Carbonyls from Some Commercially Important Fishes and Shell Fishes of Tropical Waters

K. AMMU, P. G. VISWANATHAN NAIR and K. DEVADASAN
Central Institute of Fisheries Technology, Cochin - 682 029

Results of a preliminary study of the hexane extractable carbonyls from three fish and one shell fish are reported. Volatile carbonyls that can be isolated from oil sardine by distillation at different temperatures in air or nitrogen atmosphere were also studied. Carbonyls were converted to their 2:4 dinitro phenyl hydrozones. These were fractionated by column chromatography and the different fractions were analysed by capillary gas chromatography. The data show wide variations in the content of different carbonyls in different fishes. Carbonyls in distillates from muscle water homogenates of oil sardine showed some differences from the carbonyls extracted with hexane from the same fish. More data is necessary to correlate the carbonyls with the characteristic flavour of each fish.

Flavour chemistry is the most complicated and perhaps one of the most fast growing branch of food chemistry and carbonyls form one of the most important groups of compounds studied in this context. Carbonyls play a vital role in determining the flavour of fresh and processed food products. A study of the carbonyls in fish is all the more important in view of the highly unsaturated nature of fish lipids. The carbonyls arising out of the easy oxidation of the highly unsaturated fish lipids influence the nutritional and functional properties of fish proteins also. The effect of various processing conditions on carbonyls in fish and how these carbonyls influence the flavour and nutritional quality of processed fishery products, is thus a problem demanding serious attention of fish processing technologists. But, unfortunately, little has been reported on these aspects in the case of tropical fishes. The picture is much better in the case of temperate fishes (Ota 1958, Mangan, 1959; Hughes, 1961; Yu *et al.* 1961; Wyatt & Day, 1963; Mendelsohn & Steinburg, 1962). In recent years, the advent of more sophisticated analytical techniques has resulted in renewed interest in this problem. A survey of the relevant literature reveals wide variations in the carbonyls found in different fish species. Josephson *et al.* (1983, 1984) studied the carbonyls of several freshwater and marine fishes. The carbonyls of fresh cod (Wong *et al.* 1967; Megill & Hardy, 1977a) and frozen stored cod (McGill, 1977b, McGill *et al.* 1974, Ross & Love, 1979) were also studied in several recent investigations. Clam (Mendelsohn & Brook, 1968) and oyster meat (Ronald & Thomson, 1964) carbonyls have also been studied in detail. Maijboom & Stroink (1972) and Ke *et al.* (1975) studied the carbonyls in relation to the oxidation of fish oils.

The basic method employed in all these studies is to convert the carbonyls into their 2:4 dinitrophenyl hydrazone (DNPH) derivatives and to separate them into major classes by column chromatography (Schwartz *et al.* 1962) or thin layer chromatography (Ruffini, 1965; Dhont & Dijkman, 1969). The conversion to DNPH derivatives can be done before or after extraction of the carbonyls (Merrit *et al.* 1969, Schwartz *et al.* 1963, Keay & McGill, 1968). After separation into the major groups, individual carbonyls can be identified and estimated by gas chromatography of the DNPH derivatives or the regenerated carbonyls (Uralets *et al.* 1980; Linko *et al.* 1978), Halverson, 1971; Gadbois *et al.* 1967 and Hoshika & Takata, 1976). High pressure liquid chromatography has also been employed by some workers (Selim, 1977) for this identification.

The growing fish processing industry of India will surely need, in the coming years, a clearer picture of the carbonyl profiles of our major food fishes, from which different processed products will be manufactured on a commercial scale. This preliminary report on the carbonyls of some typical freshwater, brackishwater and marine fishes and shell fishes of India, forms a part of an exhaustive study on the carbonyls profile of our major food fishes. Oil sardine *(Sardinella longiceps)*, pearl spot *(Etroplus suratensis)*, rohu *(Labeo rohita)* and prawns *(Penaeus indicus)* were selected.

