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THE EFFECT OF GAMMA RADIATION ON PRERIGOR MORTIS FILLETED COD

by

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INTRODUCTION

Fish is a perishable foodstuff giving rise to numerous preservation problems which have not been satisfactorily solved by conventional methods. The use of low doses of gamma radiation in conjunction with refrigeration may solve many marketing difficulties by increasing the shelf life of fresh fish by a factor 2—3 (Shewan, 1966). Further advantages are reduced spoilage losses and reduced transportation and handling costs (Ketchum et al, 1965). Moreover, fish pasteurised by irradiation may, in many parts of the world, be more convenient to introduce than fish preserved by other methods. The pasteurising of fish by low doses with ionising radiation has been studied at numerous laboratories (IAEA, 1970, IAEA, 1971, and Goldblith, 1970). However, relatively few studies have been undertaken using fresh fish of marine origin.

The aim of the present investigation was to study the effect on prerigor mortis cod fillets of gamma radiation on some of the common parameters used for characterising fresh fish.

MATERIAL AND METHODS

Sampling and packaging. Cod, caught on the western coast of Norway was brought to the laboratory alive. Immediately after slaying the fish was filleted and skinned under aseptic conditions, at low temperature.

The fillets were cut into pieces of about 50 g and packed in samples of 500 g in plastic bags (mylothene 58/S). The samples were stored under nitrogen at 0°C.

Irradiation. The samples were irradiated in a cobalt-60 source at the Institute of Atomic Energy, Kjeller, at doses of 25—50—90 and 180 krad. The doses were controlled by Fricke dosimeters and perspex (red-400) dosimeters (Thomassen, 1970).

Determination of proteolytic activity. One part of ground muscle was homogenized with 4 volume distilled water with 5 % toluene added. Aliquots of the homogenate were adjusted to different pH with 1 N HCl. The proteolytic activity was measured as the formation of trichloroacetic-acid-soluble peptides by using the method of Eggstein & Kreutz (1955).

For the calibration curve, trichloroacetic acid-soluble peptide-extracts were used, the nitrogen-content being determined according to Ma & Zuazaga (1942).

Taste, odour and consistence. A taste-panel of a staff trained in controlling fresh and frozen fish examined the irradiated products.

Measurement of drip. The amount of drip exuding from the stored samples, was measured directly in the fillet (ml/kg).

Total bacterial count. Five grams of fillets were mixed with 45 ml saline to give homogeneous suspensions, from which 10-fold dilutions were made. From each dilution 0.1 ml was spread on nutrient agar (Oxoid). The plates were incubated at 20 °C for 72 hrs.

Trimethylamine (TMA), ammonia and total volatile basis (TVB). The analyses of TMA, ammonia and TVB were carried out, in principle, according to the method of Conway (1950). Fish muscle (100 g) was homogenized with 200 ml distilled water. The pH was adjusted to 5.2 with 1 N HCl and 15 ml colloidal iron hydroxide were added and filled up to 500 ml with water. The solution was then heated to 70 °C and filtered.

Volatile acids (VA). The content of VA in irradiated muscle was rather low, and the method published by Conway (1950) had to be modified.

Fish muscle (25 g) was homogenized with 25 ml distilled water and the precipitate removed by centrifugation. To 20 ml of the supernatant, 6 ml 20 % phosphotungstic acid were added, and the precipitate again removed by centrifugation. 1 ml protein-free extract, 0.2 ml 3 N sulphuric acid, and ca. 3 g of anhydrous sodium sulphate were placed in the central chamber and 4 ml N/500 sodium citrate in the outer chamber. After the diffusion of the volatile acids into the outer chamber, the sodium citrate solution was transferred to a small beaker and titrated with N/200 barium hydroxide. The pH 7.9 was found to be the most convenient endpoint according to our titration-curves.

Hypoxanthine was determined by a slight modification of the method described by Burt et al (1969), where hypoxanthine is changed into uric acid. By addition of a redox indicator dye, 2,6-dichlorophenol-indophenol, the content of hypoxanthine above the acceptable limit of 1.91 μmol/g could be registered. This value is normally found in cod after storage for 13—14 days at 0 °C. As the presence of oxygen disturbs the readings, the solutions should be evacuated before the measurements are carried out.

Glutamate — Oxalacetate — Transaminase (GOT). The determinations were carried out by the colorimetric method of «Biochemica-Test-colorimetrie».

