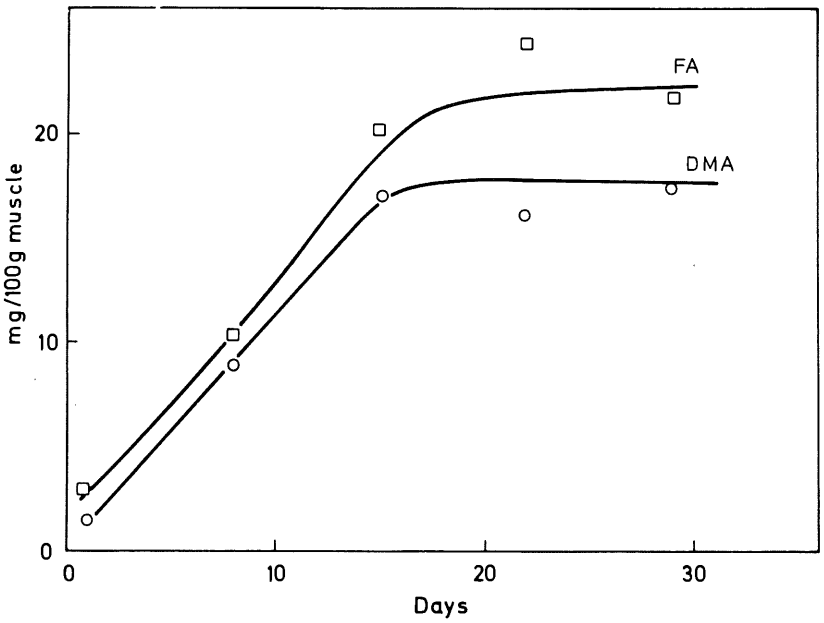

Fig. 4. Dimethylamine (o) and formaldehyde (□) in 100 krad irradiated saithe fillets.

The formaldehyde (FA) concentration in irradiated and non-irradiated samples is shown in Table 5. As for DMA, the FA concentrations were constantly low in non-irradiated samples, while the FA concentration in irradiated samples increased during storage. The highest concentration, 41.4 mg/100 g, was found in fillets irradiated with 50 + 100 krad. The close relation between DMA and FA (100 krad) is illustrated in Fig. 4. Table 6 shows the amount of drip measured. Here there seems to be no difference between non-irradiated and irradiated fillets.

An organoleptic evaluation was carried out by a panel of tasters. It was found that the appearance and consistence of irradiated fillets were almost unchanged, even after four weeks storage. A moderate crablike

Table 6. The amount of liquid "Drip", extended from non-irradiated and irradiated saithe ml/kg fish muscle

Storage (days)	Non-irradiated	Irradiation dose (krad) 50	100	150	50 + 50	50 + 100
1	56	100	95	90		
8	68	123	100	106	100	131
15	111	141	140	135	134	131
22	145	—	132	173	128	148
29	160	—	125	152	116	126
36	168	—	—	—	137	124

odour was noticed immediately after irradiation. The odour diminished after cooking. After a while the odour also vanished from raw fillets when stored on ice. Fillets receiving 100, 150 and 50 + 100 krad were organoleptically acceptable for about four weeks, compared to less than two weeks for non-irradiated fillets.

4. DISCUSSION

Micro-organisms are most sensitive to radiation in their logarithmic growth phase (Liston et al 1969). As different micro-organisms do not enter into their logarithmic growth phases simultaneously, the chances of hitting the micro-organisms in their most sensitive phase is thus increased by giving two doses of irradiation separated by a week's interval. This theory is supported by the present results which indicate that the double doses, especially 50 + 100 krad, were more effective in reducing the total bacterial count than a comparable single dose treatment, although the effect was considerably smaller than that observed by Liston et al (1969) for cod. This difference might be due to a higher initial bacterial count or to a slight difference in the bacterial flora of the saithe fillets used in the present investigation as compared with the starting material used by others.

The proteolytic activity in non-irradiated and single dose irradiated saithe seems to be in good accordance with the total bacterial count (Fig. 2), and is thus in agreement with earlier observations where the irradiation of pre-rigor cod was studied (Jebsen et al 1972). Although double doses had some additional effect on the total bacterial count (compared with a corresponding single dose) no measurable additional effect on the proteolytic activity, at least during the first three weeks storage, could be observed. This might indicate that for splitted doses a relatively greater proportion of the proteolytic micro-organisms survive than when a corresponding single dose is given. Masurovsky et al (1963) found that pseudomonas species in particular grew out in haddock irradiated with low doses and that the spoilage flora consisted to a considerable degree of these bacteria. An initial dose of 50 krad might thus be too small to keep the spoiling flora sufficiently low in the time interval between the two irradiation treatments. Our results further indicate that if the second radiation dose is to have a prolonged retarding effect on the spoilage processes it should be at least 100 krad.