Oil sardine is quantitywise the largest single fishery of India. As such, for ensuring its full utilization, catches of oil sardine will have to be processed into different products. As most of these processes will involve heat processing, the changes in the carbonyl profile of this fatty fish during heat treatment at different temperatures in air and in an inert atmosphere merits more detailed study to understand the flavour changes in this fish during these processing treatments. So in the case of oil sardine, the carbonyls released during distillation at various temperatures in air and under nitrogen were studied in detail separately. The monocarbonyl fraction was investigated more thoroughly on account of their known flavour potential.

Materials and Methods

Oil sardine, pearl spot and prawns, in prime condition, were procured from local fish landing centres. Rohu used in the study was brought fresh from a nearby fish farm. The analyses of the samples were done in all cases with minimum possible delay to avoid changes due to prolonged storage.

Carbonyls from the samples were extracted with carbonyl free hexane by the method of Thomas *et al.* (1971). Repeated treatments with DNPH and distillations were found necessary to make the hexane (LR/BDH) completely carbonyl free. The carbonyls in the hexane extract were then converted to their 2:4 DNPH derivatives by the method of Lawrence (1965). The samples were then applied on a Celite 545/Mangnesia column and the lipids removed by hexane elution. The DNPH derivatives of the different major classes of carbonyls (Methyl ketones saturated aldehydes, 2-enals and 2:4 dienals) were then progressively eluted from the same column by hexane containing increasing concentrations of chloroform (15%, 30%, 60%) and finally by pure chloroform. The dicarbonyls were retained in the column. The individual components of methyl ketone and saturated aldehyde fractions were then identified and estimated by capillary gas chromatography using fused Silica WCOT column (SE, 3012.5 M) on a Varian 3700 model gas chromatograph, with s VISTA 402 data system. Operating conditions were column, initial temperature 150°C, programmed 5°C/min,, final temperature 230°C and carrier gas, nitrogen at 2.5 ml/min. The reference hydrazones were prepared from high purity sample of the respective carbonyls as per the method of Vogel (1978). Only those components for which reference standards were available had been identified and reported.

For comparing the release of carbonyls from muscle of oil sardine under various conditions, muscle from fresh fish was minced and homogenised with water and the carbonyls distilled off according to the method of Matsuto *et al.* (1967), at selected temperatures (30°C, 65°C, and 85°C). The distilled carbonyls were trapped in 2:4 dinitrophenyl hudrazine and the reaction was allowed to proceed over night at room temperature. The 2:4-DNPH derivatives formed were then extracted with chloroform. The monocarbonyls and discarbonyls were separated by column chromatography on a Magnesia/Celite 545 column. Dicarbonyls were retained in the column and were estimated by difference. The content of total carbonyls and monocarbonyls were estimated by measuring the absorbance at 358 nm. The concentration was calculated using E= 22,500 (Schwartz *et al.* 1963). The monocarbonyl fraction was further separated into the major classes according to Schwartz *et al.* (1962). The absorbance of each class was read at the respective wave lengths and values converted to u moles/100 g muscle using appropriate E values.

Results and Discussion

The individual aldehydes and ketones identified and estimated in the hexane extracts

from oil sardine, rohu, pearlspot and prawns *(P.indicus)* are presented in Tables 1 and 2 respectively.

45% of the total aldehydes present in oil sardine was valeraldehyde. Heptaldehyde was also one of the major constituents in sardines. Octaldehyde, pelargon-aldehyde, acetaldehyde etc. were the other aldehydes seen in sardine muscle. Isovaleraldehyde and propionaldehyde were also present in small amounts. The lean freshwater fish rohu had significantly higher amounts of aldehydes in its muscle. Acetaldehyde and valeraldehyde were the major constituents in this case. Propionaldehyde also was present in high amounts. As different from oil sardine, rohu had formaldehyde, isobutyraldehyde and hexaldehyde in detectable quantities in its muscle. Isovalerdehyde contents were comparable in the two fishes. The brackishwater lean fish, pearlspot, was somewhat poor in total aldehydes. Octaldehyde was the major aldehyde (73%) in this case with a small amount of valeraldehyde and traces of propionaldehyde and acetaldehyde. Prawn muscle was noted for its content of lower aldehydes as different from fishes. Acetaldehyde accounted for most of the aldehydes in prawns (62%). Propionaldehyde and formaldehyde were also present in notable amounts. Butyral-

