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**Research Article**

# Characterization of Carotenoid Extracts Obtained from Mogi Velvet Shrimp, *Metapenaeopsis mogiensis* (Rathbun, 1902) of Mandapam Coast, Tamil Nadu, India

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## Abstract

Shrimps are produced in large quantities and in many processing industries crustacean wastes are one of the important sources of natural carotenoids. The present study was carried out to assess the total carotenoid composition in the mogi velvet shrimp, *Metapenaeopsis mogiensis* collected from Mandapam coast, Tamil Nadu. The sample was assessed using organic solvents in their different body components (Head, meat and shell waste). The yield was significantly higher in the head waste followed by meat in both male and female. Carotenoid content was higher in head waste of male (64.77 µg/g) and the lowest was observed in the meat of both male and female. The waste constituted the highest carotenoid content (98.18 µg/g and 61.65 µg/g) in male and female respectively. FT-IR study on the carotenoid characterization showed the major peaks C-H, O-H, $CH_2$, C-O amino group and aromatic C-H and C=C bonds which are the characteristic bands of the major pigments, astaxanthin, canthaxanthin and astaxanthin mono and diesters.


**Keywords:** Shrimp, processed waste, Total carotenoids, FT-IR

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## Introduction

Indian marine foods are of great importance for their excellent protein, low fat and tremendous amount of the major source of carotenoids. But on the other side, the processing of the marine foods has made the environment dumped with the discards in the form of solid shell wastes. It has been estimated that waste generation from processing of Indian shrimps ranges 48 – 56 % leading to 1, 25, 000 - 1, 50, 000 tons per year [1]. But these are enriched with minerals, proteins, carotenoids etc. The arthropod shells (exoskeletons) of marine crustaceans including shrimps and crabs are the easily accessible sources of the naturally abundant xanthophyll, carotenoids. The global annual production of crustacean shell waste harvested is estimated as 1.44 million metric tons [2]. Shrimp is one of the marine lives with high economic value, generally exported in frozen form and the processing activities produce approximately 60 - 70 % of discarded shrimp waste (head and tail). This solid waste is a great environmental issue.

Colour is one of the major factors determining the acceptability as well as the marketability of seafood. During quality evaluation of seafood, any change in natural colouration is viewed negatively by consumers, thereby reducing the marketability of the product. Carotenoids and carotenoproteins are responsible for natural pigmentation in aquatic organisms. Each animal species has its own carotenoid requirements and each tissue appears to have specificity in the assimilation of carotenoids because some are deposited unchanged, while some others are converted before being deposited. Crustaceans are incapable of *de novo* synthesis of carotenoids[3, 4], but they have the ability to transform the pigments of their diet to endogenous forms [5]. Depending on the source of their origin, crustacean carotenoids are of two types; esterified and non-esterified. The esterified forms are generally present in all organs and tissues, but in most crustaceans, protein complexes contribute to a wide variety of colourations.

Carotenoids are tetra-terpenoid pigments of either aliphatic or acyclic structure composed of isoprenic residues exhibiting diverse functions in plant and animal cells. These pigments in micro-organisms and algae perform a protective function with respect to photosensitizing effect of oxygen on cells. They are biofunctional molecules. The carotenoids and their relative proportions occurring in different organs and tissues fluctuate according to the species. The nature and concentration of pigments may also vary according to the geographic distribution of animals. The carotenoids from food pass through the gut wall into the body fluids and are distributed to other tissues and organs. Major factors that affect the pigmentation pattern in crustaceans include ecological conditions, food and physiological status of the animal. Astaxanthin is a red-pigment carotenoid occurring naturally in a wide variety of living organisms and classified as a xanthophyll. It has a chemical structure similar to that of the familiar carotenoid β-carotene. It is commonly found in crustaceans (e.g. shrimps, crawfish, crabs and lobster) and produced by microalgae. It is a naturally occurring carotenoid pigment with excellent antioxidant effect, documented to protect against free radicals and promote numerous health functions. Various dosage forms have applications in nutraceutical, cosmeceuticals, food and feed industries.

Large quantities of the byproducts are being wasted as processing wastes resulting in the loss of valuable bio-components and also polluting the environment. The carotenoid content of Indian shrimps is found to be varying from 35-153 µg/ g depending on the species [1]. The cheapest raw materials for the carotenoid recovery cannot be equalized with the synthetic feeds available in the market. Thus the present study on the effective utilization of the shrimp waste from the marine velvet shrimp, *Metapenaeopsis mogiensis* collected from the Mandapam coast.

