PART II

SCIENTIFIC AND TECHNICAL

STUDIES ON RADIATION PASTEURISATION OF MEDIUM FATTY FISH

I. CONTROL OF RADIATION INDUCED OXIDATIVE CHANGES IN WHITE POMFRET (*STROMATEUS CINEREUS*) BY VACUUM PACKAGING

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Marked differences were observed in proximate biochemical compositions of the skin and muscle of white pomfret. The skin showed comparatively higher content of extractable lipids and was more susceptible to radiation-induced oxidative changes like development of rancid odours and yellow discolouration than the muscle. Irradiation of skin samples under vacuum suppressed these changes.

INTRODUCTION

Fatty fish generally do not lend themselves to irradiation treatment owing to the development of rancidity independently or concurrently with discolouration (Coleby and Shewan, 1965). Combination procedures integrating irradiation with vacuum packaging have been developed for controlling rancidity in radiation pasteurised petrale sole (Spinelli *et al*, 1965) and trout (Hansen and Jorgensen, 1967) as well as in radiation sterilized halibut steaks (Sinnhuber *et al*, 1966). Learson *et al* (1969) observed that the development of rancidity in radiation sterilized cod, halibut, ocean perch and flounder fillets could be suppressed by vacuum packaging; browning of these fish during storage at ambient temperatures, however, could not be arrested. Studies of Yu *et al* (1969) indicated that leaching of the fish with water was effective in controlling brown discolouration of radiation sterilized cod patties during storage at ambient temperature. These investigations suggest that the effiicacy of vacuum packaging or any other processing treatment may vary with composition of the fish.

White pomfret (*Stromateus cinereus*) constitutes an important variety of medium fatty fish available on the west coast of India. Two major limitations in preservation of this fish either by freezing (Pawar and Magar, 1966; Kamasastri *et al* 1967) or by irradiation (Kumta and Sreenivasan, 1966) arise from the development of rancidity and onset of typical yellow discolouration on skin surface of the fish. Glazing treatments have been employed with limited success for

the control of this discolouration (Pawar and Magar, 1966). This suggests that the skin of the fish may be more susceptible to such deteriorative changes. It was therefore of interest to investigate the proximate composition of skin and muscle of white pomfret and susceptibility of these tissues to irradiation treatment, in terms of sensory alteration and chemical parameters of oxidative changes.

The present paper also reports on the efficacy of vacuum packaging in controlling oxidative rancidity and yellow discolouration in white pomfret skin subjected to irradiation and subsequent storage at 0 - 2°C.

Experimental

Preparation of fish samples:

Fresh white pomfrets available from local market were brought to laboratory in ice. The fish were washed clean with water before and after evisceration and filleted. Skin and muscle were then separated from a lot of five pomfrets, cut into fine pieces, mixed thoroughly to get uniform sample and stored in ice until used. The experiments relating to the assessment of chemical composition and evaluation of organoleptic and chemical changes were repeated thrice with the three different lots of the fish. Each assay was carried out on duplicate samples. The average values on the three lots of the fish have been reported in the text.

Chemical composition:

The skin and muscle samples were analysed separately for their proximate composition.

Moisture:

Moisture content was determined by heating the fish samples (10 g each) for 18 hr in hot air oven at 105°C.

Lipids:

Lipids were isolated from the skin or muscle of fish by the method of Bligh and Dyer (1959). Fractionation of lipids into triglycerides, free fatty acids and phospholipids was effected as per Crider *et al* (1964). Triglycerides, free fatty acids and lipid phosphorus were determined using the methods of Van Handel (1952), Duncombe (1963) and Fiske and Subba Row (1925) respectively.

Proteins:

Sarcoplasmic and fibrillar protein fractions were extracted from the fish muscle by the procedure of King (1966). Sarcoplasmic protein fraction was extracted by mincing 5g fish muscle with 95 ml 0.01 M phosphate buffer (pH 7.5) in Sorvall Omnimixer cup. Slurry so obtained was then spun in Sorvall RC_2 centrifuge at 7000 rpm to separate protein solution from solid residue. The residue was then repeatedly washed with the same extractant and then treated with 5% NaCl in 0.01 M phosphate buffer (pH 7.5) for isolating fibrillar protein fraction. Water soluble and salt soluble protein fractions were also isolated from the skin tissue of the fish in a similar way. Protein nitrogen was estimated by nesselerisation (Umbreit *et al* 1957) in all the samples.

