ON THE CAROTENOID PIGMENTS IN INDIAN PRAWNS

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Changes occurring in carotenoid pigments of prawns during different types of processing and storage has been a matter calling for a scientific solution for which a knowledge of their fundamental nature is essential. A detailed account of the methods employed in isolating the individual pigments and the results achieved in their identification are presented in this paper.

INTRODUCTION

All species of prawns, when subjected to heat or any other treatment resulting in denaturation of protein, will develop a characteristic colour on the surface, the intensity and composition of which will vary depending on the species, maturity, ground from where they are caught etc. However, this colour is not evident in the fresh state as the pigment is reported to be present in a state of loose combination with the surface protein (Karrer and Jucker, 1950; Goodwin, 1954). The denaturing effect breaks the protein-pigment link thereby liberating the free pigment when the colour becomes apparent (Goldblith *et al.*, 1963). The characteristic red colour in prawns and that met with other fishes like salmon, rainbow trout etc. has roused considerable interest among scientists working in related fields.

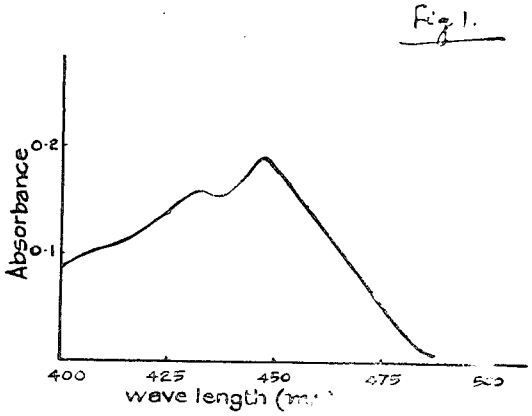
It is a common experience that dry prawns on storage lose the natural carotenoid colour with the appearance of some other undesirable colour, thereby the consumer acceptability of the commodity getting reduced. This, to a greater extent, is controlled by such storage conditions as the type of packaging material employed, exposure to and protection from light, and air, vacuum etc. in the package (Anon, 1964). Lusk, Karrel and Goldblith (1964) have reported on the astacene pigment loss occurring in freeze dried shrimp and salmon as a function of storage variables like packing in nitrogen, air, etc. Similar problems of loss of colour has been observed with respect to canned prawns as well. Though not visibly evident to such extents similar deteriorative changes occur in frozen prawns also. Faulkner and Watts (1955) have measured the loss of astacene pig-

ment in shrimp during frozen storage. Colour, being a principal factor ranking along with texture and flavour in determining the consumer preference of the products, has an important bearing on the marketability. Thus pigments in prawns present a problem of importance from scientific and commercial points of view.

Karrer and Jucker (1950) have reported the presence of astaxanthin in prawns besides a number of other carotenoids in smaller amounts, astaxanthin being the principal pigment. On boiling shrimp the pigment tetraxanthin and/or astaxanthin is liberated and provides characteristic colour to the blanched meat (Wood, 1951). Rousseau (1960) has studied the effect of storage variables on the pigment content of shrimp waste meal used as a dietary supplement to hatchery-raised trout to give the fish the natural pink colour, as the shrimp waste acted as a source for dietary intake of natural astaxanthin.

It is, thus, an established fact that astaxanthin is one of the major components contributing to the natural colouration of prawns. In addition, other carotenoids in varying amounts, melanoid pigments etc. may take a part in the colouration. The nature, amount and the form of existence of these pigments will be significant parameters in a research programme on this aspect for providing valid information on the above aspects and to study their changes during different types of processing like drying, canning, freezing etc. and subsequent storage.

Most of the reported accounts on



Absorption spectrum of epiphasic pigment

the nature of the different pigment fractions in prawns dealt with extraction of total pigments with solvents like acetone or chloroform and measuring the optical density at 470-475 mμ (Rousseau Jr, 1960; Lusk *et al.*, 1964; Stewart, James. E, 1970). These methods suffer from the defect that pigments other than astaxanthin absorbing in the above region also will contribute to the total absorption and be measured as astaxanthin. Other methods have been reported for the extraction of pigments and identification by the method of column chromatography (Goodwin and Srisukh, 1949; Tsukuda *et al.*, 1966; Nakamura *et al.*, 1967) in connection with identification of pigments of locusts, fish roe and some red fishes etc. The method adopted by Goodwin and Srisukh (1949) with the necessary modifications at all stages to suit the purpose was employed in this study and the present communication is aimed at reporting the procedural details of the methods employed and a qualitative account of the major components of carotenoid pigments in prawns used for study.