The formation of volatile acids seems to be dependent on the microbiological activity. Non-irradiated samples with a relative high bacterial

count are observed to have a volatile acid concentration which is significantly higher than in irradiated, low bacterial loaded samples. However, the favourable effect of double doses on the total bacterial count is not reflected in a corresponding reduction in the volatile acid concentrations over that of samples irradiated with a single dose.

In the present investigation the initial TMAO concentrations varied between 33 and 44 mg/100 g. This is lower than the concentration (70 mg/100 g) reported by Dreyer (1952). In non-irradiated fillets a rapid degradation of TMAO occurs, being almost completed in 14 days. This degradation of TMAO is significantly retarded by the irradiation. The difference in degradation rate can most probably be ascribed to the importance of bacterial enzymes taking part in this process of which TMA is the most important among the reaction products.

As indicated above, the formation of TMA is dependent on the presence of TMAO reducing bacterial enzymes. This is supported by results shown in Table 3 where the TMA concentration in non-irradiated samples increased during the first few weeks of storage, whilst the TMA concentration for the irradiated samples was constantly low during the storage period. The change in the micro flora from a predominance of pseudomonas species in non-irradiated fish to a predominance of achromobacter species in irradiated fish, affects simultaneously the species capable of reducing TMAO (Laycock & Rieger, 1970). Those authors found only one species constantly capable of reducing TMAO to TMA in irradiated fish.

In irradiated samples TMAO degrades mainly to DMA and FA. It can be seen from Table 4 that the DMA concentration in irradiated samples increases during storage while the concentration in non-irradiated samples is generally low and varies little throughout the storage period. Obviously degradation of TMAO to TMA and DMA can occur in saithe, but at different rates, the former being dependent on bacterial enzymes, while the formation of DMA and FA seems to be an autolytic process taking place at a slower rate.

The results of the FA determinations in stored pre-rigor saithe show a considerably higher amount of extractable FA in irradiated as compared with the non-irradiated samples. The latter results are in agreement with other values found in fish products (Lunde et al 1934, Soudan 1962, Yamada & Amano 1954—65—69, Amano & Yamada 1963—64—65, Castell et al 1961).

The correlation between the DMA and FA formed in the irradiated samples should be noted (Table 4, 5 and Fig. 4). In this investigation an almost stoichiometric formation of FA and DMA is observed.

The decrease in DMA and FA after 4—5 weeks of storage may be

due to reactions with proteins which would make FA in particular less extractable. In this period there is also an increase in the number of bacteria which could also influence the TMAO—TMA reaction.

The present investigation shows that TMAO is mainly reduced to DMA and FA when the dose is 50—100 krad and higher. The concentration of FA may thus reach considerable values. The analyses show that most of the FA in saithe is present in the fish muscle either as free or loosely bound FA. This supports theories put forward by Amano & YAMADA (1965).

Another wholesomeness aspect is the relatively high concentration of DMA. Together with nitrosing agents DMA may form nitrosamines. Some of these are toxic. Further studies should be carried out on this point.

The crab like odour noticed shortly after irradiation may be due to the formation of volatile sulphur compounds, probably methylmercaptane, during the irradiation. However, the odour disappeared slowly and had small influence on the taste. The panel of tasters found raw and cooked irradiated saithe acceptable for about four weeks. Towards the end of that period the fillets were a bit dry. This is probably due to the relatively great amount of drip exudating during storage. Non-irradiated saithe fillets passed the acceptable limit in the course of two weeks.

5. SUMMARY

Saithe, caught on the western coast of Norway, was filleted immediately after killing, packed in plastic bags and irradiated using a cobalt-60 source at the Institute of Atomic Energy, Kjeller. The doses were 50, 100, 150, 50 + 50 and 50 + 100 krad.

All samples were analysed for their total bacterial count, proteolytic activity, ammonia, total volatile acids, trimethylamine oxide, tri- and dimethylamine and formaldehyde. The organoleptic quality was evaluated by a panel of tasters.

Double doses were more effective in reducing the total bacterial count than an equivalent single dose. A close relation was found between the bacterial count and the proteolytic activity.

The trimethylamine oxide concentration was found to vary between 33 and 44 mg/100 g muscle, decreasing rapidly in non-irradiated fillets to 3 mg/100 g. In non-irradiated fillets trimethylamine oxide was mainly reduced to trimethylamine and in irradiated fillets to dimethylamine and formaldehyde.

A split dose of 50 + 50 krad may have a favourable effect on the total bacterial number, but the analyses of proteolytic activity, the volatile

acids and the trimethylamine indicated that a higher proportion of spoilage bacteria survive.

The low proteolytic activity and the high content of DMA/FA prove that split doses of 50 + 100 krad as compared with single dose of 150 krad, are particularly favourable in the case of saithe.

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