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PROXIMATE, AMINO ACID AND FATTY ACID COMPOSITION THE MARINE CRABS FROM THE SOUTHEAST COAST OF INDIA

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Abstract

Marine crabs, *Portunus pelagicus* (Linnaeus, 1766), *Portunus gladiator* (Fabricius, 1798) and *Charybdis lucifera* (Fabricius, 1758) are a valued decapods species captured mainly in the Mandapam coast, Tamil Nadu, India. In this work the species differences in terms of proximate composition, amino acids, and fatty acid content were compared. The proximate composition (%) results showed significant difference ($P<0.05$) between the various species. Higher amount of protein (22.57 %) and carbohydrate (1.17 %) contents were detected in *C. lucifera* when compared than *P. pelagicus* and *P. gladiator*. While, lipid content was higher in *P. pelagicus* (2.15 %) and lower recorded in *C. lucifera*. Totally twenty amino acids (g/100g) recorded in all species except *C. lucifera*. Essential amino acid composition (EAA) and non-essential amino acid composition (NEAA) has been highly found in *C. lucifera* (3.92 and 4.81 g/100g) but cystiene only absent. The ratio of EAA and NEAA had only minimum differences between the species. The fatty acid profile (g/100g) was determined by GC-MS and recorded major fatty acid components. Significant differences in fatty acid composition were also found among different species ($P<0.05$). The results showed, *P.pelagicus* having high amount of saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) than the other two species. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were found in all Portunid crabs. The results obtained from the present study indicate that the *P.gladiator* and *C.lucifera* crabs meat contain well balanced composition of amino acids as well as *P.pelagicus* had good source of fatty acids. It is concluded that all the three species are the healthiest crustacean sea foods.

Keywords: sea slug, *K. ornate*, nutritional potential, PUFAs, minerals

INTRODUCTION

Seafood and its products have attracted a considerable attention as an important source of nutrients in the human diet. Apart from their delicacy, crustacean species such as shrimp, crab, lobster consist of amino acids, peptides, protein and other useful nutrients (Heu *et al*., 2003). In addition, crustaceans are highly appreciated and are considered as luxury seafood items. Although their frequent consumption is not advisable in general, either because of allergic reactions or their supposedly high cholesterol content, there is a growing number of studies promoting crustacean consumption (Rosa and Nunes 2003a; Chen *et al*., 2007). Nutritional benefits of crab's consumption include high protein content, essential macro and trace elements, fatty acids and amino acids, as well as low fat and cholesterol contents, which are specifically present in the crab muscle (Barrento *et al*., 2010). The marine crabs are one of the valuable seafood items of great demand both in the domestic and export industry of India. In the Global level, countries such as China, France, Indonesia Japan, Philippines, Spain, Thailand and United States (Manisseri and Radhakrishnan, 2003) consider the crab varieties to be of primary economic importance. The crab fishery in India is fast developing and there are 44,586 t of crabs landed in the coast during the year 2013-2014. In this way, the crabs rank next to shrimps among the crustaceans (CMFRI, 2014). The commercially important crabs, which are found in the southeast coast of India are *Scylla serrata, Scylla tranquebarica, Portunus sanguinolentus, Portunus pelagicus, Podopthalamus vigil, Charybdis feriata, Charybdis lucifera, Charybdis natator, Charybdis granulata* and *Charybdis truncata* (Johnsamuel *et al*., 2004). Crabs are a large group of invertebrates and, due to the high palatability of their meat, they are the prime focus of wide commercial fisheries (Latyshev *et al*., 2009). Various edible crab products, including the traditional hard shell and soft shell crabs, cocktail claws and also the processed varieties such as canned, refrigerated and pasteurized crabmeat are consumed worldwide (Sumpton, 2005).