Packaging of the skin samples:

Skin samples (5 g each) were sealed under vacuum in polycell pouches after sepeated evacuations and flushings with nitrogen gas. The pouches were subrequently packed under vacuum in metal cans to maintain strict anaerobic conditions during irradiation and storage. Samples packed in presence of air in a similar manner served as control.

Irradiation:

The fish samples (fillets, skin and muscle) were irradiated at various dose levels ranging from 0.1 to 1.5 M rad at a dose rate of 0.3 Mrad/hr. in Food Package Irradiator IR_{11} (1,00,000 curies,

Co^{60} source). During irradiation, temperature was maintained at 0-2°C. Organoleptic and chemical evaluations of the irradiated samples were carried out immediately after irradiation along with unirradiated controls.

Organoleptic evaluations:

A 10 point reference scale, based on colour, odour and appearance (Miyauchi *et al* 1964) was used for organoleptic assessment of the fillets, skin and muscle samples of the fish.

Chemical evaluation of oxidative changes:

The fish samples were examined for oxidative changes in terms of peroxide, carbonyl, 2-thiobarbituric acid (TBA) and free fatty acid (FFA) values as per methods of Wagner *et al* (1947), Henick *et al* (1954), Witte *et al* (1970) and Duncombe (1963) respectively.

Assessment of yellowing:

Quantitation of yellowing produced on skin of white pomfret was rendered difficult as the pigment could not be extracted with common organic solvents like chloroform, ether, methanol, ethanol, pyridine, tetrahydrofuran etc or inorganic solvents like mild (N) alkalis or acids. 6 N solution of NaOH, however, could extract the yellow pigment but the coloured alkali solution did not show any absorption maximum peak in the visible region. Solvent system consisting of acetone-HCl-methanol (100 : 4 : 0.1) used by Koizumi *et al* (1968) for extracting green pigment of tuna was adopted for extraction of the yellow pigment from the white pomfret skin. The pigment extracted by this way gave two peaks at 360 and 470 $m\mu$ respectively.

Extraction of the yellow pigment was effected by swirling at 0-2°C, 2 g pigmented skin tissue with 10 ml mixture of acetone-HCl-methanol in Sorvall Omnimixer cup for 3 min and filtering the mixture through glass wool. Residue was further extracted twice with the same extractant and filtered. The filtrates were then pooled and made up to a known volume. OD of the extracted pigment was measured at 470 $m\mu$.

Results

Chemical composition:

Assessment of major chemical constituents of skin and muscle of white pomfret revealed marked differences in composition of the two tissue systems. Table I incor-

TABLE I CHEMICAL COMPOSITION OF SKIN AND MUSCLE OF WHITE POMFRET

Tissue* component	Moisture %	Total extractable proteins %	Sarcoplasmic proteins %	Fibrillar proteins %	Total extractable lipids %	Phospholipids %	Neutral lipids %	Free fatty acids %
Skin	63.0	1.8	0.60	1.20	16.20	2.50	11.00	0.40
Muscle	75.0	15.8	3.65	12.15	4.25	0.95	2.35	0.08
Whole fish	74.6	15.1	3.50	11.60	4.85	1.00	2.78	0.10

* Skin and muscle components constitute respectively 5 and 95% of beheaded, eviscerated and deboned white pomfret.

porates data on distribution of proteins, lipids and moisture in skin and muscle of white pomfret. Skin tissue of the fish contained as high as 16% lipids against 4.25% in the muscle. Concentrations of triglycerides and free fatty acids in skin were four times more than those in the muscle, while phospholipid content of the former was twice as much as that of the latter. Muscle tissue mainly contributed towards extractable protein fractions.

Sensory changes:

Table 2 describes radiation induced changes in sensory attributes of colour and odours of skin and muscle tissues and also of the fillets of white pomfret. Exposure to 0.5 Mrad imparted rancid odours to the fillets, formation of which accentuated with the increase in radiation dose upto 1.5 Mrad. A slight but distinct yellow discolouration could also be seen on skin irradiated at 1.5 Mrad.

Skin and muscle samples, when irradiated separately, exhibited considerable differences in their colour and odour changes. Development of rancid odours (0.5 Mrad) and onset of yellow discolouration (1.5 Mrad) were conspicuous with the skin tissue while the muscle samples were characterized by burnt type of odours. These alterations in sensory attributes of colour and odour led to consistent decrease in organoleptic score (O. S.) of the irradiated skin and muscle samples.

