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STUDIES ON THE RADIATION PRESERVATION OF FISH I.

The Effect on Certain Vitamins in Fresh Fillets of Cod and Dogfish and in Smoked Fillets of Cod and Herring

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INTRODUCTION

Radiation preservation of food has been studied rather extensively in later years, and increased keeping qualities of several food items have been reported. The wholesomeness of the irradiated food has been given particular attention. The developments up to 1962 have been covered in reports and discussion at an international meeting (Report FAO/WHO/IAEA, Rome 1962).

With the approval by the U.S. Food and Drug Administration of the use of Cobalt 60 gamma irradiation for the preservation of canned bacon (Fed. Reg. 1963a) and the use of gamma radiations for the control of insect infestation in wheat and wheat product (Fed. Reg. 1963b), the practical application of radiation preservation of foods approached realization. Sea foods are considered among the most promising foods for preservation by ionizing radiation. Radiation doses of less than one megarad were shown to destroy the Pseudomonas and similar organisms (LERKE *et. al.* 1961). After irradiation the samples could be stored at temperatures above freezing and below 4° C in order to prevent germination of the spores which survive this radiation dose. Thus significant extention of cold storage life could be obtained and «fresh» seafoods could reach markets further away from the sites of production (GOLDBLITH, 1963; Anonymous, 1963; MAZUROVSKY *et. al.*, 1963).

Studies on the effect of radiation on individual food components, have shown that certain vitamins are among the radiosensitive nutrients (DAY *et. al.*, 1957; RICHARDSON *et. al.*, 1961; FORD *et. al.*, 1962). The extent to which vitamins were lost by irradiation depended upon the nature and physical state of the medium and the amount of radiation energy applied. Thus most vitamins were reported to exhibit different degrees of radio-sensitivity when irradiated in dry form, in pure solutions or in the naturally occuring forms in foods (RICHARDSON *et. al.*, 1958; GRONINGER & TAPPEL, 1957).

ZIPORIN *et. al.* (1957) reported that at radiation doses of 2.79 and 5.58 megarad riboflavin was reduced in turkey flesh but not in beef, ham, bacon, powdered milk, peaches and beets. Niacin was reduced in peaches only whereas thiamine was extensively destroyed in all foods tested except powdered milk. RICHARDSON *et. al.* (1961) reported that vitamin B_6 and vitamin K continued to decrease during storage of irradiated samples of beef, spinach, broccoli and cabbage.

Only a few data are available on the effect of irradiation on the vitamins in fish and other sea foods. GRONINGER *et. al.* (1956) and GRONINGER & TAPPEL (1957) studied the effect of irradiation on thiamine in several species, and found extensive destruction. HATA & ONISHI (1960) studied the effect of radiation on vitamin A in dogfish liver oil, and the influence of vitamin E on the rate of destruction.

The possible future of irradiation for processing sea foods and the scarcity of data on the radiostability of vitamins in this group of foods prompted the present study. This paper deals with the effect of radio-pasteurization (0.3 megarad) and radio-sterilization (3.0 megarad) on the content of certain vitamins in two types of fresh fish fillets, cod (*Gadus morrhua*) and dogfish (*Squalus acanthis*), and in smoked cod and smoked herring (*Clupea harengus*). The effect of storage of the irradiated samples at appropriate temperatures was also investigated.

METHODS

Samples. The cods were collected alive from well boats, and the cod fillet samples thus refer to absolutely fresh fish. The dogfish was obtained as fresh as possible from the wholesale marked. Both species were filleted upon arrival at the laboratory. Fillets from more than ten fishes were combined and homogenized in a rapid meat chopper/mixer. To avoid possible errors as a result of the presence of dark meat with high vitamin concentrations (BRÆKKAN, 1959), only the white meat was used. The ground fillets were filled in aluminium cans (1/4 dingly), which were sealed at a local canning factory. The cans were then frozen and stored at c. $-20°$ C.