## Experimental

### *Sample Collection*

Shrimps were collected from the coast of Mandapam (9° 16' N; 79° 7' E) (Southeast coast) during the month of November, 2015. They were transported to the laboratory in ice-boxes and were taxonomically identified according to the characters outlined by Perez Farfante and Kensley [6] and morphometric measurements were taken.

### *Processing and Yield (%) calculation of the different components*

Shrimps were processed by removing the head and the body shell (carapace), and yield (%) of meat, head and carapace was determined. They were kept under storage at -20° C until use. The material was thawed in running water before use and homogenized in a laboratory mixer. The processed samples were selected to study the total carotenoid content in the head and body shell waste of both male and female.

### *Determination of Total Carotenoid Content (µg/ g)*

Different body components of shrimp from processing of shrimp in each batch of 6 g each in duplicates were homogenized in a waring blender. The samples selected were the head waste, carapace and meat (edible tissue) of shrimp (15- 20 g) in triplicates was homogenized in a laboratory mixer respectively. Carotenoids were extracted from homogenized samples and the total carotenoid content was determined [7] as explained by Sachindra *et al*., [1]. The homogenized samples were extracted with 10 ml of acetone in order to assess astaxanthin recovery. It was repeated and filtered till the acetone extract becomes colourless. The solvent extracts were pooled together and they were phase separated with an equal quantity of hexane using separating funnel. The resulting carotenoids concentrate was taken up in hexane and made up to 100 ml and the absorbance of the appropriately diluted extract was measured at 468 nm using a spectrophotometer (Spectronic 21). The amount of the total carotenoids was calculated as astaxanthin (Simpson and Haard, 1985) using the formula:

$$\text{Carotenoid content } (\mu\text{g astaxanthin/g sample}) = A_{468nm} * V_{extract} * \text{Dilution factor}/0.2 * W_{sample}$$

where, A = Absorbance, V = Volume of extract, 0.2 = $A_{468}$ of 1 µg/ ml of standard astaxanthin and W = Weight of sample.

***Fourier Transform-Infra Red (FT-IR) Spectral Analysis for Total Carotenoid Content***

FT-IR spectroscopy of total carotenoids extracted from the waste (head and shell waste) and tissue of *M. mogiensis* shell was relied on an AVATAR 330 Spectrometer. Sample (10µg) was mixed with 100µg of dried Potassium Bromide (KBr) and compressed to prepare a salt discs (10mm diameter) for reading the spectrum.

***Statistical Analysis***

Total carotenoid content was carried out in triplicates and the data were analyzed for significant ($P < 0.05$) differences by ANOVA (ANalysis Of VAriance) and mean separation was accomplished by Duncan's Multiple Range Test (DMRT) using STATISTICA software [8].

## Results Discussions

***Taxonomic Classification***

The genera classification proposed by Perez Farfante and Kensley [6] has been adopted for classification of genus Metapenaeopsis. The penaeid shrimp selected was *Metapenaeopsis mogiensis* [9]. The length and weight of the selected species constituted between 8.3 – 10 cm and 3 – 10 g respectively.

***Morphometric Measurements***

Sex identification was made through stereo-microscopic inspection of the first and second pairs of pleopods and the species obtained were equal females and males. Eight Morphometric measurements were made on each specimen species [Total length (TL), carapace length (CL), carapace height (CH), first segment width (FSW), first segment length (FSL), sixth abdominal segment length (SISL)] and tabulated (**Table 1**).

**Table 1** Morphometry of Metapenaeopsis mogiensis.

| S. No | Morphometric Measurements | Male | Female |
|---|---|---|---|
| **1.** | Carapace length (cm) | 1.1 - 2.5 | 0.6 - 3.2 |
| **2.** | Carapace width (cm) | 0.1 - 1.1 | 0.5 - 1.3 |
| **3.** | First segment length (cm) | 0.4 - 0.7 | 0.3 - 1.3 |
| **4.** | First segment width (cm) | 0.6 - 1.1 | 0.6 - 1.2 |
| **5.** | Sixth segment length (cm) | 0.6 - 0.9 | 0.6 - 1.2 |
| **6.** | Sixth segment width (cm) | 0.4 - 0.9 | 0.6 - 0.9 |
| **7.** | Total length (cm) | 5.1 - 7.5 | 5.1 - 8.0 |
| **8.** | Total weight (g) | 3.54 - 27.04 | 11.9 - 34.42 |

