Biological Forum – An International Journal **15(2): 712-718(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Studies on Gas Chromatography-Mass Spectrometry (GC-MS) and Thin Layer Chromagraphy (TLC) Analysis of Three Spotted Crab (*Portunus sanguinolentus)*

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(Received: 03 January 2023; Revised: 15 February 2023; Accepted: 17 February 2023; Published: 20 February 2023)
(Published by Research Trend)

ABSTRACT: Crabs are decapods crustaceans of the family Portunidae. Carbs obtain carotenoids from an external sources like medium and food materials. It has been suggested that carotenoids are strong antioxidants that shield membrane lipids from harmful peroxidation processes. The peculiar spectrum characteristics of carotenoids were frequently used for detection and quantification; these chemicals' conjugated polyene architectures give them their distinct light absorption spectra, high molar absorptivity, and remarkable lower limits of detection. In the present study, carotenoids are extracted from the three spotted crabs. I collected from the South East Coast of India. The results revealed that carotenoid compounds were distributed a varied level in the carapace and muscle. Increasing variability was observed in carotenoid groups between individuals concerning age. The quantities of carotenoids of hexane extract were determined by GC-MS and TLC respectively. The experimental crab species seems to contain a comparable concentrations of carotenoids.

Keywords: Three spotted crab, Carotenoids, FAME, GC-MS, and TLC.

INTRODUCTION

The long structure of alternating double and single bonds seen in carotenoids is known as a polyene chain (Jumaah *et al*., 2016). These vital anti-oxidants are also produced by plants and photosynthetic bacteria and serve as immunostimulating, anti-mutagenic, and tumor-preventing agents. Animals get them through nutrition (Li *et al.,* 2005). There are more than 700 distinct carotenoids known. Several edible fruits, vegetables, fungus, flowers, spices, shellfish, and some animal items contain a far smaller amount of them (Pfander, 1992; Edge *et al*., 1997). These pigments' dark hue is also a feature of the many basic species that naturally make carotenoids, including yeast, fungi, and algae (Kaczor *et al*., 2016). Carotenoids cannot be produced by animals. They obtain them from their diets (Katrangi *et al.,* 1984). According to Goodwin (1984), crustacean carotenoids are typically found as free carotenes, unesterified carotenols, fatty acid esters of carotenols, or caroteno proteins, which are proteins that are soichiometrically attached to xanthophylls. There has been a lot of interest in the chemical composition of individual carotenoids (Schiedt *et al*., 1991) and carotenoproteins (Zagalsky, 1994), but little is known about the fatty acid moieties of the carotenol esters. Members of the terpenoid family, carotenoids are distinguished by their polyunsaturated structure. The carotenes, lycopene, and certain alcohol derivatives such xanthophyll, zeaxanthin, lycophytes, and cryptoxanthin are the most prevalent terpenoids in the heart (Amorim-Carrilho *et al.,* 2014). Not all carotenoids are enzyme-convertible to retinol or vitamin A; that is, not all carotenoids are provitamin A. Because each molecule of carotene produces two molecules of vitamin A through oxidative cleavage, only -carotene is regarded as 100% provitamin A. (retinol). All other carotenoids are inactive as precursors to vitamin A, while the carotenes and cryptoxanthin constitute 50% provitamin A (Katrangi *et al.*, 1984). Because of the structural diversity of carotenoids, which are present throughout all kingdoms of living things, they serve a variety of crucial activities. An important class of aquatic creatures known for having a high concentration of carotenoids are the crustaceans. The carapace of crustaceans contains free and esterified forms of carotenoids (Šesták, 2004). The presence of carotenoids in crustaceans is mostly caused by the pigments ingested in plant-based foods depositing as such or biologically converting to keto or hydroxyl derivatives. Astaxanthin and similar carotenoids are frequently found in significant quantities in many