Oxidative changes:

Radiation-induced oxidative changes in skin and muscle of white pomfret and their relationship with organoleptic score are shown in Fig. 1. It is apparent from these results that the oxidative changes, measured in terms of peroxide, carbonyl, TBA and FFA values, showed a consistent rise with the increase in radiation dose. At a given dose of radia-

TABLE II ORGANOLEPTIC EVALUATION OF IRRADIATED FILLET, SKIN AND MUSCLE OF WHITE POMFRET

Radiation dose: Mrad	Fish sample	O. S.	Quality characteristics
Unirradiated	Fillet	10	Colour and odour of fresh fish
	Skin	10	,,
	Muscle	10	,,
0.1 & 0.25	Fillet	10	,,
	Skin	10	,,
	Muscle	10	,,
0.5 & 0.75	Fillet	7	Development of rancid odours
	Skin	7	Colour remains same
	Muscle	8	Slight burnt type of odours
1.0	Fillet	6	Strong rancid odours, colour unchanged.
	Skin	6	,,
	Muscle	7	Appreciable burnt type of odours
1.5	Fillet	5	Very strong rancid odours and yellowing on the skin
	Skin	5	Very strong rancid odours and yellow discolouration
	Muscle	6-7	Strong burnt type of odours.

tion, the levels of these indices were higher for the skin than for the muscle, thus indicating that the former was more readily susceptible to radiation-induced oxidation than the latter. The oxidative changes showed an inverse relationshipwith organoleptic score (O. S.) in both the tissue systems.

Influence of vacuum packaging:

Results incorporated in Table 3 and Fig. 2 describe the effects of irradiation under vacuum or in presence of air on organoleptic changes and chemical indices of oxidation respectively. Skin samples packed under aerobic condition showed a sharp rise in peroxide, carbonyl, TBA and FFA values with the increase in radiation dose and were also prone to the development of rancid odours and yellow discolouration as described earlier. The vacuum packed skin samples, however, did not show appreciable rise (over the initial levels) in the indices of oxidative changes as a function of radiation dose and remained free from development of rancid odours and yellow discolouration. These samples retained their original high O. S. Figures 3 and 4 show the data on TBA values and yellow discolouration respectively of the skin samples irradiated at 0.3 or 0.5 Mrad under vacuum or in presence of air and subsequently stored at 0-2°C. Skin samples irradiated under aerobic conditions exhibited dose dependent rise

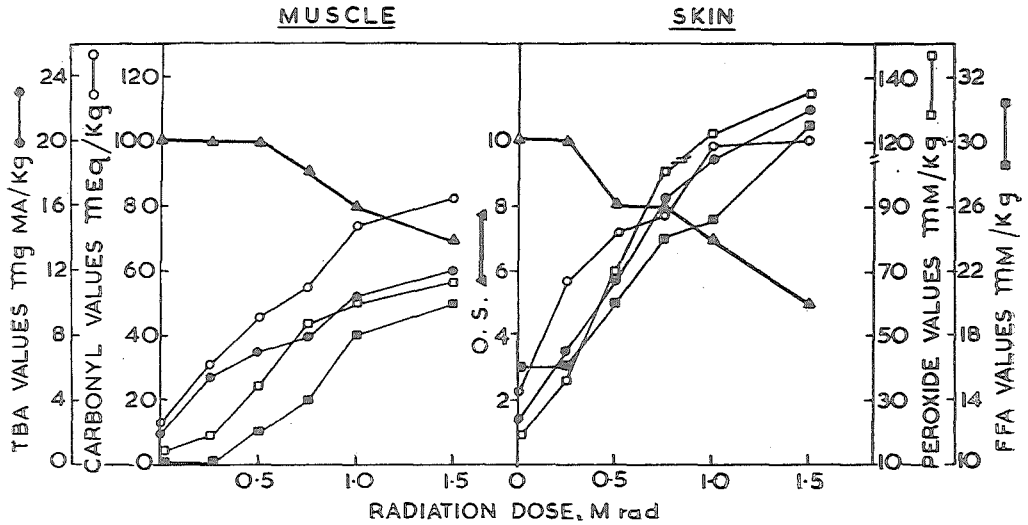

Fig. 1: Differences in radiation sensitivity of skin of white pomfret. and muscle Skin and muscle tissues isolated from white pomfret were irradiated at different dose levels in the range of 0.25 to 1.5 Mrad and subsequently analysed for oxidative charges in terms of peroxide, carbonyl, TBA and FFA values.

Skin tissue of the fish was more susceptible to radiation-induced oxidative changes than the muscle.