***Yield of different Body Components (%)***

The processed shrimp (having 10 g weight each) was initially determined for the yield (%) of the meat, head and carapace between the sexes respectively. From **Table 2**, it is evidenced that the meat yield was 39.3 and 31.5 % in both male and female. In both sexes the head waste was 42 % followed by 15 % and 17 % of shell waste respectively. The yield of waste (57.45 and 59.57 %) has shown similar in both sexes when compared to meat yield (39.39 and 31.59 %).

**Table 2** Yield (%) of body components of shrimp.

| Body Components | Yield (%) *Metapenaeopsis Mogiensis* | |
|---|---|---|
| | **Male** | **Female** |
| **Meat** | 39.39 | 31.59 |
| **Head** | 42.20 | 42.50 |
| **Carapace** | 15.25 | 17.01 |

Among aquatic animals, carotenoids are responsible for the colour of many important fish and shellfish. From the study on the yield (%) of the species, there was no difference between sexes. But the processing waste (head and carapace) (57-59 %) were more than the edible meat yield (31-39 %). According to [1, 10] the yield of waste harvested from the Indian shallow waters was reported to be in the range of 40 - 50 %. The results show a higher waste yield. Generally the head constitutes 34 - 45 % and body shell 10 - 15 % in tropical shrimp [11]. From the study on the deep-sea shrimp by [12], the higher yield of waste is due to the higher yield of head. Similar result was observed in the present study. The wastes were dominated by the head waste (42 %).

### *Carotenoids in different body components (µg/ g)*

The amount of carotenoid in different body parts showed a significant difference ($P < 0.05$) between the body parts and sexes (**Table. 3**). Total carotenoid content was higher (64.77 µg/ g) in the head of male *M. mogiensis*.

**Table 3** Total Carotenoid content (µg/g) of shrimp.

| S. No | Body Components | Total Carotenoid Content (µg/ G) | |
|---|---|---|---|
| | | **Male** | **Female** |
| 1. | **Meat** | $9.24 \pm 0.08^{a}$ | $10.99 \pm 0.97^{a}$ |
| 2. | **Carapace** | $33.41 \pm 0.59^{b}$ | $24.54 \pm 1.30^{b}$ |
| 3. | **Head** | $64.77 \pm 1.54^{c}$ | $37.11 \pm 2.97^{c}$ |

Values are expressed as mean ± standard error with significant difference at $p<0.05$

The species showed significant difference in carotenoid content of head and carapace and was highest in head waste respectively. The carapace of the male species showed 33.41 µg/ g of carotenoid content and the meat had 9 - 10 µg/ g of the content. Thus from the study in both the sexes the carotenoids are in the higher order of, head waste followed by carapace and meat.

The reported values for carotenoid content (µg/ g) in different body components of shrimp from shallow waters of India were 10.4 - 17.4 for meat, 35.8 - 156.1 for head and 59.8 - 104.7 for carapace [1]. The study reveals that the bright colour of the shrimp is directly relating its body pigment (carotenoids). Astaxanthin and its esters (mono and di-ester form) were the major carotenoids of shallow and deep sea shrimps. The distribution of astaxanthin and its esters ranged from 63.5 - 92.2 % of total carotenoids [12].

Astaxanthin and its esters are known to have storage function in crustacean epithelium which is the predominant form of carotenoid [3, 13, 14]. The total carotenoid content in crustaceans has been found to vary with species [15]. However, reports on the carotenoid content in crustaceans from tropical waters are limited. The carotenoid content in tiger prawns (*P. monodon*) from waters of the Indo-Pacific region varied from 23 to 331 µg/ g in the exoskeleton [14].

The IR spectra showed a strong absorption band at 2920, 2930, 2960 and 2926 $cm^{-1}$ due to symmetric $CH_2$ stretching vibrations (**Fig. 1, 2 and 3**). The head showed a strong band at 1713 $cm^{-1}$ indicated the keto C=O stretch. The band at 3431, 3424 and 3433 $cm^{-1}$ is due to O-H and amine N-H symmetrical stretching vibrations. 1070, 1054 and 1075 $cm^{-1}$ was assigned to the skeletal vibrations involving C-O stretching. Also the bonds showed the respective functional groups of alcohols, phenols, carboxylic acids, esters, aliphatic amines, primary and secondary amines. **Table 4** shows the presence of the functional groups representing the major carotenoids, astaxanthin, canthaxanthin and their mono and diester forms that can be confirmed by further qualitative and quantitative analysis.

