Effect of Preprocessing Storage Conditions on the Carbonyls of Oil Sardine

K. AMMU and K. DEVADASAN
Central Institute of Fisheries Technology, Cochin - 682 029

Changes in the total as well as major individual carbonyls of oil sardine muscle during storage at room temperature for 24 h and in crushed ice upto 6 days are reported. Carbonyls extracted with hexane were converted to their 2:4 dinitrophenyl hydrazone (DNPH) derivatives and were separated into major classes by column chromatography on celite/magnesia. Individual carbonyls were then identified by capillary gas chromatography of these derivatives. Though absolute values for carbonyls exhibited wide variations depending upon the degree of freshness, the pattern of changes in the carbonyls during storage of fish under different conditions gave an insight into the influence of carbonyls on flavour. The significance of the findings is discussed.

Fish oils are well known for their high content of highly unsaturated fatty acids which confer special nutritional and pharmacological properties on them. However, susceptibility of these unsaturated fatty acids to easy oxidation leading to the formation of various carbonyls which affect the flavour of fish is a relatively less studied field. Even minor charges in processing and preservation conditions can cause variations in the nature and content of cabonyls. Moreover, carbonyls can also originate from amino compounds, sugars, lactic acid etc. Irrespective of the source, carbonyls are known to influence the flavour of fish and fishery products, and a systematic study of these compounds is therefore important in product development. Ammu *et al.* (1986) reported the nature and content of carbonyls isolated from several species of fish and shellfish under different conditions of extraction/isolation. Subsequent studies showed that even for a given species of fish, the carbonyl profile can show wide variations depending upon the location of catch, degree of freshness and storage conditions. Therefore, it was felt meaningful to study the pattern of changes in the carbonyl profile for a given fish species during different processing conditions, rather than correlating it with the species concerned. The abundant fatty fish of the southwest coast, oil sardine, was taken for this study. This paper reports results of a study on the changes in the nature and content of different carbonyls in oil sardine muscle during storage at room temperature (28–30°C) and in crushed ice.

Materials and Methods

Oil sardine *(Sardinella longiceps)* was procured in fresh condition from local fish landing centre. Initial sampling was done immediately. A portion of the fish was kept in crushed ice in an insulated box and samples were drawn for analysis after 2, 4 and 6 days. Another portion was kept at room temperature for 24 h.

10 g portions of minced muscle of fresh sample were ground with sufficient anhydrous sodium sulphate and repeatedly extracted with carbonyl free hexane to estimate total carbonyls. This method of extraction was found to give better extraction than the method used in the previous studies (Ammu *et al.*, 1986). Conversion of the extracted carbonyls to the dinitriophenyl hydrazone (DNPH) derivatives, separation of the total carbonyls into major classes by column chromatography on celite 545/magnesia and identification of individual carbonyls by capillary gas chromatography were done by the methods reported earlier (Ammu *et al.*, 1986). For estimating the fraction with absorption maximum at 380–385 nm the E value was taken as 22,500. Reference dinitrophenyl hydrazones of available common carbonyls were

prepared using carbonyl free reagents and solvents as per the method of Vogel (1978). Standard DNPH derivatives of saturated aldehyde (C_{10}, C_{12}, C_{13}, C_{16} and C_{18}), unsaturated aldehydes (2-pentenal, 2-hexenal, 2-heptenal and 2-octenal), saturated methyl ketones (C_{10}, C_{13}, C_{15}, C_{17} and C_{19}), saturated ethyl ketones (diethyl, ethyl propyl, ethyl hexyl and ethyl octyl) and unsaturated ketones (methyl heptenone, and3 decene 2 one) were obtained from the Dairy Research Laboratory of the Division of Food Research of the CSIRO, Australia. However, due to lack of authentic reference standards of required purity, some peaks in the chromatograms, could not be identified.