Smoked samples of cod and herring were purchased at the fish market. The fish had been filleted by cutting along the back, leaving the backbone attached to one side and both halves joined by the abdomen. Both species had been cold smoked. Care was taken to obtain corresponding samples for irradiation and control. Equivelant section f rom opposite sides of the same fish provided such samples in the present

study. The samples were vacuum-packed in plastic bags, labelled and stored at 2—4° C.

Irradiation. Within twenty four hours from preparation, the samples were shipped by air for irradiation at Wantage Research Laboratory. The canned fillets were shipped in a specially made insulated container with dry ice. The smoked samples were packed in a plain wooden box. Upon arrival to the irradiation centre, the samples were thawed overnight at + 3—5° C. They were then irradiated in the Spent Fuel Rod Assembly in the presence of crushed ice. The dose rate was 1.4 megarad per hour. Two irradiation doses were employed: a pasteurization dose of 0.3 megarad and a sterilization dose of 3.0 megarad. After irradiation the cans were frozen at —20° C, and smoked samples returned to the cold room. The next day the samples were returned to the containers and returned by plane to Bergen. It took six days from the purchase of the products to the return for analysis. The canned fillets were held at c. —20° C and the smoked samples at 2—4° C untill analysis. Irradiated and control samples were treated simultaneously in the same manner.

Storage. To study the effect of storage after irradiation, series of samples were treated as follows: Sterilized samples (3.0 megarads) were stored at room temperature (17—20° C). Pasteurized samples (0.3 megarads) were stored in the cold room at 2—4° C. Non-irradiated control samples of the canned fillets were stored at c. —20° C, whereas the control samples of the smoked products were treated in the same manner as the irradiated samples.

Thiamine was determined microbiologically with *Lactobacillus fermenti* (ATCC 9338) as test organism. The method described by MAGNASR (1957) was employed with a modification in the preparation of the medium to avoid the formation of cloudiness which was found to interfere with the growth of the organism. Calculated on dry matter basis c. 1.0 gm of homogenized sample was extracted by steaming with 25 ml of 0.1 N sulfuric acid for 30 minutes. After cooling the pH was adjusted to 4.5 with 2.5 M sodium acetate and 20 mgm papain + 20 mgm takadiatase were added. The mixture was incubated overnight at 37° C under toluene. The incubate was steamed for 30 minutes, diluted to 100 ml and filtered. An aliquot of the filtrate was adjusted to pH 5.5 with sodium hydroxide and diluted to contain approximately 0.005 μg thiamine per ml. After incubation for 18 hrs. at 37° C, the growth response was measured turbidimetrically at 660 $m\mu$ in a Beckman Model B Spectrophotometer.

Riboflavin was determined micro-biologically by the acidimetric method described in Pharmacopoea Nordica (1960), using *Lactobacillus casei* (ATCC 7469) as test organism. The experimental volume was

reduced to 2.0 ml, and the response measured potentiometrically.

Niacin was determined microbiologically by the acidimetric method described in Pharmacopoea Nordica (1960) using *Lactobacillus plantarum* (ATCC 8014) as test organism. The experimental volume was 2.0 ml, and the response measured potentiometrically.

Vitamin B_{12} was determined microbiologically according to the method of THOMPSON *et. al.* (1950) employing *Lactobacillus leichmannii* (ATCC 4797) as test-organisms. The vitamin was extracted by autoclaving for 15 min. at 120° C with 50 ml M/15 sodium acetate buffer of pH 4.5 + 5 ml 1% potassium cyanide per g sample. The incubation was carried out for 20—22 hours at 37° C in a water bath. The response was measured turbidimetrically.

Vitamin A was determined essentialy as described in U.S. Pharmacopoeia (1960) with purification of the non-saponifiable matter on alumina according to GRIDGEMAN *et. al.* (1948).

α-Tocopherol was determined according to the procedure described by BRÆKKAN *et. al.* (1963).