**Table 4** Wavelength of the main bands obtained for the carotenoid extracted from different componenets of *M. mogiensis*

| Vibration modes | Shell ($cm^{-1}$) | Head ($cm^{-1}$) | Tissue ($cm^{-1}$) |
|---|---|---|---|
| O-H stretch, H-bonded | - | 3431 | 3433 |
| O-H stretch | 3011 | 2930 | 2587 |
| | 2926 | 2960 | 2852 |
| | 2856 | - | - |
| O-H bend | - | 971 | - |
| Symmetric $CH_2$ stretch | 2920 | 2926 | 2930 |
| C-H stretch | - | 2856 | 2852 |
| | 2856 | 2960 | 2852 |
| | 2926 | 2930 | 2920 |

| | | | |
|---|---|---|---|
| C-H bend | 1463<br>963<br>840<br>719 | 1460<br>971<br>825<br>720 | 1463<br>722 |
| C≡C stretch | - | 2174 | - |
| C-O stretch, C-N stretch | 1227<br>1054 | 1229<br>1149<br>1070 | 1226<br>1075 |
| C=O stretch | 1713 | - | 1715 |
| C-O stretch (amide III) | 1380 | 1379 | 1383 |
| N-H bend | - | 1639 | 1628 |
| Free amino group | 1226<br>1075 | 1227<br>1054 | 1229<br>1070 |
| Aromatic CH stretch | 722 | 719 | 720 |
| Aromatic CH, C=C bending | 1715 | 1713 | 1639 |

