NUTRITIONAL ANALYSIS OF SOME SELECTED FISH AND CRAB MEATS AND FATTY ACID ANALYSIS OF OIL EXTRACTED FROM PORTUNUS PELAGICUS

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Abstract: The meat of male *P.pelagicus*(n=3) was analyzed for protein, fat, moisture and ash contents by proximate analysis. The flesh of shark (*Caracharhinus* spp.)(n= 3),thalapath (*Istiophorus* spp.)(n= 3) were also analyzed for water-soluble protein content. Peptide-mapping was also carried out for the water-soluble protein fraction of all three types of samples. Moreover,the sub-samples (n=3) of the crab meat samples and commercial fish oil samples (n=3) were also analyzed for fatty acid profile and content, using gas chromatography. The results of proximate analysis revealed a composition of, 89.4±0.072 %(mean ±SE) moisture, 75.7±0.069% protein, 13.0±0.002% ash and 02.2±0.047% fat, in crab meat. The analysis water-soluble fraction revealed species-specific patterns on SDS-PAGE demonstrating greater amounts of myosin heavy chain and fimbrin compared to that of shark and thalapath flesh. As it was expected, a high content of polyunsaturated fatty acids were found in crab oil, representing 40.68 % of the total fatty acid content. The fatty acid profile exhibited greater percentages of oleic acid (28.03%) and eicosapentaenoic acid (EPA) (12.12%) when compared to existing data related to that of commercial fish oil. In conclusion, the study revealed high protein content and a low fat content in the meat of *P. pelagicus*. The water-soluble protein profile, meat of P. pelagicus could possibly be differentiated by peptide mapping which shows thick bands for the myosin heavy chain (MHC) and fimbrin. Oil of *P. pelagicus* carries relatively greater amounts of EPA and oleic acid. Thus, the consumption of crabs would help to prevent nutritional deficiencies in the future.

Keywords: Crab meat, Fatty acid, Protein, Proximate composition

1 INTRODUCTION

According to Chen et al. (2007), muscle meat contributes mainly for protein and amino acids, which accounts around 80% of the total whole body protein. Ke et al. (1990) reported that crab body meat contains 82.9% water, 16.0% protein, 0.86% fat, and 1.7% ash and Krzynowek et al. (1982) reported that Jonah crab meat contains about 78% moisture, 16.2% protein, 1- 2% fat, and 1.5% ash. The analysis of fatty acids has become increasingly important, because more people have become aware of their nutritional and health implications.Lovern (1962) compared oils from special parts of fish and marine animals and found that large amounts of fatty acids are associated with phospholipids, glyceryl ethers (alkoxydiglycerides) and wax esters, depending on the source of oils and lipids. Seafood lipids are rich in polyunsaturated fatty acids such as EPA and DHA. These fatty acids have a peculiarity of health benefits, including prevention of sudden cardiac death (Leaf et al, 2003)and chemopreventive effects of cancer (Akihisa et al., 2004). Linolenic acid (LNA, 18:3n-3), linoleic acid (LA, 18:2n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were essential for the evaluation of nutritional quality of lipids (Gil, 2002). Omega 3 fatty acids can aid in reducing diseases of coronary artery (Skonberg and Perkins, 2002), inflammation (Harwood and Caterson, 2006; Gil, 2002; Shoda, et al., 1996), coronary heart disease (Harper and Jacobson, 2005), cancer (Roynette, et al., 2004), neurological disorders (Falinska et al., 2012), and can guarantee good health and normal development (Horrocks and Yeo, 1999; Innis, 2000; Voigt et al., 2000) and immune system (Sijben et al., 2009). Byrem and Strausburg (2000) mentioned about thin filaments consisting of four proteins: actin, tropomyosin, troponin and nebulin. The thick filaments are composed primarily of myosin, though titin is also often associated with the thick filaments. The protein bands in the 18 to 20 kDa range in the gels and wash supernatant were correlated with myosin light chain and troponin (Thorarinsdottiret al., 2002).The band at 190 kDa was probably the myosin heavy chain (Thorarinsdottiret al., 2002). Also crabs, among numerous other invertebrates are considered as an essential shell fishery product (Nalan et al., 2003). Thus, the objective of this study was to define the nutritive value and thereby to encourage an increase in the consumption and utilization of these species in Sri Lanka.

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2 MATERIALS AND METHODS

Determination of nutrient composition of meat of *Portunus pelagicus* (blue swimming crab) to compare its protein composition with that of some selected fish and to analyze the fatty acid composition of oil extracted from *P. pelagicus.*

2.1 Sample Collection

Mature, male *P.pelagicus* crabs were collected from Negombo and Kalpitiya Fishing area in Sri Lanka. The meats of Crabs (Crunchy chest/ Walking legs/ Tissue) were taken and mixed to prepare samples. The morphometric measurements [carapace length (CL), carapace width (CW) and weight] of all samples were done. The morphometric measurements of crab carapace were done using a vernier caliper and ruler.