RESULTS AND DISCUSSION

The results are summarized in tables 1—3. In Table 1 are given the results for the contents of the B-vitamins in fresh, irradiated and stored fish fillets. Table 2 reports the corresponding values for the smoked products. In Table 3 are finally given the data on vitamin A and α-tocopherol in both types of samples. It was found more convenient to discuss each vitamin, than to compare the conditions in each type of samples.

Thiamine was found to be the most radiosensitive of the vitamins studied in the present investigation. From Tables 1 and 2 can be seen that the pasteurization dose of 0.3 megarad caused approx. 40 percent loss, whereas the sterilization dose of 3.0 megarad caused total destruction of thiamine. This was true regardless of the type of sample. None of the samples of smoked herring contained measurable amount of thiamine. This may be explained by total destruction of thiamine by the thiaminase found in herring (MELNICK, 1945).

The non-irradiated samples of fresh cod and dogfish showed a decrease in their thiamine content after 30 days storage in aluminium cans at −20° C, the losses were 47 and 27 percent respectively. The pasteurized sample showed no further loss upon storage for one month at 2—4° C. Thus at the end of the storage period the level of thiamine in the irradiated and control samples was the same. This indicated that either the physical or chemical properties imparted on fish by irradiation

Table 1. The contents of certain B-vitamins in fresh, irradiated and stored fish fillets*.

Sample of:	Thiamin μg/g		Riboflavin μg/g		Niacin μg/g		Vitamin B_{12} μg/g	
	Non-stored	Stored	Non-stored	Stored	Non-stored	Stored	Non-stored	Stored
Fresh cod fillets	0.36	0.19	0.91	0.34	24.0	26.5	—	0.005
Fresh cod fillets 0.3 megarad	0.21	0.18	0.82	0.32	24.1	27.9	—	0.005
Fresh cod fillets 3.0 megarad	0	—	0.60	0.34	19.7	21.2	—	0.005
Fresh dog fish fillets	0.55	0.40	0.94	0.83	53.3	62.9	—	0.012
Fresh dog fish fillets 0.3 megarad	0.37	0.37	0.73	0.67	58.6	65.2	—	0.011
Fresh dog fish fillets 3.0 megarad	0	—	0.89	0.61	50.5	60.2	—	0.011

* Samples irradiated with 0.3 and 3.0 megarad were stored for 30 days at 3—5 and 17—20° C respectively. Non-irradiated samples were stored frozen at — 20° C.

Table 2. The contents of certain B-vitamins in smoked, irradiated and stored cod and herring.*

Sample of	Thiamin μg/g		Riboflavin μg/g		Niacin μg/g		Vitamin B_{12} μg/g	
	Non-stored	Stored	Non stored	Stored	Non-stored	Stored	Non-stored	Stored
Cod — control	1.03	0.35	0.97	0.75	16.6	14.9	0.011	0.014
- — 0.3 megarad	0.64	0.15	0.98	0.68	15.6	13.9	0.012	0.013
- — control	0.67	—	0.96	1.26	15.6	13.3	0.009	0.012
- — 3.0 megarad	0	—	0.84	0.57	17.0	12.2	0.007	0.011
Herring — control	—	—	1.86	1.97	28.1	32.1	0.085	0.111
- — 0.3 megarad	—	—	1.97	2.24	22.2	28.2	0.100	0.112
- — control	—	—	1.80	—	28.0	—	0.085	—
- — 3.0 megard	—	—	2.04	2.03	19.6	29.0	0.084	0.141

* Samples irradiated with 0.3 and 3.0 megarad and their respective controls were stored for 30 days at 3—5 and 17—20° C respectively.

favoured thiamine stability or that irradiation did not equally destroy all forms of thiamine in fish tissues. The latter assumption would indicate that the form of thiamine which is most radiosensitive is the same which is destroyed upon storage.