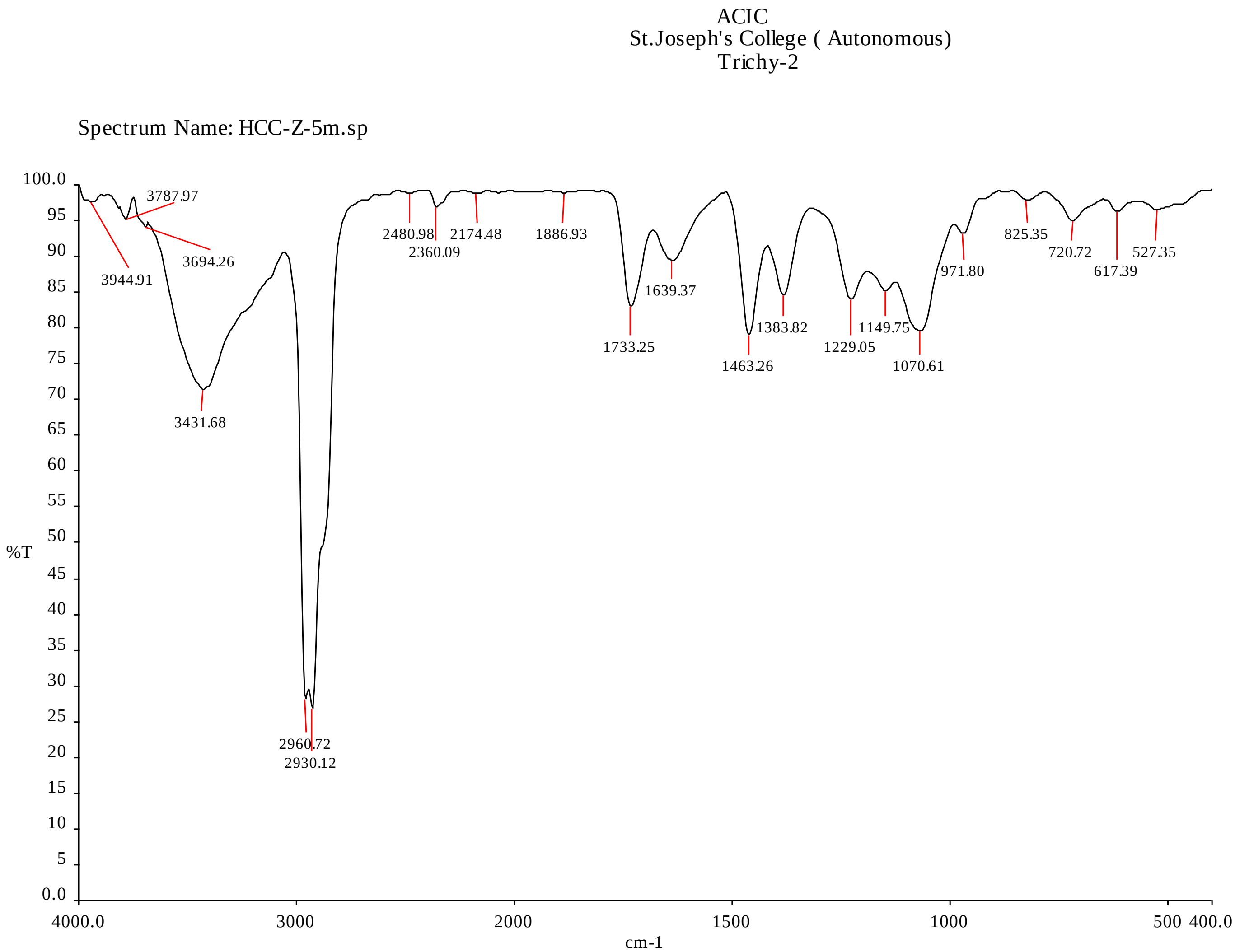


**Figure 1** FTIR Spectrum of Total Carotenoid Extract from Head Waste.

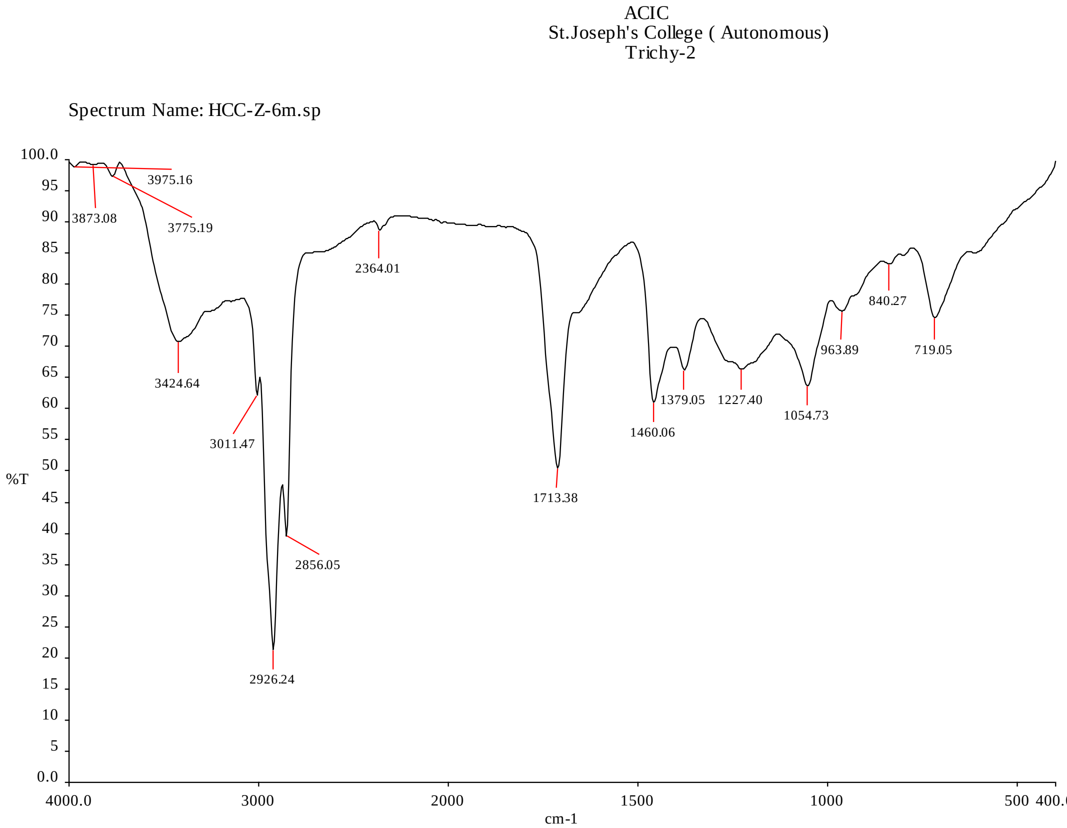


**Figure 2** FTIR Spectrum of Total Carotenoid Extract from Shell Waste.

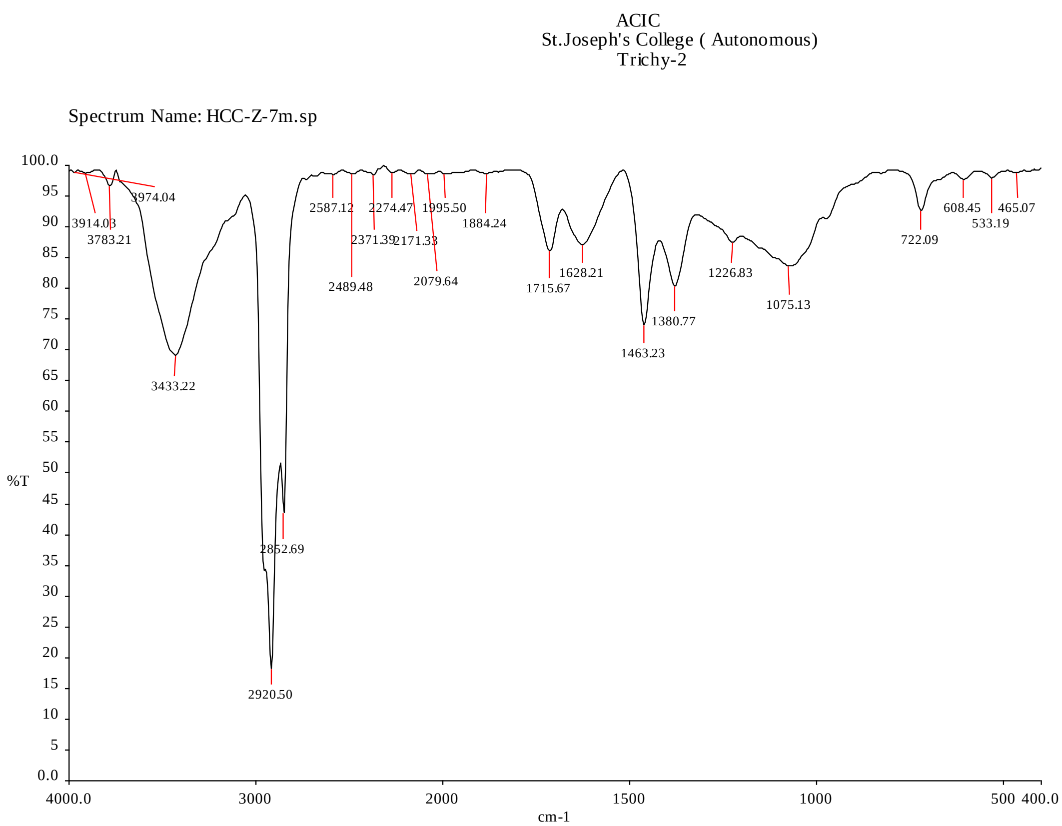


**Figure 3** FTIR Spectrum of Total Carotenoid Extract From Tissue.

According to Sachindra *et al*., [1] the commercially important shrimp species harvested from Indian waters contain a variable level of carotenoids. The body colour of each species also varied with carotenoid content. For example, *P. indicus*, having a low carotenoid content, showed a whitish exoskeleton, while *P. stylifera*, having a higher carotenoid content, showed a dark orange/red exoskeleton. As the highest carotenoid contents were observed in the head and carapace of *P. stylifera*, with the waste generated from the processing of this species also being higher, it would be a rich source of natural carotenoids. Thus the present work highlights the importance of the seafood processing discards.

## Conclusion

Thus the processed waste (Shell + Head Waste) has the higher pigment than in the tissue in both sexes. Further the results are to be continued with the composition and fatty acid profiling of the different body components. The preliminary work on carotenoid content revealed with higher concentration of carotenoids, the waste generated from the processing of shrimp could be a good source of carotenoids particularly that of astaxanthin and its esters. Thus the pigment composition in the shrimp taken for the study evinced that the species included in the Genus Metapenaeopsis is having more pigment composition in their waste as well as in the edible parts (tissue). So these may be used in as natural colourants than using the synthetic products and the tissue as a pigment rich diet.

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