marine invertebrate creatures, such as shrimp, crabs, and lobsters. When consumed raw, these carotenoids are typically green, purple, or blue, but when cooked, they become denatured and turn red (Britton, 1995). Researchers have looked into the biological functions of carotenoids in crustaceans (Bendich and Olson 1989). Studies have been done on carotenoids and their role in fish and crustacean nutrition (Amorim-Carrilho *et al.*, 2014) Crustacean-processing waste or by-product has traditionally been added to animal feeds. These by-products contain pigments, flavor components, calcium, and other minerals, as well as high-quality protein and can also be used to create value-added products (Lage-Yusty *et al.*, 2011). Crab shells contain various nutritional and valuable components, including proteins, minerals (rich in calcium), chitins, and carotenoids, and they have been recycled and valorized as nutraceutical chitin/chitosan, animal foods, natural pigments, etc. Murphy *et al*. (2003); Jun *et al*. (2019) reported on the mineral content of the red snow crab shell to utilize as a coagulant for tofu. The conditions for preparation of a calcium rich extract from the crab shell and their effects on the yield, and textural and sensorial properties of the tofu were investigated. The digestive gland, muscle and ovaries, cuticle and tail muscle of the mature female crayfish, *Cherax quadricarinatus*, are examples of tissues with high carotenoid concentration and distribution. In many ways, analysing carotenoids is a difficult undertaking. The compounds degrade due to the instability of the lengthy polyene chain. Moreover, these substances are light- and heat-sensitive, which speeds up cis-trans isomerization (Boon *et al.*, 2010). Carotenoids in the samples have been examined using a variety of separation techniques. Carotenoids have been separated using techniques like TLC and Gas Chromatography-Mass Spectrometry (GC-MS).Many experts recommend using gas chromatography/mass spectrometry (GC/ MS) to identify volatile substances in complicated combinations. The uses of carotenoids as food additives (colourants, antioxidants) and in medicine and cosmetics are widely recognised. The goal of the current study was to use chromatographic analysis to ascertain the fatty acid and carotene makeup of three spotted crabs.

MATERIALS AND METHODS

Three spotted crabs (*Portunus sanguinolentus*), a marine crab species, were taken as samples from the Mannapad Estuary in the Tuticorin area and transported to the lab in ice. Before being employed for GC-MS separation, all of the chemicals and reagents used for the investigation were of AR grade.

Extraction of carotenoids using solvents

Crab shell scraps from a deep-sea species of *Portunus sanguinolentus* were the raw materials used for the current study. This deep-sea species was chosen to produce the greatest amount of astaxanthin pigment. The viscera from the gills were removed, and the yield of flesh and shell was calculated through weighing. In a mortar and pestle, fresh crabs were ground to a fine powder after being dried (in the dark, at 40°C) to 85% of their dried weight.10g of the carapace and 5g of the abdominal muscle were removed. The extractant (1:1 hexane/acetone) was added in three volumes and vigorously stirred for around 15 minutes. Using Whatman no. 1 filter paper, the solvent combination was filtered under vacuum. Cleanly separating the filtrate from the filter paper, the extraction was carried out repeatedly using lower amounts of extractant until the filtrate was colourless. The saponified solution (methanol, 40% w/v KOH) was added, and the mixture was agitated for about 45 minutes at 56 °C. A separatory funnel was used to hold the saponified extract before one volume of the salting out solution (10% w/v Na_2SO_4) was added. The top layer was rinsed three times with 10ml of water after the bottom layer was removed. Whatman no. 1 filter paper was used to filter 3gm of anhydrous sodium sulphate. For the qualitative and quantitative assessment of carotenoids using thin-layer chromatography and GC-MS, the resulting filtrate was employed as the sample.

Purification of carotenoid by Thin layer chromatography (TLC). Chemically concentrated carotenoids were extracted using silica gel and 60 F MERCK TLC paper. On the TLC plate, a thin line was drew 1.5 cm above the bottom. On the line, a small amount of the concentrated carotenoid extract was applied, and it was let to dry. The part on the same website was then repeatedly added after that. Hexane and acetone were combined in the developing chamber in a beaker at a 3:2 ratio (Lorenz, 1998). The developing chamber was filled with the TLC plate, and the top was covered. On the plate, the solvents were allowed to rise until they were 1.5 cm from the top. The Rf was then determined after it was removed.

Purification of carotenoids by GC-MS. The individual's filtrate was ultimately subjected to GC-MS analysis at the Instrumentation Center of Ayya Nadar Janaki Ammal College in Sivakasi, specifically for GC and MS analysis, using 90% acetone. Using GC-MS QP 2015 (SHIMADZU) and a VF-5MS column with a diameter of 30.0 mm and a film thickness of 0.25 mm, fatty acid methyl esters (FAME) were measured. Using an auto-sampler, samples were injected (0.2–0.8 l injection volume) onto the same GC column that was used for GC. In 0.45 seconds, full scans from m/z 50 to m/z 400 of mass spectra were obtained. The National Institute of Standard and Technology (NIST) database, which contains more than 62,000 patterns, was used for mass-spectrum GC-MS interpretation. The spectrum of the unknown components was compared with the range of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained using CAS number. NIST and FAME software were used as MS library for FAME identification and analysis (Elumalai *et al.*, 2014).