Fig. 1. *Mature, male Portunus pelagicus crab.*

2.2 Sample preparation and storage

Carapace and meat were sampled separately and meats were stored at -80℃. Muscle meat and edible viscera were separately homogenized and stored at -80℃ prior to further analysis of proximate compositions, protein and fatty acids profile and contents. Total fatty acid content and fatty acid composition were determined simultaneously in the crab oil samples (n=3) and Fish oil sample (n=3). Crabs oil samples were stored at -20℃. Fatty acid analysis performed in triplicates consisted of two consecutive steps; preparation of fatty acid methyl ester (FAME) and chromatographic analysis. In this work, FAME was prepared by the following two procedures.Were evaluated separately, Crabs meats with three seafood types, namely,the flesh of shark (Caracharhinusspp.)(n=3), thalapath (Istiophorusspp.)(n= 3) were also analyzed for water-soluble protein content

2.3 Laboratory Analysis

2.3.1 Proximate Analysis

Proximate analysis of *Portunus pelagicus* meat was carried out. It consisted of analysis of moisture content, analysis of crude protein contentusing Micro-Kjeldahl method, analysis of crude fiber and analysis of oil using Soxhlet method. The methods described by Pearson (1976) were carried out. The ash content was determined using the method of Pomeranz and Meloan (1994) Dry matter, ash, crude fat, and crude protein content of meat of *Portunus pelagicus* were measured according to the AOAC (2005). Prior to proximate analysis *P.pelagicus* crabs were dried at 60 °C, until constant weight was reached, using a drying oven (Yamato, Japan).Then the samples were ground into powder using a grinder (MX-151SG1, Panasonic, China). Finally samples were packed air-tight and were stored in a desiccator until analysis (AOAC, 2005).

2.3.2 Oil Extraction Procedure from crab meat

Total lipids of triplicate samples of each finely ground meat samples were extracted using chloroform: methanol (2:1, v/v) according to the method of Folch et al. (1957). Tissue samples were thawed, all visible fat and connective tissue were removed. The sample was then ground and a 0.5 g of sub sample was weighed into a glass tube (16×150 cm) pre-rinsed with heptane. To the sample was then added 2.75 ml of methanol and 1 ml of internal stranded (prepared by dissolving 100 mg of heneicosanoic acid (C21:0) (Sigma-Aldrich, UK) in 100ml of chloroform was vortexed for 90 seconds in 30 second-long intervals with a 1 min gap between each vortexing. To the tube was then added 3.75 ml of chloroform, vortexed for 2 min and placed in an ultrasonic water bath (Branson 2510, Danbury, USA) for 30 min. After adding 3.75 ml of 2% NaCl solution (prepared by dissolving 20 g NaCl in 1 liter of distilled water) and 3.75 ml chloroform, the sample was shaken vigorously in a shaker (Kika-labortechnik, Germany). The sample was then centrifuged (Beckman Avanti, UK) at 1700 g for 20 min and the chloroform layer at the bottom was removed into an extraction tube (16×150 cm), using a glass Pasteur pipette. The sample was then filtered through a glass wool column into an extraction tube as follows: a glass Pasteur pipette was cut to remove the top contoured piece, plugged with approximately 1 inch glass wool pushing the wool into the area immediately above the narrowed pipette, and adding approximately 1 inch of anhydrous sodium sulphate. This process is time-consuming, however, yields a higher percentage of impurities in the oil. Centrifugation speeds up the process and creates a more pure crab oil product.

2.3.3 Procedure of preparing fatty acid methyl esters

Fatty acid profiles of fat extracted from the blue crab samples were determined by gas chromatography (GC) of methyl esters. Methyl esters were prepared by under the following method,100mg of crab oil and fish oil sample was weighed into a separate glass tube with the sample was then added Add 3 ml of 5% H_2SO_4in methanol. Heat for 1 hour in oven at 100 ^{0}C and it was gently shaking for every 15 min in 30 second. After one hour, Samples place on Cool in cold water/crushed ice layer and the tube was then added 2 ml distilled water + 3 ml hexane. The sample was then Shake thoroughly and allow separating of the two layers and now Draw 2 ml of the upper layer (can store at -80^{0}C).in to the glass tube draw upper layer Evaporate using N_2 gas and finally Adding 150 µl hexane. The sample was then now ready for GC analysis.