RESULTS

The proteolytic activity in the nonirradiated cod fillets was determined in two successive experiments. The results are shown in Tables 1 and 2. From the tables it can be seen that the pH-optimum for the proteolytic enzymes varied between pH 3 and 4. At this pH interval the proteolytic activity was usually a factor of 3—6 greater than at pH 7. From Table 2 it can also be seen that the proteolytic activity in fillets stored at 37 °C was roughly a factor of 5—6 higher than in fillets stored at 0 °C.

The proteolytic activity in irradiated cod fillets is shown in Table 3. It was observed that, even after 29 days storage at 0 °C, the proteolytic activity in fillets receiving 180 and 90 krad was still low. Doses below 90 krad were insufficient to keep the proteolytic activity at the same low level throughout the storage period. The lowest dose used, 25 krad, caused also a certain reduction in the proteolytic activity.

Table 4 shows the proteolytic activity at 0 °C and 37 °C in irradiated fillets homogenised after irradiation. No protease inhibiting compounds seemed to be formed during irradiation, and no change in the pH-optimum of the enzymes was observed. The effects of proteolytic enzymes produced by micro-organisms was hindered by the addition of toluene.

The total bacterial counts in irradiated and nonirradiated fillets are shown in Table 5. Especially at the two highest doses, 180 and 90 krad, a large reduction in the bacterial count was observed. When 180 krad was used the bacterial count reached $4.8 \cdot 10^6$ in fillets stored at 0 °C after 35 days. Correspondingly, $3.8 \cdot 10^6$ bacteria per gram were reached after 29 days when the radiation dose was 90 krad. The lower doses, 50 and 25 krad, had only moderate effects on the total bacterial counts.

During storage a milklike liquid, drip, exuded from the fish muscle. Table 6 shows the drip from irradiated and nonirradiated fillets. Measurement on the 14th day of storage at 0 °C showed that the drip decreased with increasing doses, being 12 ml per kg from 180 krad irradiated fillets and 56.5 ml per kg from nonirradiated fillets. Later during the storage the difference between irradiated and nonirradiated samples seems to disappear.

No «gaping» effect was observed in the irradiated fillets.

The formation of trimethylamine (TMA), total volatile base (TVB), ammonia, volatile acid (VA) and the change in pH in irradiated and nonirradiated fillets are shown in Table 7. The formation of TMA and TVB during storage was much greater in nonirradiated fillets as compared to irradiated with 90 and 180 krad. By the method used for the TMA analyses (Conway 1950) it is not possible to differentiate between TMA and dimethylamine (DMA). However, preliminary

results of the formaldehyde concentration (FA) indicated that the content of DMA in these fillets was low compared with TMA. Ammonia and pH changed very little during storage. There was no difference between irradiated and nonirradiated samples. From the table it can also be seen that in nonirradiated and in fillets irradiated with 25 krad, the concentrations of volatile acids increased considerably during storage while in fillets receiving 180 and 90 krad the concentration remained low for 35 days.

The formation of hypoxanthine in irradiated and nonirradiated cod fillets is shown in Table 8. Two plussigns (++) indicate hypoxanthine concertrations exceeding the acceptable upper limit 1.91 μmol/g fish (Burt et al, 1969). In nonirradiated fillets, and fillets receiving 25 krad, this limit was exceeded after 14 days, while in fillets receiving 50 and 90 krad the acceptable concentration limit was not reached until 1—2 weeks later, i.e. after 3—4 weeks storage at 0°C.

Measurement of glutamate-oxalacetate-transaminase (GOT) in some of the samples indicated a lower concentration in irradiated fillets as compared to nonirradiated (Table 9).

Radiation doses of 100—200 krad led to the development of slight crab-like odour, especially during the first days after irradiation. Fillets receiving 90 and 180 krad were still organoleptically acceptable up to about 27 days storage at 0 °C. The test panel found the nonirradiated fillets unacceptable after 14 days storage.

DISCUSSION

Deterioration of fish is caused mainly by proteolytic enzymes of bacterial origin and less to autolytic activity (Liston, 1965). The proteolytic enzymes have different pH-optima. As shown in the present investigation, the enzyme mixture of nonirradiated fresh and stored cod fillets had a pH-optimum at pH 3—4. Siebert (1962) found the maximum activity of pure cod muscle cathepsins to be at pH 4.3, while Makinodan & Ikeda (1969) have reported a pH-optimum of pH 2.6. The radiation doses used, did not cause any change in the enzymes' pH-optimum. The results confirm that the proteolytic activity in nonirradiated fish is mostly due to proteolytic enzymes of bacterial origin (Table 3 & 5). The proteolytic activity in irradiated fillets was very low throughout the storage period. This observation can be correlated with the low total bacterial count found in irradiated fillets, and thus a proportionally lesser formation of bacterial proteolytic enzymes. As shown by Siebert (1962), Dahle & Underdal (1971), Rossebø & Underdal (1972) genuine tissue enzymes as well as bacterial proteases show a high degree of

radiation resistance. The radiation doses used in the present investigation would thus have little, or no, inactivating effect on the enzymes present.