RESULT AND DISCUSSION

A crustacean sample of the three spotted crab was collected from the Mannapad Estuary. The crude

extract and dry matter of the three spotted crabs were analyzed for carotenoid. The presence of astaxanthin and its esters in this work has been validated by a carotenoid extract from the *Portunus sangunilentus* that was expected to contain astaxanthin. In marine crustaceans, the main carotenoid is astaxanthin and its esters (Shahidi *et al.,* 1998). On a chromatographic column, a macerated extract of three spotted crab specimens in acetone was separated. In comparing the outcomes of these investigations with the data that has been released (Matsuno *et al.,* 1974). It was discovered that the -carotene, reported by Matsuno *et al.* (1974), differed from the findings of the aforementioned author. According to (Coral-Hinostroza and Bjerkeng 2002), endogenous factors like development stage and physiological status and exogenous factors like nutrition can both contribute to variations in carotenoid content and even composition within the same species (Sjodahl *et al.*, 2002). In the marine crab, accumulation of astaxanthin β- carotene and zeaxanthin has been reported. The crab's Fatty acid Methyl Ester and astaxanthin mono and diesters were analysed in detail. In each of these, as well as in several preliminary samples, seven pigments were detected by Gas Chromatography–Mass Spectrometry Analysis (Table 1). The present GC-MS results are similar to the GC-MS chromatograph of *Haematococcus pluvialis* in which Astaxanthin esters occurs at the retention time (Elumalai *et al.*, 2014). When in comparison with all the fatty acids Hexadecenoic acid (C:16:2), Oleic acid (C:18:2, 9-Octadecenoic acid,(E) (C:18:2), Cyclotetradecane (C:14:0), 1,2-Benzenedoil, 3,5-bis(1,1-dimethylethyl)-(C:14:2), Octasiloxane,,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (C:16:7:),n-Propyl11-octadecenoate (C:21:2), 2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- (C:14:2),1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester (C:23:6), Fumaricacid, 4-heptyltridecylester (C:24:4) based on their UV/visible spectra (max 460 and 452 nm, respectively). Common carotenoids were compared in order to confirm the fatty acids' identities. Using a commercial standard of -carotene, the carotenoids from three spotted crabs were quantified by GC-MS. For confirmation, the same crab extracts were also treated to determine their lipid content using TLC analysis (Nm *et al.*, 2005). Around 90% of the total lipid content was made up of hexadecenoic acid, Z-11-, oleic acid, 9-octadecenoic acid, (E), and cyclotetradecane (linoleic, oleic, palmitic, and stearic acids, respectively). Tetra, penta, and heptadecanoic acids, as well as eicosanoic and docosanoic acids, were just slightly detected.

Fig. 1. Three spotted crab.

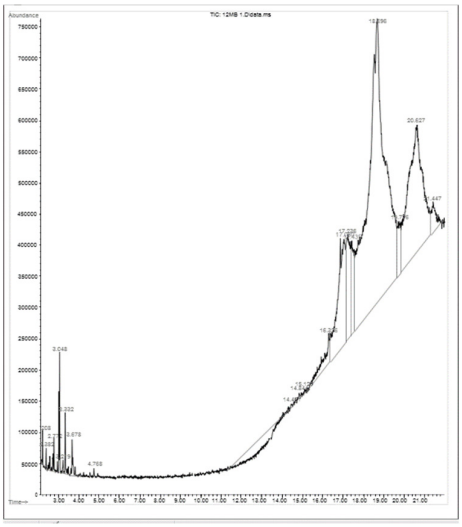
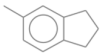
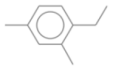
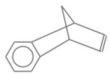
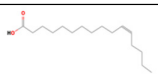
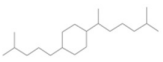
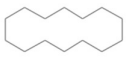
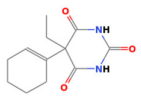
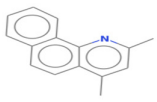
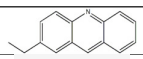
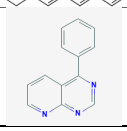
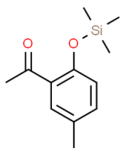
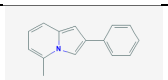
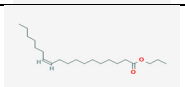
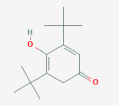
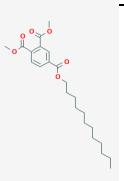
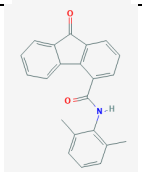
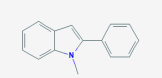
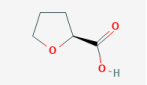
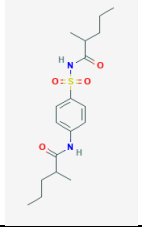
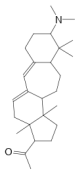
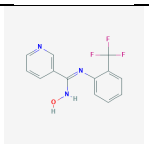
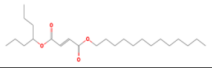