Results and Discussion

Changes in total extractable carbonyls and major groups namely, saturated monocarbonyls, 2-enals, dicarbonyls and the fraction having absorption maximum at 380–385 nm (presumably unsaturated ketones and 2:6 dienals) in the muscle of oil sardine during storage at room temperature (28–30°C) for 24 h and in crushed ice upto 6 days are presented in Table 1. Changes

Table 1. *Changes in the total and major classes of carbonyls in oil sardine during storage (μ moles/100 g)*

Sample	Total carbonyl	Saturated aldehydes and ketones	2 enals —	Compound with absorption at 380–385 nm	Dicarbonyl by difference
Fresh	36.0	21.0	—	—	15.0
Stored at room temperature for 24 h	62.0	42.0	—	—	20.0
Stored in ice					
2 days	58.0	30.0	—	—	28.0
4 days	93.0	34.0	4.0	4.6	50.4
6 days	48.0	23.0	—	—	25.0

Table 2. *Changes in the content of individual carbonyls during storage at room temperature and in ice (% increase or decrease from the values for fresh fish)*

Carbonyl compound	Stored at room temperature for 24 h	Stored in ice 2 days	4 days	6 days
Formaldehyde	+ 142.0	+ 307.0	+ 593.0	+ 257.0
Acetaldehyde	+ 397.0	+ 1.35	— 100.0	+ 43.0
Propionaldehyde	+ 323.0	+ 260.0	+ 485.0	+ 383.0
Isobutyraldehyde	— 100.0	+ 160.0	+ 213.0	— 100.0
Isovaleraldehyde	+ 28.0	+ 111.0	+ 308.0	+ 285.0
Valeraldehyde	+ 3379.0	+ 297.0	+ 429.0	+ 167.0
Octaldehyde	+ 59.0	—	—	—
Pelargonaldehyde	— 100.0	— 100.0	— 100.0	— 100.0
Acetone	— 100.00	+ 49.00	+ 4.2	— 100.0
Methyl ethyl ketone	— 37.2	— 100.0	— 100.0	— 100.0
2 pentanone	+ 1269.0	+ 463.0	— 100.0	— 100.0
3 heptanone	— 95.0	+ 81.0	+ 368.0	+ 458.0
Octanone	— 100.0	— 85.0	— 100.0	— 100.0
Hepthyl methyl ketone	+ 79.7	+ 290.0	— 100.0	— 100.0

+ = % increase; — = decrease

in the individual identified carbonyls during storage of the fish at room temperature and in ice are given in Table 2. As the absolute value show fluctuations, percentage increase/decrease in the content of individual carbonyls only is given.

In the fresh fish, 58% of the total carbonyls were saturated monocarbonyls which are known to influence flavour. Dicarbonyls account for the balance 42%. 2-enals, unsaturated ketones, 2:4 dienals, 2:6 dienals etc. were not detected.

Storage of fresh fish at room temperature (28–30°C) for 24 h resulted in 72% increase in total carbonyls. 63% of the total carbonyls in this sample was saturated monocarbonyls, balance being dicarbonyls. 2-enals or 2:6 dienals were not detected in this case (Table 1). Valeraldehyde showed a tremendous increase. 2-pentanone, 2-heptanone acetaldehyde, propionaldehyde, formaldehyde etc registered steady increase whereas pelargonaldehyde,acetone, octanone, 3-heptanone, isobutyraldehyde etc disappeared after 24 h at room temperature. Eventhough valeraldehyde showed a very high increase, it formed only 50.5% of the total aldehydes. Ammu *et al.* (1986) reported that even when it formed 45% of the total aldehyde it did not impart any off flavour to the fish sample. But it is likely that 2-heptanone along with the amines formed may be responsible for the spoiled odour observed in sardines kept at room temperature for long (Table 2). The impact of the high increase in valeraldehyde on the flavour is not clear.