Nowadays, the tendency of benefiting from seafood for an essential protein supply is increasing worldwide

rapidly (Jhaveri *et al*., 1984). Crab tissue proteins contain 20 different amino acids of significant nutritional value. Such amino acids include, threonine, valine, methionine, arginine, isoleucine, leucine, phenylalanine, lysine, histidine and tryptophan are the Essential amino acids (EAA) as well as aspartic acid, glutamic acid, cystiene, tyrosine, alanine, asparagine, glycine, proline, serine and taurine are Non-essential amino acids (NEAA). The taste, nutritional quality and health benefits of seafood products, including marine crabs is, to a large extend, associated with their essential amino acid (EAA) contents (Chen *et al*., 2007) and it's essential nutrients for human growth and for functions such as physiology, biochemistry, and immunity (Maria *et al*., 2007). High levels of amino acid may promote the pathogenesis of many diseases, such as Crohn's disease (Shoda *et al*., 1996) and inflammatory disease (Gil, 2002). It is essential for the treatment of rheumatoid arthritis, allergies, ulcers anemia and protect nerve cells. It is needed for the production of both red and white blood cells, protection of the body from radiation damage, lowering blood pressure and aids in the removal of heavy metals from the body (Bruce Barber, 2013).

Marine lipids are also vital nutrients for human health, but decline in seafood stocks (FAO 2010) are currently threatening food security for human populations on a global scale (Parrish *et al*., 2008). The omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were believed to be protective for human health in many ways. The consumption of PUFA reduces the risk of coronary heart disease and cancer and thus has both anti-atherogenic, anti-thrombotic effects, control of rheumatoid arthritis and hypertension. It also reduces the risk of diabetes and prevents cardiac arrhythmias (Mahaffey, 2004; Schmidt, 2003; Sidhu, 2003; Simopoulos, 2001). EPA and DHA are also the precursors of several metabolites that are potent lipid mediators, considered by many investigators to be beneficial in the prevention or treatment of several diseases (Serhan *et al*., 2008). PUFAs were mainly acquired from seafood and thus human obtain EPA and DHA by consuming aquatic invertebrates like crustaceans (Schmidt *et al*., 2005). The crab breast meat and claw meat have higher amounts of EPA and DHA and they are beneficial to human health (Celik *et al*., 2004).

So many studies have been reported the nutritional quality of different crab species (Latyshev *et al*., 2009; Marques *et al*., 2010; Cherif *et al*., 2008; Naczk *et al*., 2004). But limited data on the nutritional composition of *Portunus pelagicus*, *Portunus gladiator* and *Charybdis lucifera* in Mandapam coast in Ramanathapuram district have been studied. Therefore, present work aims to determine the biochemical composition (protein, carbohydrate, lipid, amino acid and fatty acid composition) of these selective marine crabs.

MATERIALS AND METHODS

Sample collection

The samples were collected from the landing centre of Mandapam, (Lat 10˚ 45ˈN; 79˚ 51E) southeast coast of India, during January, 2014. Adult male crabs (20 in nos) were collected and the samples were washed with water to remove contamination. They were transported to the laboratory in ice- boxes. The species were taxonomically identified as *Portunus pelagicus*, *Portunus gladiator* and *Charybdis lucifera*.

Sample preparation

The crabs were dissected to obtain body and claw meat from all crabs was carefully removed, the edible tissues of each species were pooled and sample was dried in hot air oven at a constant temperature 60° C until the wet sample dried completely. Then dried sample were homogenized using mortar and pestle to make powder form and stored for biochemical analysis.

Estimation of Proximate composition (%)

In the present study, biochemical composition viz., protein, carbohydrate and lipid content were analyzed by using standard methods. The protein was estimated by Lowry *et al.,* 1951, carbohydrate by Dubois *et al*., 1956 and lipid by Folch *et al*., 1957.

Estimation of Amino acid Composition (g/ 100g)

Samples (5g) were used in estimating the amino acid composition using in the High-Performance Liquid Chromatography (HPLC) (Merck Hitachi L-7400) following the method of Baker and Han, 1994.