It should also be mentioned that in irradiated fish the surviving bacteria are generally nonproteolytic (Kumta et al, 1970, Spinelli et al 1965). Laycock & Regier (1970) found that gram positive organisms which constituted only a small percentage of the preirradiated flora appeared as a larger proportion of the immediate postirradiation flora. It can thus be assumed that the proteolytic activity in irradiated fish fillets can be mainly ascribed to the cathepsins. The present results record low proteolytic activity and thus support observations by Chung et al (1963) that the autolytic activity in cod muscle is generally low. In an earlier investigation, the proteolytic activity in cod muscle was found to be approximately constant throughout the year (Jebsen & Siebert, 1965, unpublished).

From a bacteriological point of view irradiation of prerigor mortis fish is to be preferred as the bacterial load at that stage is low. On the other hand due to the rigor contraction in prerigor mortis filleted fish muscle, the drip is greater than in postrigor mortis filleted muscle. However, the irradiation seems to have a favourable, depressing effect on the drip formation, most probably due to general decreased proteolytic degradation.

The much lower TMA-concentration found in irradiated fillets as compared to nonirradiated, support the theory of Beatty & Gibbons (1937) that in sterile muscle press juice trimethylamine oxide (TMAO) are not reduced to TMA. This is also in good agreement with Tarr (1939) who discussed the possibility that TMAO is reduced to TMA by bacterial enzymes.

The considerably lower concentrations of volatile acids, and to some extent, hypoxanthine in irradiated fillets compared to that of nonirradiated indicates that formation of these degradation products is greatly influenced by bacterial enzymes. Jones (1965) is of the opinion that the first steps in the degradation of adenosinetriphosphate to hypoxanthine are governed by autolytic processes and the next steps partly by bacterial enzymes.

Glutamate-oxalacetate-transaminase (GOT) is released from the mitochondria when the mitochondrial membrane is damaged. From the present investigation where the GOT concentration was in fact found to be lower in irradiated fillets than in nonirradiated, it can be concluded that the applied radiation doses have had no damaging effects on the mitochondrial membranes. Our observations further support Hamm et al (1969) who indicated a positive correlation between bacterial growth and the release of GOT from skeletal muscles.

The present investigation has shown that irradiation doses from 90 to 180 krad retard many degradation processes in cod muscle. Small concentrations of some of the degradation products, such as hypoxanthine and volatile acids, contribute, no doubt, to the normal taste and odour of fish. A supression of the formation of these and other products, and also absorption of flavourinitiating compounds, by formaldehyde, might thus give rise to a more or less «neutral» tasting product. This was stressed by the panel. The characteristics of irradiated stored cod fillets were thus not a strong «off»-taste and odour, but rather a lack of the typical fish odour. It should, be emphasized, however, that the test panel found irradiated fillets acceptable for consumption after 27 days storage while the nonirradiated fillets passed the acceptable limit after 14 days storage at 0 °C.

SUMMARY

The effect of gamma radiation on cod fillets was studied. Cod caught off the west coast of Norway were immediately filleted after slaying, packed in plastic bags and irradiated at the Institute of Atomic Energy, Kjeller. The doses were 25, 50, 90 and 180 krad.

Irradiated and nonirradiated fillets were stored at 0 °C and analysed at intervals with respect to proteolytic activity, total bacterial count, trimethylamine (TMA), total volatile base (TVB), volatile acids (VA), hypoxanthine and glutamate-oxalacetate-transaminase (GOT).

In irradiated cod fillets the proteolytic activity was considerably lower than in nonirradiated fillets. The proteolytic activity is correlated with the bacterial count, which was significantly decreased by irradiation. Correspondingly, TMA, TVB, VA and hypoxanthine formation were retarded by irradiation. The GOT concentration was lower in irradiated fillets than in nonirradiated.

During the first days after irradiation a crab-like odour could be registered in fillets receiving 90 and 180 krad. This special odour disappeared gradually and irradiated fillets were found to be organoleptically acceptable after 27 days storage at 0°C, compared to 14 days for nonirradiated fillets.