Table 1. *Major aldehydes in the hexane extracts from muscle of oil sardine, rohu, pearl spot and prawns (P. indicus) (ug/100g muscle)*

Aldehyde	Oil sardine	Rohu	Pearl spot	Prawns *(P. indicus)*
Formaldehyde	—	137.8	—	179.5
Acetaldehyde	214.5	2179.8	26.0	1237.9
Propionaldehyde	72.0	665.1	80.5	403.0
Isobutyraldehyde	—	158.0	—	28.2
Butyraldehyde	—	—	—	70.1
Isovalaraldehyde	77.0	86.3	—	—
Valeraldehyde	997.3	1998.8	229.5	—
Hexaldehyde	—	37.6	—	62.9
Heptaldehyde	537.1	—	—	—
Octaldehyde	173.2	—	1132.5	—
Pelargonaldehyde	154.1	—	—	—

— not detected

Table 2. *Major ketones in the hexane extracts from muscle of oil sardine, rohu, pearl spot and prawns (P. indicus)* (ug/100g muscle)

Ketone	Sardine	Rohu	Pearl spot	Prawns *(P. indicus)*
Acetone	59.2	519.8	32.0	195.8
Methyl ethyl ketone	44.7	763.0	44.5	56.6
2 Pentanone	—	83.5	13.9	—
2 Hexanone	80.8	909.3	58.5	32.2
4 Heptanone	—	—	—	32.6
3-Heptanone	1428.1	236.8	—	85.0
2-Heptanone	1286.1	1443.9	1286.5	31.5
Octanone	248.9	2399.3	981.5	171.7
Heptyl methyl ketone	596.9	—	—	41.5

— not detected

dehyde, isobutyraldehyde and hexaldehyde were present in traces.

Ketonic components were more in all fishes compared to aldehydes. Here again, rohu was the richest among the fishes studied in the content of ketones, 2-heptanone, 3-heptanone and 2-octoanone were in general the major ketones in all the fishes. Sardine had a high content of heptyl methyl ketone also. Rohu had high amounts of acetone, methyl ethyl ketone and 2-hexanone in its muscle. Pearlspot was generally poor in ketonic components also. Prawn muscle had all ketones except 2-pentanone, though quantitywise generally the ketonic components were less important in prawns.

From the data it is seen that the carbonyl profiles of these tropical fishes have many similarities to those of temperate water fishes like herring (Hughes, 1961) salted cod (Yurkowshi & Bordeleava, 1965), halibut (Matsuto *et al.* 1967), white fishes (Josephson *et al* 1983) and also clams (Gadbois *et al.* 1967). According to Caporaso (1977) methyl ketones with an odd number of carbon atoms are the most important carbonyls in foods. In the case of fish, in addition to acetone, 2-pentanone, 3-heptanone and 2-heptanone, 2-octanone was also present in high amounts. The hexane extracts from all the fishes studied were noted for the absence of 2-enals and 2:4-dienals.

Table 3 presents data on the total carbonyls, monocarbonyls and dicarbonyls distilled at three different temperatures, namely, 30°C (room temperature), 65°C and 85°C from oil sardine muscle in air and under nitrogen. At 30°C, the total carbonyl content did not show much variation irrespective of the atmosphere (air or nitrogen) under which the carbonyls from the muscle-water homogenate were distilled. But at 65°C distillation in air produced more than double the quantity of carbonyls produced under nitrogen. Further rise in temperature to 85°C, resulted only in a marginal increase of carbonyls when distillation was done in a nitrogen atmosphere. Increase in carbonyls at higher temperature in the presence of air has been reported in beef and pork muscle also (Hornstein & Crowe 1960). A notable point was that distillation in air encouraged formation of more dicarbonyls.

