Research Article

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY STUDY OF SPINY LOBSTERS FROM VISAKHAPATNAM COAST, ANDHRA PRADESH, INDIA

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ABSTRACT

The purpose of the present study was to find out the functional groups present in the spiny lobsters (Panulirus homarus, Panulirus polyphagus and Panulirus versicolor) muscle tissue by using Fourier Transform Infra-Red (FTIR) Spectroscopy method. FTIR analysis was carried out by (Bruker, a ALPHA- 1). The analysis of spectrum shows variations of functional groups in the muscle tissue samples at a Wavenumber region of 4000–550 cm⁻¹. FTIR spectroscopic investigation showed the presence of distinctive peak values with different useful combination of functional groups, which were carbonyl group, alcohol, alkane, alkene, amine, amide, ethers, ether, alkyl group and aromatics. The FTIR results exhibited the three species of spiny lobsters 12, 11 and 12 functional groups were identified. The highest peak of 3348.42 cm⁻¹ in P. versicolor, 3395.48 cm⁻¹ in P. homarus and 3281.37cm⁻¹ in P. polyphagus were observed in the FTIR spectra. The present study concluded that the tissue sample of P. polyphagus possessed strong functional groups, when compared with the P. homarus and P. versicolor.

Keywords: Spiny Lobsters, Functional Groups, FTIR, Visakhapatnam Coast, Bay of Bengal.

INTRODUCTION

Seafood is recognised as a good source of affordable protein and delicacy around the world, includes different kind of finfish and shellfish species. Seafood is drawn from a wide range of phyla of the animal kingdom like mollusc to crustacean to chordates, that is, from both invertebrates and vertebrates1-3.

Lobsters are one of the most valuable and highly priced crustaceans in India, as well as an important export commodity recent years. These species widely distributed along the Indian coasts like Bay of Bengal, Arabian Sea and Indian Ocean. Major fisheries are located on the north-west, south-west, and south-east coasts6-11. (Radhakrishnan & Manisseri 2003). They are usually inhabitants of hard substrates associated with coral reefs, rocky shores and hard bottoms layers. They are common throughout tropical and subtropical seas12 and form some of the most important commercial fisheries of the world.

The aquatic environments are particularly sensitive to the toxic contaminants, because a considerable amount of the chemicals used in industry, agriculture and urbanization enters the marine and other aquatic environments13. The discharge of potentially toxic race metals into the marine and fresh water environments has become a global problem. Continuous exposure of fresh water organisms to a low concentration of heavy metals may result in bioaccumulation, causing changes in several liver enzymes14. Fish, as living biotinindicator species, play an increasingly important role in the monitoring of water pollution, because they respond with great sensitivity to changes in the aquatic environment15,16. Infrared spectroscopy is a powerful method for studying molecular structure and intra molecular interaction in biological tissues and cells17. Several authors have studied infrared spectroscopy on biological substances like muscle tissues. The biological macromolecules provide us the most sensitive expression of the relationship between molecular structure and chemical and physical properties of a substance18. IR spectroscopy is a promising technique both to define the biochemical basis of cell viability more clearly with quantitative information about chemical functional groups in cells and to identify those characteristics specific to viable cells (19) (G. Jagadeesan). FTIR allows measurement of the entire spectrum simultaneously, providing a means to collect spectral information accurately and rapidly20.

Infrared (IR) spectroscopy has the potential to provide biochemical information without disturbing the biological sample. Consequently, the spectroscopic study of biological cells and tissue is an active area of research. It is one of the most widely used methods to identify the chemical constituents and functional groups of compound structures21. Fourier transform infrared spectroscopy has been used extensively to probe structural changes in proteins and lipids22,23. FTIR has played an important role in pharmaceutical analysis in present years 24.

The present study, attempts have been made to analyse the functional groups and bio molecular composition in muscle tissue samples of the spiny lobsters species from Visakhapatnam coast, by using Fourier Transform Infrared Spectroscopy (FTIR).

MATERIALS AND METHODS

Sample collection

The lobsters, Panulirus homarus (Scallop spiny lobster), Panulirus polyphagus (Mud spiny lobster), and Panulirus versicolor (Painted spiny lobster) were used for this study. The lobsters were purchased from the boat owners who go for single day fishing soon after they arrived at Visakhapatnam fishing harbour, Andhra Pradesh, India. The samples were immediately transported to the laboratory in ice containing boxes and stored in Ziploc bags. They were dressed (removal of shell, viscera, head
and fins) and maintained under deep refrigeration for until further use.

**Sample Preparation**

After collection of the samples from refrigeration the muscle tissue samples of the lobsters were dissected. The separated muscle tissue samples were cut into small pieces and were dried at constant temperature of 80°C for 48 hr. Dried samples of individual muscle tissues were ground into fine powder using mortar and pestle. Dried powder samples were weighed accurately 0.05 gms for analysis.