Storage of oil sardine in crushed ice initially lead to an increase in the extractable carbonyl content of the muscle upto 4 days (Table 1). During this period there was an increase of 158% but after this, it registered a decrease. After 6 days in ice the value was only 33% more compared to the initial. Saturated monocarbonyls also showed an initial increase, but after 6 days in ice was almost the same as in the fresh fish. During iced storage, dicarbonyls also registered an initial increase followed by a decrease as in the case of total carbonyls (Table 1). The fresh fish after storage in ice for 4 days showed the presence of small amounts of 2-enals and 2:6 dienals also, but during continued storage these compounds again disappeared. Formaldehyde, propionaldehyde, isovaleraldehyde, valeraldehyde, 2-pentanone, heptylmethyl ketone and 3-heptanone were the major carbonyls that showed significant increase in the initial stages. Of these, all carbonyls except 3-heptanone, recorded a decrease along with the decrease in total carbonyls. Pelargonaldehyde, acetone, isobutyraldehyde, methyl ethyl ketone, 2-pentanone, 2-heptanone, Octanone and heptyl methyl ketone disappeared after 6 days' storage in ice (Table 2). In the case of ice stored fish, free amino acids, ammonia, amines etc. will be formed in significant quantities which may react with the carbonyls leading to their nonavailability. Isobytyraldehyde, pelargonaldehyde, acetone, methyl ethyl ketone, octanone, heptyl methyl ketone etc. appear to be reactive as they tend to disappear easily from the fresh samples during most of the methods of processing and preservation. This disappearance in most cases coincides with a general loss of flavour. Pelargonaldehyde can be easily formed from all unsaturated fatty acids, but the reaction obviously goes on leading to the disappearance of this aldehyde and resulting in the formation of other carbonyls. Loury *et al.* (1965) demonstrated how n-decanal formed by oxidation of oleic acid can be degraded stepwise to methanol. Propionaldehyde found in significant quantities in ice stored sample may be coming from the lactic acid that accumulates in fish muscle during postmortem storage. Lipase and lipoxygenases present in fish muscle also contribute to the formation of various carbonyls during iced storage. Lipoxygenase catalyses the peroxidation of methylene interrupted polyunsaturated fatty acids with cis double bonds which are present in good amounts in fish oils.

Hughes (1963), Ota (1958) and Mendelsohn and Steinberg (1962) reported that during chill storage there is a general increase in the concentration of carbonyls in fish. The present observations also support this view. But after the initial increase, carbonyl content registers a steady decrease presumably due to loss of carbonyls in the ice melt water or interaction with other reactive muscle constituents. Howgate (1977) observed that fish held in ice for 3–5 days shows a

marked decrease in fish aroma just before the onset of putrid and spoiled odour. This may be due, at least partly, to the loss of carbonyls at this stage as observed in this study. An initial increase in carbonyls followed by a decrease when spoilage sets in was observed by other workers also (Diemer, 1964). But Tokunaga *et al.* (1981) observed that while fish held in ice showed a decrease in its aldehyde content, fish held in water containing ice did not show such phenomemon throughout the storage period.

The authors are thankful to Shri M.R. Nair, Director, Central Institute of Fisheries Technology, Cochin for his encouragement and permission to publish this paper. Thanks are due to the Dairy Research Laboratory of the CSIRO., Australia for providing the DNPH derivatives of several carbonyls for use as reference standards.

References

Ammu, K., Viswanathan Nair, P. G. & Devadasan, K. (1986) *Fish. Technol.* 23, 134

Diemer, W. (1964) *FAO Symposium on the Significance of Fundamental Research in the Utilisation of Fish.* Husum, Germany. Paper No. WP/4/9

Howgate, P. (1977) *Proceedings of a Joint Symposium,* University of Aston, Jan. 6-7, p. 41

Hughes, R. B. (1963) *J. Sci. Food Agric.* 14, 893

Loury, H., Lecharlic, G. & Fenney, M. (1965) *Rev. Franc. Corpe. Gra.* 12, p. 253

Mendelsohn, J. M. & Steinberg, M. A. (1962) *Food Technol.* 16, 113

Ota, F. (1958) *Bull. Jap. Soc. Sci. Fish.* 24, 334

Tokunaga, T., Iida, N., Nakamura, K. & Ota, Y. (1981) *Bull. Tokai Reg. Fish. Res. Lab.* 104, 67

Vogel, A. I. (1978) *Text Book of Practical Organic Chemistry* 4th edn., p. 1112, Longman, London and New York