Table 4 gives the proportions of the different major classes of monocarbonyls distilled from sardine muscle at two different temperature, when distillation is done in air and

Table 3. *Total carbonyls, monocarbonyls and dicarbonyls distilled from the muscle of oil sardines at temperatures in air and under nitrogen* (μ mol/100g muscle)

Distillation condition	Total carbonyls	Mono carbonyls	Dicarbonyls (estimated by difference)
30°C/N2	1.38	0.804	0.576
30°C/Air	1.35	0.640	0.710
65°C/N2	2.19	1.250	0.940
65°C/Air	5.86	3.040	2.820
85°C/N2	3.30	2.040	1.260

Table 4. *Proportion of different classes of monocarbonyls in the distillate from muscle of oil sardine at different temperatures in air and under nitrogen (% total monocarbonyls)*

Distillation conditions	Methyl ketones	Saturated aldehydes	2-enals	2:4-dienals
30°C/N2	26	74	—	—
30°C/Air	12	88	—	—
67°C/N2	5.95	88.45	5.60	—
67°C/Air	6.90	75.70	17.40	—

under nitrogen. At all remperatures, saturated aldehydes accounted for the major part of monocarbonyls in sardine. 2 enals were detected only when distillation was done at higher temperatures. Again distillation on air tends to increase the percentage of 2-enals compared to distillation under nitrogen. 2:4 dienals were absent in distillates also.

Table 5 gives the proportion of individual monocarbonyls identified from the distillate from oil sardine muscle (distillation in nitrogen atmosphere), at two temperatures, namely 45°C and 100°C. Acetaldehyde and propionaldehyde were the most important carbonyls at both temperatures. Butyraldehyde and acetone were also present in appreciable amounts. There was a substantial

Table 5. *Individual carbonyls identified from the distillate from oil sardine muscle at 45°C and 100°C under nitrogen (% total monocarbonyls)*

Carbonyl	Distilled at 45°C under nitrogen	Distilled at 100°C under nitrogen
Formaldehyde	6.90	0.62
Acetaldehyde	31.10	23.80
Acetone	8.10	2.30
Propionaldehyde	41.40	67.30
Isobutyraldehyde	0.70	0.38
Methyl ethyl ketone	2.80	0.38
Butyraldehyde	3.50	3.00
Valeraldehyde	3.50	0.38
Isovaleraldehyde	—	1.10
Dipropyl ketone	0.60	0.76
Hexaldehyde	1.00	—
2–Heptanone	0.60	—

increase in the release of monocarbonyls during distillation as the temperature of distillation was increased ie from 0.6 μg/g tissue at 45°C to 5.32 μg/g at 100°C (9 times) where as increase in the proportions of acetaldehyde and butyraldehyde were four and five times respectively. Increase in the distillation temperature does not appear to have any appreciable effect on the formation or release of valeraldehyde and formaldehyde, as there was an apparent decrease in their proportions.

A point of interest that was noted was that the carbonyls profile showed significant variations when the two different methods were used namely, (1) distillation of carbonyls from muscle-water homogenates and (ii) extraction of carbonyls from the muscle using hexane. This was observed in the case of halibut by Matsuto *et al.* (1967) also. Sanderson *et al.* (1966) indicated that the carbonyls involved in meat flavour are primarily lipid soluble. That may be the reason for the difference in carbonyls obtained from water homogenate and hexane extract. This, no doubt, cautions against drawing conclusion from the data collected, on the contribution of individual carbonyls to the flavour of each fish. Much more data on all these aspects must be collected to permit any conclusion in this regard. The preliminary data presented here do not permit any definite conclusions on the role of these carbonyls in determining the characteristic flavour of each fish. It is however possible that, the relative amounts of these compounds ultimately determine the characteristic flavour of each fish.

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