**Infrared (IR) Spectroscopic Analysis**

For the analysis, 5 mg of tissue sample and 100 mg of KBr were taken in a mortar. They were mixed thoroughly while grinding with the mortar and pestle. Just enough amount of prepared mixture was placed to cover bottom in pellet die. After that it was placed in KBr press and then pressure was increased upto 7 tons. The pressed sample was removed carefully from die and placed in the FTIR sample holder. The pressed disc was almost clear. The spectra were recorded in the range of 4000-550 cm-1 was used. Absorbance was seen through the plot of Transmittance (%) against Wavenumber cm-1 and possible to directly relate the intensities of the absorption bands of the corresponding functional groups. The analysis of functional groups was analysed by using Fourier Transform Infrared Spectroscopy (FTIR - Bruker, α ALPHA- t) which was available in the College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

**Table 1: IR Interpretation of compounds of *P. homarus***

<table>
<thead>
<tr>
<th>S. no</th>
<th>Wave number cm⁻¹ (Test sample)</th>
<th>Wave number cm⁻¹ (Reference article)</th>
<th>Bond</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3395.48</td>
<td>3300-3500</td>
<td>N-H stretch</td>
<td>Amine</td>
</tr>
<tr>
<td>2</td>
<td>2963.49</td>
<td>2850-3000</td>
<td>C-H stretch</td>
<td>Alkane</td>
</tr>
<tr>
<td>3</td>
<td>1634.95</td>
<td>1620-1680</td>
<td>N-H Bending stretch</td>
<td>Amine</td>
</tr>
<tr>
<td>4</td>
<td>1404.37</td>
<td>1400-1600</td>
<td>C-H bending</td>
<td>Alkane</td>
</tr>
<tr>
<td>5</td>
<td>1082.01</td>
<td>1050-1150</td>
<td>C-N stretch</td>
<td>Amine</td>
</tr>
</tbody>
</table>

**Table 2: IR Interpretation of compounds of *P. polyphagus***

<table>
<thead>
<tr>
<th>S. no</th>
<th>Wave number cm⁻¹ (Test sample)</th>
<th>Wave number cm⁻¹ (Reference article)</th>
<th>Bond</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3281.37</td>
<td>3200-3600</td>
<td>O-H stretch</td>
<td>Alcohols</td>
</tr>
<tr>
<td>2</td>
<td>2961.79</td>
<td>2850-3000</td>
<td>C-H stretch</td>
<td>Alkane</td>
</tr>
<tr>
<td>3</td>
<td>1641.42</td>
<td>1640-1690</td>
<td>C=O stretch</td>
<td>Amide</td>
</tr>
<tr>
<td>4</td>
<td>1546.66</td>
<td>1515-1560</td>
<td>N-H bending</td>
<td>Amide</td>
</tr>
<tr>
<td>5</td>
<td>1084.14</td>
<td>1050-1150</td>
<td>C-N bending</td>
<td>Amide</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

The present study was carried out to analyze the molecular structure and functional groups present in the muscle tissues of the spiny lobsters species using Infrared (IR) Spectroscopic method. Fourier transform infrared (FTIR) spectroscopy is a vibration spectroscopic method that helps the infrared radiation to vibrate molecular bonds absorbs it within the sample. Most of the examples have different sub-atomic bonds or distinctive setups of sub-atomic bonds, FTIR allows to get compound data on particles inside the specimen. Figures 1-3 shows the FTIR spectra of the muscle tissue of spiny lobsters in the Wavenumber range of 4000-550 cm\(^{-1}\). The spectrum is relatively complex and contains several bands appearing from the contribution of different functional groups belonging to the biomolecules like protein, lipids, and carbohydrates.

The table.1 and figure.1 demonstrates the presence of 5 functional groups recognized from the tissue sample of *P. homarus*. The strong instance peaks were identified at 3395.48 and 1634.95 cm\(^{-1}\) which are assigned to the N-H-bonded stretch and bending vibration. The peaks at 2963.49, 1404.37 and 1082.01 cm\(^{-1}\) which were assigned to the carbonyl compound frequency vibration. That means some of the carbonyl compounds are existed in the *P. homarus* muscle tissue sample. Amine and Alkane functional groups were identified in the *P. homarus*.

Analysis of infrared spectra for *P. polyphagus* was shown in table.2 and figure.2 allows the allocation of the following absorption bands for lobsters species. A strong band at 3281.37 cm\(^{-1}\) corresponding to the vibrations of free OH stretching of alcohol functional group. The 2961.79 band of C-H stretching and alkane functional group was present. A band at 1641.42 C=O stretching with the functional group of amide. The peaks at 1546.66 and 1084.14 cm\(^{-1}\) N-H bending and C-N bending which were assigned to the aromatic compound frequency vibration. These two peaks were identified as amide functional group.

The functional groups identification was through by FTIR analysis and the active components based on the peak value in the region of infrared radiation. The tissue sample of *P. polyphagus* was passed into the FTIR spectroscopy and the functional groups of the components were separated based on the peak values.

CONCLUSION

In conclusion, the present study examined that the FTIR analysis shows the clear distinction between the three lobsters species. The comparison of the three selected lobsters *P. homarus*, *P. polyphagus* and *P. versicolor* were high in macromolecules and identified the various functional groups were observed in lobsters species. FTIR spectroscopic investigation showed the presence of characteristic peak values with different useful mixtures of functional groups such as carbonyl group, alcohol, alkane, amine, amide, esters, ether, alkyl group and aromatics. Seafood consumption has a potential protective role against coronary heart diseases. Nowadays, health, food and nutrition quality is one of the major concerns in all developed countries. The lobsters were responsible for good nutritional values and medicinal properties present in the lobsters species. Further advanced research is needed for spectroscopic investigations to the identification of structural and bioactive compounds as well as medicinal properties present in the lobsters species.

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