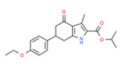
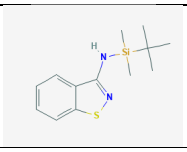
Fig. 2. Representative chromatogram of Three spotted crab.

Table 1: Compounds found in real samples by GC-MS as per NIST library.

Sr. No.	Retention time	Name of the compound	Molecular formula	Molecular weight	Chemical structure	Peak area %
1.	2.210	Benzene,1,2,3-trimethyl-	C_9H_{12}	120.1916		0.11%
2.	2.380	Benzene, 1-ethyl-3-methyl-	C_9H_{12}	120.1916		0.09%
3.	2.380	Benzene, 1,2,4-trimethyl-	C_9H_{12}	120.1916		0.09%
4.	2.768	o-Cymene	$C_{10}H_{14}$	134.2182		0.46%

5.	2.768	p-Cymene	$C_{10}H_{14}$	134.2182		0.46%
6.	3.051	Benzene, 1,2,4,5-tetramethyl-	$C_{10}H_{14}$	134.2182		0.91%
7.	3.051	Benzene, 1,2,3,5-tetramethyl-	$C_{10}H1_4$	134.2182		0.91%
8.	3.222	Benzene,2-ethyl-1,4-dimethyl-	$C_{10}H_{14}$	132.2023		0.13%
9.	3.222	3-Phenylbut-1-ene	$C_{10}H1_2$	132.2023		0.13%
10.	3.222	1H-Indene, 2,3-dihydro-5-methyl-	$C_{10}H_{12}$	132.2023		0.13%
11.	3.335	Benzene, 1-ethyl-2,4-dimethyl-	$C_{10}H_{14}$	134.2182		0.42%
12.	3.675	Naphthalene	$C_{10}H_{18}$	128.1705		0.43%
13.	3.675	Azulene	$C_{10}H_{18}$	128.1705		0.43%
14.	4.772	Benzocycloheptatriene	$C_{11}H_{10}$	142.1971		0.05%
15.	4.772	1H-Indene,1-ethylidene-	$C_{11}H_{10}$	142.1971		0.05%
16.	4.772	1,4-Methanonaphthalene,1,4-dihydro-	$C_{11}H_{10}$	142.1971		0.05%
17.	**14.454**	**Hexadecenoic acid, Z-11-**	**$C_{16}H_{30}O_2$**	**254.4082**		**4.27%**
18.	**14.454**	**Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-**	**$C_{20}H_{40}$**	**280.5316**		**4.27%**
19.	**14.454**	**Oleic acid**	**$C_{18}H_{34}O_2$**	**282.4614**		**4.27%**
20.	**14.842**	**9-Octadecenoic acid, (E)**	**$C_{18}H_{34}O_2$**	**282.4614**		**0.21%**
21.	**14.852**	**Cyclotetradecane**	**$C_{14}H_{28}$**	**196.3721**		**0.21%**
22.	**15.126**	**1,2-Benzenedoil, 3,5-bis(1,1-dimethylethyl)-**	**$C_{14}H_{22}O_2$**	**222.3233**		**0.19%**
23.	17.234	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236.2670		5.24%
24.	17.234	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	207.2704		5.24%
25.	15.126	2-Ethylacridine	C15H13N	207.27		0.19%
26.	15.126	Pyrido [2,3-d] pyrimidine, 4-phenyl-	C13H9N3	207.23		0.19%