Estimation of Fatty acid Composition (g/ 100g)

Total lipid was extracted from (5g) sample using the chloroform: methanol (2:1, v/v; containing BHT 0.1 mg/100 g) method (Folch *et al*., 1957). For determination of fatty acid composition, in order to have more representative samples, lipid extracts from crab samples were pooled together for preparation of fatty acid methyl esters (FAME) and two such pooled samples were analyzed. The lipids were transmethylated using 2 M methanolic sodium hydroxide followed by 2M methanolic hydrochloric acid to obtain FAME. FAMEs were analyzed by gas chromatography (Shimadzu GC 2014, Japan) for identifying the individual fatty acids. FAME dissolved in hexane was analyzed using Omegawax TM 320 fused silica capillary column (30 m × 0.32 mm × 0.25 μm). The conditions used for GC analysis

was injection temperature of 250° C, detector (FID) temperature of 260° C and column temperature of 200° C for 60 min. The carrier gas was hydrogen or helium for using gas chromatography. The peaks were identified by comparing with authentic standards. The fatty acid analyses were conducted in triplicate.

Statistical analysis

The results obtained were subjected to descriptive statistics and tested using analysis of variance and Duncan's multiple range tests using SPSS version 16 Statistical Package for Windows (Differences were considered to be significant when $p<0.05$).

Table. 1 Proximate composition (%) of different marine crabs

Proximate composition	*Portunus pelagicus*	*Portunus gladiator*	*Charybdis lucifera*
Protein (%)	20.15±0.00^{b}	18.41±0.02^{c}	22.57±0.03^{a}
Carbohydrate (%)	0.54±0.05^{c}	0.83±0.0 ^{b}	1.17±0.08^{a}
Lipid (%)	2.15±0.01^{a}	1.79±0.03^{b}	1.65±0.00^{c}

Results are the mean value in triplicates ± Standard deviation (SD) with significant difference at P<0.05. Identical lower case superscripts denote similar values horizontally.

Table 2 Amino acid composition (g/100g) in different marine crabs

Amino acids (g/100g)	*Portunus pelagicus*	*Portunus gladiator*	*Charybdis lucifera*
Threonine	0.48±0.08^{a}	0.39±0.03^{b}	0.57±0.00^{ab}
Valine	0.22±0.02^{b}	0.19±0.00^{c}	0.63±0.01^{a}
Arginine	0.50±0.02^{a}	0.40±0.05^{b}	0.54±0.03^{a}
Methionine	0.19±0.00	0.69±0.00	0.51±0.00
Isoleucine	0.31±0.00^{a}	0.22±0.01^{b}	0.20±0.01^{c}
Leucine	0.29±0.01^{a}	0.20±0.02^{b}	0.21±0.00^{b}
Lysine	0.19±0.02^{b}	0.31±0.01^{a}	0.13±0.01^{c}
Phenylalanine	0.11±0.00^{c}	0.41±0.04^{b}	0.59±0.04^{a}
Histidine	0.39±0.04^{a}	0.33±0.00^{a}	0.41±0.06^{a}
Tryptophan	0.11±0.00^{c}	0.19±0.01^{a}	0.13±0.00^{b}
Total EAA	**2.79**	**3.33**	**3.92**
Aspartic acid	0.49±0.03^{b}	0.49±0.10^{b}	0.80±0.12^{a}
Glutamic acid	0.50±0.02^{a}	0.29±0.10^{b}	0.61±0.07^{a}
Glutamine	0.19±0.04^{c}	0.39±0.01^{b}	0.60±0.08^{a}
Cystiene	0.20±0.00^{a}	0.19±0.04^{a}	0
Tyrosine	0.19±0.00^{c}	0.40±0.04^{a}	0.30±0.00^{b}
Alanine	0.11±0.02^{c}	0.22±0.02^{b}	0.59±0.02^{a}
Asparagine	0.19±0.01^{c}	0.39±0.08^{b}	0.95±0.00^{a}
Glycine	0.30±0.00^{b}	0.22±0.00^{c}	0.34±0.01^{a}
Proline	0.30±0.05^{b}	0.28±0.05^{b}	0.50±0.02^{a}
Serine	0.20±0.07^{a}	0.19±0.00^{a}	0.12±0.00^{a}
Total NEAA	**2.67**	**3.06**	**4.81**
EAA/ NEAA	**0.81**	**1.08**	**1.04**