Material And Methods

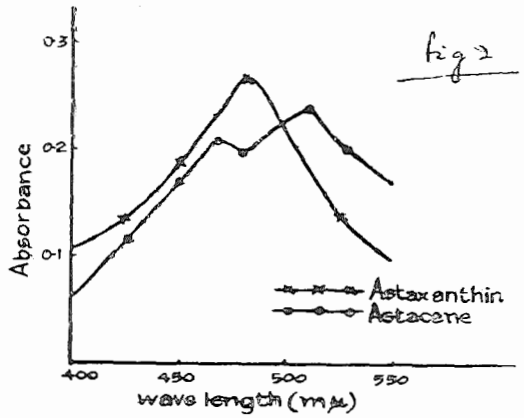
Prawns belonging to the species *Parapenaeopsis stylifera* was used mostly in this study. Prawns caught by the trawlers operated by the Institute in the sea off Cochin from a depth of 15-20 fathoms and belonging to the size group 14 to 16 cm. in length were used. As soon as caught prawns were held in ice and brought to the laboratory, headed, peeled and deveined and the meat alone used for study. Extraction of the pigment was carried out at room temperature by keeping the peeled meat immersed in acetone and drawing off the extractant at intervals and repeating the process until no colour was noted in the acetone extract. The extracts were combined and treated with light petroleum ether (bp 40-60°C) in a separating funnel and shaken gently to avoid emulsification. The pigments were absorbed in the upper petroleum ether layer. The lower acetone layer was drawn off and extracted with another portion of petroleum ether in another separating funnel. This process was repeated until all the pigments were transferred to the petroleum ether. The combined petroleum ether layers was washed free of acetone with small quantities of distilled water, each time drawing off the lower water-acetone layer and repeating the process. Washing, particularly towards the final stages, has to be carried out extremely carefully as, other-wise, emulsification with difficulty to break will occur. The washed petroleum ether extract was dried over anhydrous sodium sulphate and concentrated under vacuum to a volume sufficient to handle for further work.

The concentrated solution of the pigment was saponified with excess 1 N. solution of potassium hydroxide in methyl alcohol at room temperature with occasional shaking followed by partition of the saponified mass between light petroleum ether (40-60°C) and 90% (v/v) aqueous methyl alcohol. The epiphase was coloured yellow while the major fraction of the pigment was hypophasic and coloured red. The hypophase was repeatedly extracted with petroleum ether, combined the petroleum ether extracts and washed with 90% (v/v) methyl alcohol to remove any hypophasic pigment that might have got into the petroleum ether and combined the extracts with the original hypophase.

The epiphase was washed repeatedly with distilled water until free from alkali, dried over anydrous sodium sulphate, concentrated under vacuum and was chromatographed on activated alumina from light petroleum ether (40-60°C).

The combined hypophasic fraction was diluted to 3 times its volume with distilled water and the pigment was extracted with light petroleum ether. The extract was washed free of alkali and alcohol, dried over anhydrous sodium sulphate and was chromatographed on deactivated alumina (deactivated by washing in methanol and subsequently drying at room temperature (Gocodwin and Srisukh, 1949).

For identification of pigments the absorption spectra of the individual fractions were determined using a Beckmann DU spectrophotometer and compared the absorption maxima with those of known pigments as also by other characteristics of the individual pigments.



Absorption spectra of hypophasic pigments

Results And Discussion

Chromatography of the concentrated pigment extracted without subjecting to saponification showed that the red pigment was adsorbed at the top of the column and a yellow pigment below that when applied from petroleum ether. Elution of the pigments with petroleum ether containing progressively increasing concentrations of acetone showed that practically all of the yellow pigment could be eluted with 2% acetone in petroleum ether and majority of the red pigment could be eluted with 10% acetone in petroleum ether which would support that these pigments are, perhaps, present in the ester form. If the pigments are in the free form, their adsorption on the column must be stable enough to prevent any easy elution by these solvents (Tsukuda and Amano, 1966). There occurred a great deal of tailing of the adsorbed pigments on elution resulting in superimposition and mixing of different bands causing difficulty in isolating the separate fractions, which again will support the above assumption. It is, therefore, in order to liberate the pigments from their esterified form that process of saponification was resorted to. Hot saponification is faster, but is likely to bring about major configurational changes, as the pigments are susceptible to major changes even during normal storage. Therefore, cold saponification with possibility of minimum of configurational changes was resorted to.