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Table 1. The proteolytic activity, expressed as trichloroacetic acid-soluble peptides (mg N/100 g), in cod fillets at different pH and storage at 37 ° C.

pH	Storage time (days)						
	0	1	2	3	5	9	12
7.....	15	101	230	252	298	436	467
6.....	61	288	459	481	505	581	689
5.....	31	329	504	596	551	949	864
4.....	122	459	711	711	757	1 040	1 239
3.....	104	291	643	643	617	964	780

Table 2. The proteolytic activity, expressed as trichloroacetic acid-soluble peptides (mg N/100 g), in cod fillets at different pH and storage at 0 °C and 37 °C.

pH	Storage time (days) 0 °C			Storage time (days) 37 °C		
	1	4	6	1	4	6
7.....	39	—	29	236	472	459
6.....	—	47	91	334	—	694
5.....	76	92	174	308	865	996
4.....	46	139	212	406	852	970
3.....	79	197	222	524	970	1 114
2.....	65	204	168	101	165	210

The substrate used in the two experiments referred to in Table 1 and 2 is from two different catches of cod.

Table 3. Proteolytic activity, expressed as trichloroacetic acid-soluble peptides (mg N/100 g), in irradiated and nonirradiated cod fillets stored at 0 °C.

pH	Radiation dose (krad)	Storage time (days)			
		2	14	22	29
6.8	180	42	58	97	69
	90	—	39	82	108
	50	—	20	100	436
	25	—	91	421	402
	0	—	285	524	428

Table 4. Proteolytic activity, expressed as trichloroacetic acid-soluble peptides (mg N/100 g), in irradiated and nonirradiated cod fillets stored as suspensions at 0 °C and 37 °C.

pH	Radiation dose (krad)	Storage time (days) 0 °C			Storage time (days) 37 °C		
		0	2	10	0	2	10
6.8	180	8	39	62	8	237	367
	90	17	35	59	17	261	367
	0	19	52	98	19	228	463
4.0	180	13	91	137	13	327	702
	90	26	61	140	26	431	1 024
	0	26	78	190	26	336	929
3.0	180	26	95	267	26	431	1 033
	90	26	95	282	26	564	—
	0	10	105	249	19	478	1 285

Table 5. Total bacterial count per gram of irradiated and nonirradiated cod fillets, stored at 0 °C.

Dose (krad)	Storage time (days)			
	14	22	29	35
180	4 400	114 300	403 000	4 800 000
90	50 000	271 000	3 800 000	62 000 000
50	131 000	3 390 000	11 970 000	41 000 000
25	128 000	11 500 000	43 000 000	38 000 000
0	132 000	9 800 000	52 000 000	31 000 000

Table 6. Drip in ml per kg from irradiated and nonirradiated cod fillets stored at 0 °C.

Dose (krad)	Storage time (days)		
	14	22	29
180	12	16	38
90	23	22	30
50	37	< 2	22
25	35	10	38
0	56	26	9

Table 7. Formation of trimethylamine (TMA), total volatile base (TVB), ammonia and volatile acids (VA) in irradiated and nonirradiated cod fillets. Measured in mg %.

Storage time (days)	Dose (krad)	TMA	TVB	NH_3-N	VA	pH
0	0	< 0.5	12	12	—	7.00
	180	3	16	13	< 2	6.80
	90	< 2	14	12	< 2	
14	50	2	14	12	< 2	6.84
	25	15	28	13	16	
	0	52	65	13	52	7.18
	180	4	17	13	< 2	6.96
	90	7	19	12	2	6.91
22	50	11	24	13	3	6.95
	25	35	69	34	34	7.34
	0	67	82	15	38	7.24
	180	5	19	14	4.5	6.93
	90	5	20	14	2.7	6.86
29	50	64	77	13	7	7.38
	25	59	71	12	16	7.12
	0	60	76	16	22	7.04
	180	12	24	12	1	7.04
	90	6	18	12	1	7.09
35	50	52	66	14	87	7.42
	25	52	67	15	18	7.22
	0	66	82	16	68	7.17

Table 8. Formation of hypoxanthine in irradiated and nonirradiated cod fillets stored at 0 °C.

+++ denotes concentration grather than 2.39 pr. mol/g
++ » » » 1.91 pr. mol/g
+ » » » 1,59 pr. mol/g
— not detectable

Dose (krad)	Storage time (days)		
	14	22	29
180	+	++	+
90	—	—	—
50	—	—	—
25	+++	+++	+++
0	+++	+++	+++

Table 9. Glutamate oxalacetate-transaminase activity in irradiated and nonirradiated cod fillets (μ/g).

Dose (krad)	Storage time (days)		
	14	22	29
180	4.20	2.20	3.38
90	3.48	3.53	4.96
50	3.44	3.58	3.29
25	6.29	7.25	2.20
0	7.94	7.61	1.75

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