TABLE III ORGANOLEPTIC SCORE OF WHITE POMFRET SKIN IRRADIATED UNDER VACUUM AND AEROBIC CONDITIONS

Radiation dose: Mrad	Aerobic packing	Vacuum packing
Unirradiated	10	10
0.25	10	10
0.50	7	10
0.75	7	9
1.00	6	9
1.50	5	8

in TBA values during earlier period of storage, followed by a gradual decline. Yellow discolouration which occurred in these samples by 10th (0.5 Mrad) and 20th (0.3 Mrad) day, increased consistently with further storage period. The samples irradiated under vacuum did not show appreciable increase in TBA values throughout the storage period

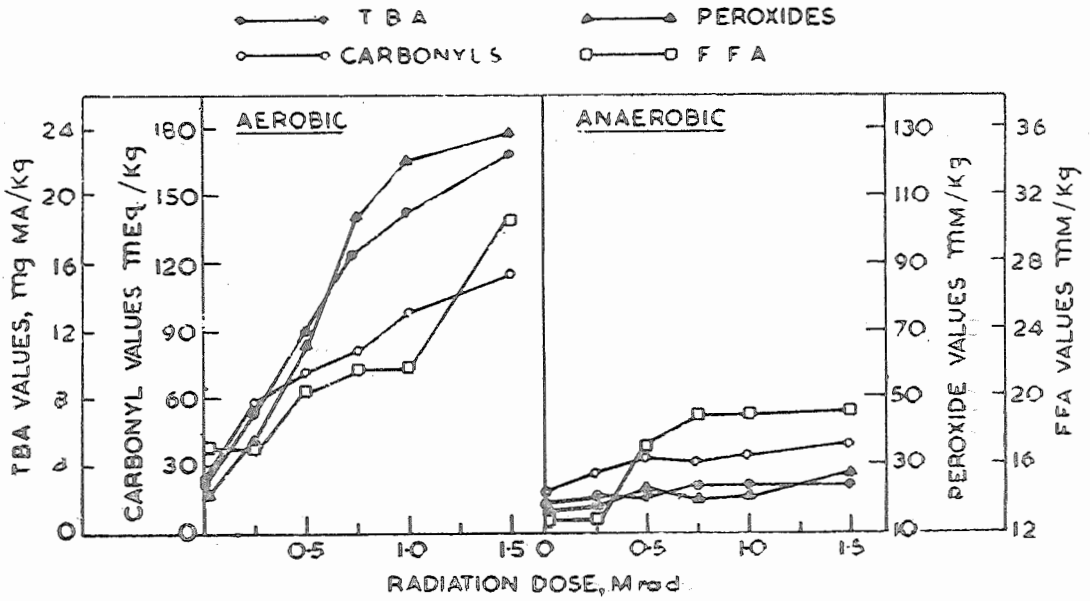

Fig. 2: Suppression of radiation-induced oxidative changes in vacuum packed white pomfret skin.

Skin tissue samples of white pomfret were packed under aerobic conditions or vacuum and irradiated at doses ranging from 0 25 to 1.5 Mrad Unirradiated samples served as control. All the samples were assessed for oxidative changes in terms of peroxide, carbonyl, TBA and FFA values.

Irradiation under vacuum minimised radiation-induced oxidative changes in the skin samples.