The epiphasic yellow pigment, when chromatographed on activated alumina from petroleum ether gave one fraction which on further spectrophotometric analysis showed an absorption maximum of 447 mμ in petroleum ether solution, (Fig. 1) characteristic of lutein (Hirao, Kikuchi and Hama, 1969). An unidentified fraction also has been encountered in certain cases.

The hypophasic fraction, which accounted for the bulk of the pigments was adsorbed at the top of the column and elution with petroleum ether and acetone could not be effective in separation of the pigments. However, a majority of the pigments could be eluted with a 2% (v/v) solution of glacial acetic acid in ethyl alcohol leaving behind a dark zone of pigment still at the top which could be eluted with a 2.5% (w/v) solution of potassium hydroxide in ethyl alcohol, in conformity with the observation of Goodwin and Srisukh (1949). The fraction eluted with glacial acetic acid in ethyl alcohol is astaxanthin, as this according to Kuhn and Sorensen (1938), is a characteristic property of astaxanthin. The glacial acetic acid solution of astaxanthin was diluted with an equal volume of distilled water and the pigment extracted with ether, was further identified by its

absorption maxima in carbon disulfide which gave a peak similar to that of crystalline astaxanthin at the region 478 mμ (Fig. 2).

The fraction eluted with alcoholic potassium hydroxide is astacene as this is a characteristic property of astacene (Kuhn and Sorensen, 1938). Goodwin and Srisukh during their investigation on the carotenoids of locusts and Tsukuda and Amano (1964) in their study of the carotenoids of red fishes have reported the presence of astacene and inferred that this might have been formed possibly by oxidation of astaxanthin during saponification process as this pigment does not occur in nature (Kuhn, Steve and Sorensen, 1939). The presence of astacene observed in prawns, therefore, might be due to similar reasons explained by them. The absorption spectrum of astacene is given in Fig. 2.

Presence of very small amounts of other pigment(s) have been occasionally noted, but their concentrations were, in no case, sufficient to make a proper identification possible.

References

Anon. 1964. *Fish Technology News letter*; **5**, 3 : 6.

Faulkner, M. B. and B. M. Watts. 1955. *Fd. Technol.*, **9** : 632.

Goodwin, T. W. and S. Srisukh. 1949. *Biochem. J.*, **45** : 263.

Goodwin, T. W. 1954. *Carotenoids, Their Comparative Biochemistry;* Chemical Publishing Co; New York.

Goldblith, S. A., M. Karrel and G. Lusk. 1963. *Fd. Technol*; **17** : 139.

Hirao, S., R. Kikkuchi and T. Hama. 1969. *Bull. Jap. Soc. Sci. Fish*; **35**, 2 : 187.

Kuhn, R., J. Steve and N. A. Sorensen. 1939. *Ber. dtsch. chem. Ges;* **72** : 1688 (not consulted in original).

Khun, R. and N. A. Soresen. 1938. *Z. angew. Chem. Ges*; **51** : 465. (not consulted in original).

Karrer, P. and E. Jucker. 1950. *Carotenoids.*, Elsevier Publishing Co; NewYork.

Lusk. G., M. Karrel and S. A. Goldblith. 1964. *Fd. Technol.*, **18**, 5 : 157.

Nakamura Masayoshi and Fukunni Tom. 1967. *Scientific Reports of the Hokkadio Fisheries Experimental Station.*

Rousseau, J. E. Jr. 1960. *Comm. Fish. Rev*; **22** : 4.

Stewart, James E. 1970. *New series circular.* No. 36, Fisheries Research Bord of Canada, Halifax Laboratory, Halifax.

Tsukuda, Nobuo and Amano, Keishi. 1966. *Bull. Jap. Soc. Sci. Fish*; **32**, 4 : 1.

Wood, T. W. 1951. *Carotenoids in Fish*; Cambridge University Press.