Results are the mean value in triplicates ± Standard deviation (SD) with significant difference at P<0.05. Identical lower case superscripts denote similar values horizontally.
EAA and NEAA represent essential and non-essential amino acids, respectively

RESULTS AND DISCUSSION

The biochemical composition (%) of marine crabs (table 1) showed significant differences ($p<0.05$) between the three species. Higher amount of protein content were present in *C.lucifera* (22.57 %) followed by *P.pelagicus* (20.15 %) and *P.gladiator* (18.41 %). When comparing three species, highest carbohydrate content were observed in *C.lucifera* (1.17 %) and lowest in *P. pelagicus* (0.54 %). Among various species *P. pelagicus* (2.15 %) had significantly higher lipid than the other two crabs species. Biochemical studies are very important from the nutritional point of view. The biochemical composition of edible tissues of marine invertebrates is influenced by their nutritional habits, age, sex, season, seawater temperature and salinity (Oliveira *et al*., 2007). In the present study, the protein content of *C.lucifera* was higher than the other studies in Chinese mitten crab (*Eriocheir sinensis*) (Chen *et al*., 2007), *Podopthalmus vigil* (Sudhakar *et al*., 2011) and *Cancer pagurus* (Barrento *et al*., 2010) as well as lower than *Callinectes pallidus* and *Cardisoma armatum* (Elegbede and Fashina-Bombata, 2013) and *Portunus sanguinolentus* (Soundrapandian *et al*., 2009). Proteins are molecular tools that perform an astonishing variety of functions. In addition, to serving as structural materials in all living organisms, they are involved in such diverse functions in catalysis, metabolic regulation, transport and defense (Trudy and James, 2010). Lipids are highly efficient as sources of energy and they contain more than twice the energy of

Table 3 Fatty acid profile (g/100g) of different marine crabs

Fatty acid profile (g/100g)		*Portunus pelagicus*	*Portunus gladiator*	*Charybdis lucifera*
SFA	Palmitic acid	4.98±0.12[a]	1.11±0.01[c]	2.04±0.01[b]
	Stearic acid	10.86±0.22[a]	1.03±0.00[c]	2.94±0.04[b]
	Margaric acid	0.91±0.04[b]	0.20±0.02[c]	1.11±0.00[a]
MUFA	Oleic acid	12.89±0.02[a]	0.40±0.05[c]	1.09±0.00[b]
PUFA	α-Linoleic acid	10.97±0.01[a]	1.04±0.04[c]	1.95±0.05[b]
	Linoleic acid	15.85±0.05[a]	0.84±0.04[c]	4.05±0.01[b]
	Morotic acid	1.39±0.06[a]	0.39±0.02 [c]	0.84±0.01[b]
	EPA	0.89±0.00[a]	0.62±0.05[b]	0.41±0.01[c]
	DHA	0.63±0.00[a]	0.49±0.00[b]	0.37±0.07[c]
	EPA/DHA	**1.41**	**1.26**	**1.10**

Results are the mean value in triplicates ± Standard deviation (SD) with significant difference at P<0.05.
Identical lower case superscripts denote similar values horizontally.
SFA, **MUFA** and **PUFA** represent Saturated, Mono unsaturated and Poly unsaturated fatty acids respectively.
EPA and **DHA** represent Eicosapentaenoic and Docosahexaenoic acid respectively

carbohydrates and proteins (Okuzumi and Fujii 2000). Lipid content of *P. pelagicus* had higher amount than the previous studies *Cancer pagurus* (Anacleto *et al*., 2011), *Eriocheir sinensis* (Chen *et al*., 2007) and *Podopthalmus vigil* (Sudhakar *et al*., 2011). The proximate composition of edible tissues generally reflects their physiological functions, metabolic needs and available diet (Rosa and Nunes, 2003b: Vinegre *et al*., 2007).