The relatively high radiosensitivity of thiamine in the present samples of fish agree with the finding reported in the literature. GRONINGER *et. al.* (1956) found that radiation doses of 0.5 to 4.0 megarads destroyed 64 to 91 percent of the thiamine in lake trout. GRONINGER & TAPPEL (1957) found the same doses to cause allmost total destruction of thiamine in salmon, halibut and tuna.

Riboflavin was only slightly reduced in the irradiated fresh fillets, with a maximum loss of 30 percent for the sample of cod fillet irradiated with doses up to 3.0 megarad. In smoked samples the present dose-levels seemed not to cause any loss. After storage, however, the fresh samples showed decreased values for the contents of riboflavin. This storage loss was particularly severe in the homogenized cod fillets, whereas the homogenized dogfish fillets showed moderate losses.

The values for niacin and vitamin B_{12} showed no decrease in any of the samples regardless of irradiation or storage. The vitamin B_{12} values for the non-stored fresh fillets were lost because of analytical errors. The values for cod after storage were in the same range as those reported by BRÆKKAN (1958). Further storage for 3 month showed essentially unchanged values.

Table 3. Vitamins A and E in irradiated dogfish fillets and smoked herring.

Sample of:	Vitamin A I.U. per g	Vitamin E μg per g
Cod fillet, control	0.1	1.0
— 0.3 megarad	«	«
— 3.0 megarad	«	«
Dogfish fillet, control	2.0	10.2
— 0.3 megarad	2.2	10.2
— 3.0 megarad	1.1	9.1
Smoked herring, control	0.43	11.3
— 0.3 megarad	0	12.4
— control	0.65	13.1
— 3.0 megarad	0	11.7

The vitamin A contents of the cod fillets were below the sensitivity of the method, thus an effect of irradiation could not be studied. In the dogfish fillets vitamin A was not affected by 0.3 megarad, but the value had dropped to 1.1 I.U. per g compared with 2.0 I.U. in the control.

The smoked samples of herring showed that vitamin A was totally destroyed already at an irradiation dose of 0.3 megarad.

For α-tocopherol the same lack of sensitivity as reported for vitamin A prevented studies in the cod samples. In dogfish fillets and the smoked herring the values after irradiation, even at the dose level of 3.0 megarad, were essentially unchanged.

The results for vitamin A and α-tocopherol deserve some further comments. Based on studies of vitamin A and E in purified systems (KRAYBILL, 1962), these vitamins have been considered among the most radio sensitive. The present results for vitamin A showed that this assumption can not be generally applied. The fairly high stability for vitamin A in the samples of fresh dogfish, show that the problem of vitamin A stability upon irradiation of different food stuffs should be carefully studied for each product. The results for α-tocopherol show a surprisingly high stability for this vitamin. α-Tocopherol as an antioxidant is considered to protect vitamin A in different products. This relation failed to be observed in the present study, thus the two vitamins may be assumed stored at different sites in the tissue of fish. This assumption needs, however, to be verified in further studies.

SUMMARY

The effect of radiopasteurization (0.3 Mrad) and radiosterilization (3.0 Mrad) on certain vitamins in canned fresh fillets of cod and dogfish and in vacuum packed smoked cod and herring, were studied. The effect of post-irradiation storage of the samples at appropriate temperatures on the vitamin contents was determined. Some differences between vitamin sensitivity to irradiation and storage among the various fish samples studied were noted. Thiamine was found to be most sensitive to both irradiation and storage of non irradiated samples. Irradiated samples lost little or no thiamine upon storage. Riboflavin was more sensitive to storage than to irradiation. Niacin and vitamin B_{12} were not appreciably affected by irradiation or storage. Vitamin A appeared to be more radiosensitive in smoked than in fresh fish. Thus a sample of dogfish retained 50 percent of the vitamin A content after irradiation with 3.0 Mrad. Vitamin E was not affected by the radiation doses employed in the fish samples investigated.

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