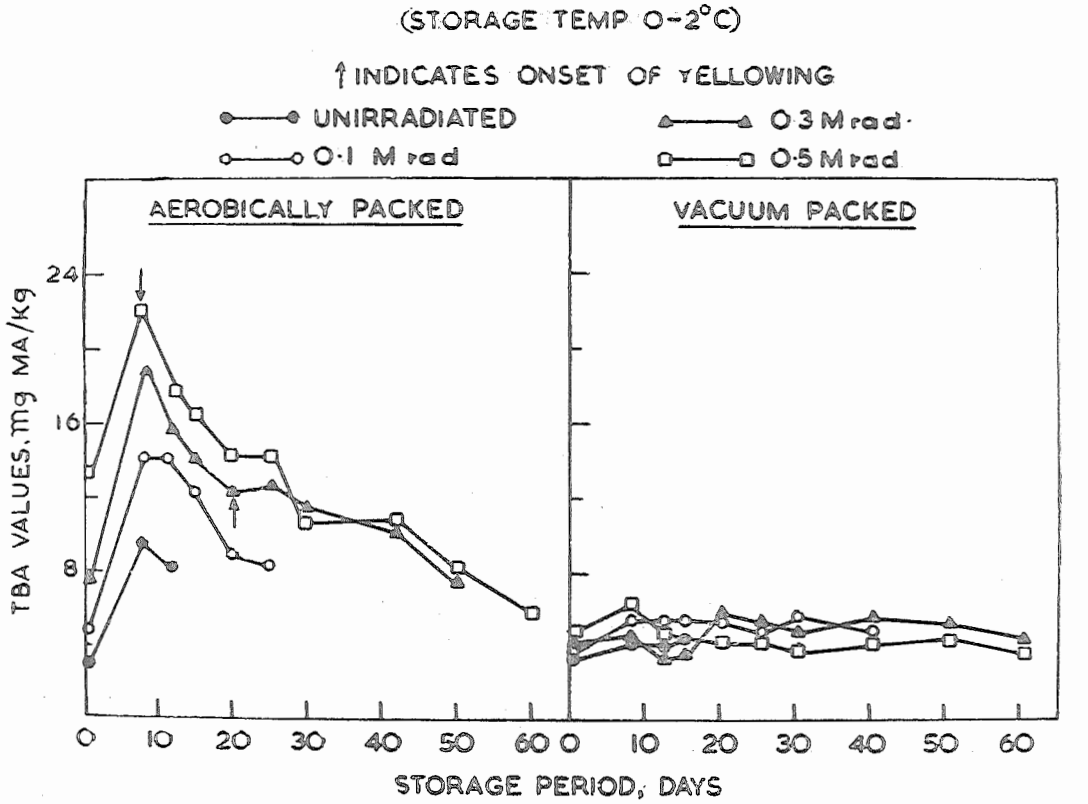

Fig 3: Oxidative changes in white pomfret skin irradiated under vacuum or aerobic condition.

White pomfret skin samples packed under vacuum or aerobic condition were exposed to radiatin doses of 0.1, 0.3 and 0.5 Mrad and subsequently stored at 0-2°C. At intervals, during storage, the fish samples were analysed for oxidative changes in terms of TBA values.

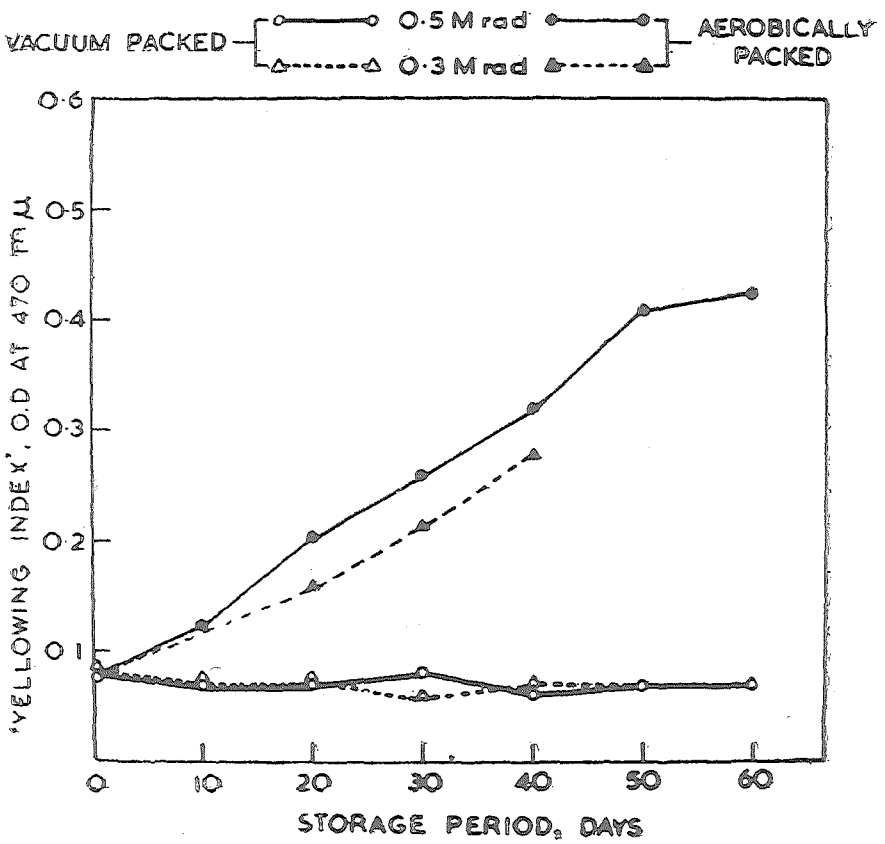

Fig. 4. Influence of vacuum packing on 'yellowing' of irradiated white pomfret skin.
White pomfret skin samples, packed under vacuum or aerobic condition, were irradiated at 0 3 and 0.5 Mrad and subsequently stored at 0 – 2°C. During storage, the samples were assessed for 'yellowing' by extracting the yellow pigment and measuring its absorption at 470 mμ.

of 60 days. Yellow discolouration too was totally absent in these samples.