Amino acids are the building blocks of proteins and also play a central role as intermediates in metabolism (Baldwin, 2003). In addition, amino acid molecules are linked together to form proteins. The kind of protein that results is indicated by the types of amino acids involved and the sequence in which the amino acids are arranged (Farr, 2002). The muscle is apparently the main protein-storage location in crustaceans. In decapods, free amino acids in the tissues reach levels ten times higher than those observed in vertebrates. From the present study, the amino acid composition (g/100g) of different crabs were represented in table 2. They showed significant differences between the species ($p < 0.05$). Totally ten essential amino acids (EAA) and non-essential amino acids (NEAA) respectively were reported in all the species. *C. lucifera* (3.92 g/100g) had higher amount EAA followed by *P. gladiator* (3.33 g/100g) and *P. pelagicus* (2.79 g/100g). They showed minimum differences between the species. Threonine (0.57 g/100g), valine (0.63 g/100g), arginine (0.54 g/100g) was dominant in *C. lucifera*. Meanwhile, metheonine (0.69 g/100g) was found to be high in *P. gladiator*. Methionine is powerful anti-oxidant and a good source of sulfur, which prevents disorders of the hair, skin, and nails, assists the breakdown of fats, thus helping to prevent a buildup of fat in the liver and arteries that might obstruct blood flow to the brain, heart, and kidneys. It helps to detoxify harmful agents such as lead and other heavy metals, helps diminish muscle weakness, prevents brittle hair, protects against the effects of radiation, beneficial for women who take oral contraceptives because it promotes the excretion of estrogen, reduces the level of histamine in the body which can cause the brain to relay wrong messages, helpful to individuals suffering from schizophrenia (Bruce Barber, 2013). From the present study, high amount of isoleucine was recorded in *P.pelagicus* (0.31 g/100g) when comparing other species. Isoleucine is necessary for the heamoglobin formation, stabilizes and regulates blood sugar and energy levels (Phyllis and CNC, 2006). Histidine was recorded in *C. lucifera* (0.41 g/100g), *P.pelagicus* (0.39 g/100g) and *P. gladiator* (0.33 g/100g). Similar result were recorded in previous study in Chinese mitten crab (*Eriocheir sinensis*) (Chen *et al*., 2007). Histidine is an indispensable amino acid involved in many metabolic functions including the production of histamines, which take part in allergic and inflammatory reactions. It plays very important role in maintaining the osmoregulatory process and is related to energy production or is used in other metabolic pathways during certain emergencies harsh conditions (Abe and Ohmama, 1987). Totally 10 NEAA were recorded in the present study. Among the various species, *C. lucifera* had significantly higher NEAA (4.81 g/100g) than the other three crabs. Glutamic acid (0.61 g/100g), alanine (0.59 g/100g), asparagine (0.95 g/100g), proline (0.50 g/100g) was maximum in *C. lucifera*. While, the high amount of tyrosine recorded in *P. gladiator*. Similarly, *P. pelagicus* had large

amount of glycine and serine. Cysteine was not recorded in *C. lucifera*. While, equal amount of aspartic acid recorded in *P. gladiator* and *P.pelagicus.* It was lower when comparing the previous studies in *Callinectes sapidus* (Kucukgulmez and Celik, 2008), *Charybdis natator* (Soundarapandiyan *et al*., 2014) and in shrimp *Aristeus virilis* (Karuppasamy *et al*., 2014). Several studies suggest that these amino acids may participate in osmoregulation and in the control of cellular volume (Chang and O'conner, 1983; Schein *et al*., 2004).