27.	**16.327**	**Octasiloxane,,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-**	**C16H48O7Si8**	**577.2**		**1.87%**
28.	16.327	5-Methyl-2-trimethylsilyloxy-acetophenone	C12H18O2Si	222.356		1.87%
29.	16.327	5- Methyl- 2 –phenyl indolizine	C15H13N	207.27		1.87%
30.	**17.083**	**n-Propyl11-octadecenoate**	**C21H40O2**	**324.5**		**9.56%**
31.	**17.083**	**2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-**	**C14H22O2**	**222.32**		**9.56%**
32.	**17.433**	**1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester**	**C23H34O6**	**406.5**		**2.72%**
33.	17.433	9H-Ffluorene-4-caboxylic acid, 9-oxo-, (2,6-dimethylphenyl) amide	C22H17NO2	324.4		2.72%
34.	17.234	1H-Indole, 1-methyl-2-phenyl-	C15H13N	207.27		5.24%
35.	18.700	Tetrahydrofuran-2-carboxylic acid, dibenzofuran-3-ylamide	C5H8O3	116.11		56.13%
36.	18.700	2-Methyl-pentanoic acid [4-(2-methyl-pentanoylsulfamoyl)-phenyl]-amide	C18H28N2O4S	368.5		56.13%
37.	18.700	B(9a)-Homo-19-norpregna-9(11),9a-dien-20-one,3-(dimethylamino)-4,4,14-trimethyl-,(3.beta.,5.alpha.)-	C26H41NO	383.31		56.13%
38	19.788	Pyridine-3-carboxamide,oxime,N-(2-trifluoromethylphenyl)-	C13H10F3N3O	281.23		1.95%
39.	**20.629**	**Fumaricacid,4-heptyltridecylester**	**C24H44O4**	**396.6038**		**22.38%**

40.	20.629	1H-Indole-2-carboxylicacid,6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-,isopropylester	C21H25NO4	355.427		22.38%
41.	21.451	1,2-Benzisothiazol-3-aminetbdms	C13H20N2SSi	264.46		1.41%

DISCUSSION

Our findings suggest that the combined concentration of and g-carotene would be adequate to play a comparable role in guarding the lipid storage reserves of fungal spores because fatty acids are stored primarily as triacylglycerides and, like carotenoids, are found in lipid droplets. The orange coloration of three spotted crab was discovered to be caused by carotenoids. The main groups used to identify astaxanthin are functional groups like hydroxyl (OH) and the keto group (C=O), which can readily react with other molecules and convert into various forms (Ambati *et al.*, 2014). There have been reports of astaxanthin, â-carotene, and zeaxanthin buildup in sea crabs (Matsuno *et al*., 1974). It was discovered that the main pigments in freshwater mullets were zeaxanthin and lutein (Matsuno *et al.*, n.d.). Crustaceans had a variety of carotenoids, including -carotene and oleic acid, as well as fatty acid methyl esters. These pigments are anticipated to have a significant antioxidant function in this organism as well, particularly in the defence of delicate biological components against reactive oxygen species like free radicals. The presence of adequate carotene in lipid droplets suggests a potential lipid-protecting function in three spotted crabs. The current work shows that the marine crab, *Portunus sangunilentus*, accumulates astaxanthin esters because it is more efficient to extract astaxanthin chemically from key carotenoid compounds. According to the findings of this study, they are an excellent source of carotenoids and fatty acids. The fatty acids and carotene (vitamin A) were investigated. It has been indicated that carotenoids can be used as biomarkers to distinguish between cancerous/malignant and healthy cells and tissues, with practical potential in medical diagnostics. However, some features of the crabs are qualitatively and quantitatively suitable for consumption about the fatty acid profiles. Therefore the muscle and carapace of the crab should be utilized and considered an alternative source for the industrial production of valuable products, such as pharmaceuticals and cosmetics.

CONCLUSIONS

Many useful bioactive compounds are found out from the crab shell extract. These compounds are play important role as well as several antimicrobial molecules were detected for further studies.

Acknowledgement. We thank to Rani Anna Government College for Women, Tirunelveli and Ayya Nadar Janakiammal College, Sivakasi**,** Tamil Nadu for the support.
Conflict of Interest. None.

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How to cite this article: K. Madhubala and T. Selvamohan (2023). Studies on Gas Chromatography-Mass Spectrometry (GC-MS) and Thin Layer Chromagraphy (TLC) Analysis of Three Spotted Crab (*Portunus sanguinolentus*). *Biological Forum – An International Journal, 15*(2): 712-718.