Discussion

Differences in chemical composition and radiation sensitivity of skin and muscle of white pomfret:

Present studies reveal marked differences in proximate composition of skin and muscle of white pomfret (Table 1). This assumes importance in view of the different responses of the two tissues towards irradiation treatment. Compositional variations in various tissue systems of fish have been well recognised. Studies of Karochinoido (1967) have pointed out that the skin of mackerels contains 94.4% carotenoids while the muscle contains only 1.8%. However, in salmon, the distribution of carotenoids in skin and muscle was 0.23 and 93.2% respectively. Dark and white muscle tissues of Baltic herring and cod (Bosund and Ganrot, 1969) have been reported to vary greatly in triglycerides, free fatty acids and cholesterol ester fractions. Various compositional differences within the fish have been reviewed by Stansby (1963).

Differences in responses of skin and muscle of white pomfret to irradiation were distinguished clearly by the types of sensory changes occurring in the tissues (Table 2) and by the higher susceptibility of the skin to radiation-induced oxidative changes than the muscle (Fig. 1).

Such differential behaviour of tissue systems towards processing treatments was pointed out by Bosund *et al*, (1969). They observed that the lipid hydrolysis in dark and white muscles of Baltic herring progressed at different rates. Brown *et al* (1966) reported that the presence of appreciable amounts of heme compounds in dark muscle of fish species render the tissue more susceptible to autoxidative changes than the white muscle.

The precise nature of precursors of undesirable odours and flavours encountered in irradiated fishery and meat products is not well understood. However, it has been postulated that rancid odours arise from the lipid components (Chipault, 1962) while degradation of amino acids and soluble proteins accounted for development of burnt feather type of odours (Hedin *et al*, 1961). Further, oxidative changes in lipids also lead to yellow or brown discolouration in certain fish species subjected to irradiation (Matsuyama, 1966). Our results show that due to high concentration of lipids (16%) in the skin of white pomfret, this fish is susceptible to radiation-induced oxidative change as well as for the development of rancid odours and yellow discolouration. Also, the similarity in overall pattern of sensory changes exhibited by the fish fillets and skin tissue (Table 2) indicated that the latter plays an important role in determining the radiation dose response of the fillets.

Influence of vacuum packaging:

When irradiated under vacuum, the skin samples did not develop rancid odours or yellow discolouration (Table 3), concomitant with suppression of the indices of oxidative changes (Fig. 2). It has been shown that the irradiation of beef, pork and fish oils under vacuum effectively decreased the levels of peroxides, carbonyls and the associated rancidity changes (Chipault, 1962).

Development of yellow to brown discolouration of the processed fishery products may be caused by i) reactivity of ribose resulting from nucleotide degradation with amino acids (Tarr, 1966), or ii) interaction of carbonyls resulting from oxidative degradation of lipids with amino nitrogenous compounds (Reynolds, 1965), or iii) a combined effect of the two agencies. In the present studies, since irradiation under vacuum arrested the development of yellow discolouration in the skin tissue of white pomfret, it appeared that the radiation-induced oxidative changes were mainly responsible for causing this discolouration.

The above observations prompted us to investigate the nature of precursor of carbonyl compounds in the skin of white pomfret and to assess the reactivity of such compounds with skin tissues of the fish in relation to the development of yellow discolouration. These investigations are reported in the following paper.

SUMMARY

White pomfret was examined for its response to irradiation treatment. Skin and muscle tissues of the fish analysed separately showed greater concentrations of triglycerides, free fatty acids and phospholipids in former than in latter. Assessment of the fish tissue samples in terms of peroxide, carbonyl, free fatty acids (FFA) and 2-thiobarbituric acid (TBA) values, indicated that the skin was more susceptible to radiation-induced oxidative changes than the muscle. Development of rancid odours (0.5 Mrad and higher doses of radiation) and yellow discolouration (1.5 Mrad) were noted in the irradiated skin as against burnt type of odours in muscle. Prepacking under vacuum effectively suppressed the oxidative changes and associated undesirable

alterations in colour and odours of the skin tissue on irradiation as well as during storage at 0-2°C.

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