Meanwhile lipids are important biochemical components of marine food webs because they are carbon rich and provide a concentrated source of energy (Parrish, 1988) and also lipids are now examined routinely as biomarkers in ecological studies and as tools to understand large-scale oceanographic processes (Budge *et al*., 2006). Fatty acid composition (g/100g) of different crab species is presented in Table-3. In all the three species the main SFA, MUFA and PUFA were analyzed. Fatty acid profile showed significant difference between the species ($p<0.05$). Among saturated fatty acids (SFA), *P.pelagicus* had the highest total SFA than the other species. Stearic acid was highly accumulated in *P.pelagicus* (10.86 g/100g). This is three fold higher than the *C.lucifera* (2.94 g/100g). Present result was higher than the other study of *Callinectus sapidus* (Celik *et al*., 2004), *Portunus pelagicus* (Wu *et al*., 2010) and shrimp *Melicertus canaliculatus* (Sri Sakthi Priyadarshini *et al*., 2015). While, Palmitic acid in *P.pelagicus* (4.98 g/100g) have been higher than the other crabs. In MUFA, oleic acids were observed in *P.pelagicus* (12.89 g/100g), *C. lucifera* (1.09 g/100g) and *P. gladiator* (0.40 g/100g). When comparing between the species *P.pelagicus* had higher amount of oleic acids. It's indicated *P.pelagicus* possessed large amount of MUFA. Among PUFA, the amount of linoleic (12.89 g/100g), α-linoleic (1.09 g/100g) and morotic acid (0.40 g/100g) in *P.pelagicus* dominant than the *C. lucifera* and *P. gladiator*. Linolenic acid of *P.pelagicus* higher than the other studies in same species meat (Wu *et al*., 2010) and eggs (Soundrapandian and Rajnish Kumar Singh, 2008).

The long-chain PUFA, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may be acquired mainly from seafoods `conditionally essential' for infant growth and development (Shahidi and Wanasundara,1998). Therefore, increased consumption of marine lipids has been suggested in order to increase the dietary intake of PUFA. Based on the present results, EPA and DHA level was highly found in *P. pelagicus* (0.89 and 0.63 g/100g). Also the ratio of EPA/DHA showed only minimum variation between the species. It indicates, all species can be suggested as a nutrient rich diet. Previous studies made on the fatty acids of crabs *Carcinus meanus* (Benjakul and sutthipan, 2009), *Callinectus sapidus* (Celik *et al*., 2004), *Cancer pagurus* (Anacleto *et al*., 2011) has shown a rich source of both eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA). Lipids and particularly polyunsaturated fatty acids (PUFA) have long been known to be essential for the maintenance of good health of any individual. Omega-3 long-chain PUFA, including EPA and DHA, are dietary fats with an array of health benefits (Su *et al*., 2008) and are essential for proper foetal development and healthy aging (Dunstan *et al*., 2007). DHA is a key component of all cell membranes and it is found in abundance in the brain (Krauss- Etschmann *et al*., 2007). EPA and DHA are also the precursors of several metabolites that are potent lipid mediators and hence considered by many investigators to be beneficial in the prevention or treatment of several diseases (Serhan *et al*., 2008). Also studies have shown that EPA and DHA prevents medical disorders in heart and circulatory diseases (Connor and Connor, 1997) and the latter is also effective in skin disorders, aids brain development and forms a good part of retina of the eye (Lee *et al*. 1985). Thus the present study to conformed crabs have good source of protein, lipid, amino acid and fatty acids.

CONCLUSION

It can be concluded that marine crabs of different species from Mandapam coast had good quality proteins, highest levels of amino acids. Further, the meat of *P. pelagicus* could be good source of fatty acids especially EPA and DHA. These indices suggest that the crab are very healthy for human consumption and is also suitable for processing into different crab products.

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