2.3.4 Gas chromatography procedure and fatty acid analysis

Fatty acids methyl esters (FAME) were analyzed on an SHIMADZU GC-14Bgas chromatography, equipped with a flame ionization detector (FID) and a SP-2560 fused silica capillary column (100 m, 0.25 mm and 0.20 µm film thickness). The initial temperature of the column was held at 140°C for 5 min, then programmed to increase to 220°C at a rate of 4 °C min−1, and then held for 10 min. Helium was used as the carrier gas at a flow rate of 0.5 mLmin−1, and the column head pressure was 280 kPa. Both the injector and the detector were set at 260°C. The identification and quantification of fatty acids were done using Gas Chromatography (Hewlett Packard 5890 model). Identification of fatty acids was carried out by comparing sample FAME peak area and relative retention times. Concentration of the individual fatty acids were calculated and expressed as mass percentages of total identified fatty acids. Peak areas of the gas chromatogram area represented their fatty acid quantity and total fatty acid.

2.3.5 Analysis of water soluble protein of crab meat

Protein analyses of crab meat (n=3) with three seafood types, namely,theflesh of shark (*Caracharhinus* spp.)(n=3),thalapath (*Istiophorus* spp.)(n= 3) were also analyzed for water-soluble protein content. Water-soluble protein fractions were extracted from all three types of samples and were further analyzed for their profile, using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Initially protein was extracted

from samples and then protein bands were identifying using gel electrophoresis. Water Soluble Protein Extracted From Sample, Following in this the Method, Weighed 0.5 g from each seafood meat sample separately and was put into a micro-centrifuge tube. Samples were then stored at 4^0 C for 24 hrs. After adding 1 ml PBS. After 24 hours, each sample was centrifuged 10000 rpm for 10 min. Finally, the supernatant was removed into a new micro-centrifuge tube. Note: supernatant mixture solution carries the water soluble protein.

3 Results and Discussion

The mean CW and CL were 15.75 cm and 7.37cm for *pelagicus* crabs. The weights of *p.pelagicus* crabs were 283.75± 43.54 g (mean ±SE) and Total Flash in the body 128.75± 18.34 g (mean ±SE).

3.1 Proximate composition

Results of proximate composition of *P. pelagicus* crab meals are as follows.

TABLE 1

CRUDE PROTEIN, CRUDE FAT, CRUDE FIBER, ASH AND TOTAL SOLIDS CONTENT OF P.PELAGICUS CRAB MEALS (75G)

Component	Percentage (%)
Moisture	89.4±0.072
Dry matter	-
Ash	13.0±0.002
Crude protein	75.7±0.069
Fat	02.2±0.047
crude fiber	-

According to the proximate composition *P. pelagicus* Crab meals have high protein contents. This study also shows that crabs have high protein (75.7±0.069 (mean ±SE) for *P.pelagicus*) and low fat contents (02.2±0.047 (mean ±SE) for *P. pelagicus*).

3.2 Fatty acid composition

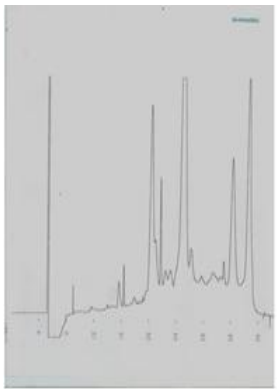

Fig3.1: *GC graph of crab oil*

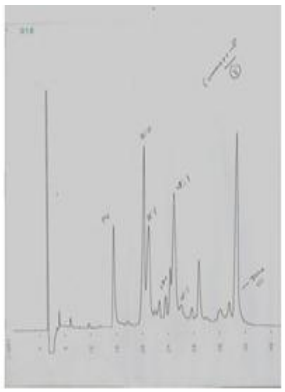

Fig3. 2: *GC graph of fish oil*

Fatty acids composition; SFAs, MUFAs, PUFAs, PUFA/SFA, n-3 acids, n-6 acids and the n6/n3 ratio of male crabs meat are presented in Table 2.

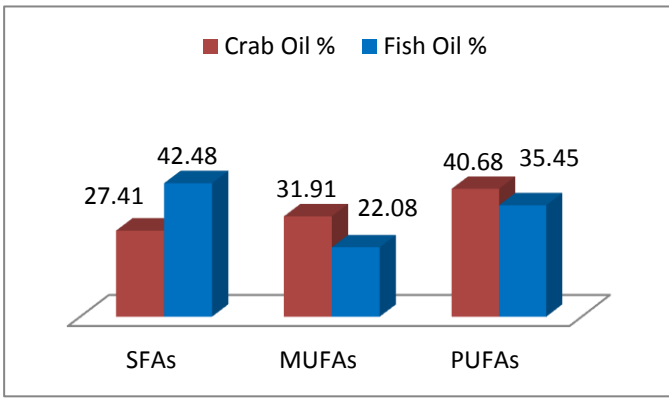

Fig. 2. *Fatty acid percentages of crab oil and fish oil*

As it was expected, a high content of polyunsaturated fatty acids was found in crab oil, representing 40.68 % of the total fatty acid content.The total PUFA value was the highest in meat of male swim crabs.The dominant SFAs were palmitic acid (20.18%) for the crab species. Oleic acid (28.03%) was the major MUFA in all crab meats, PUFAs followed by EPA (Eicosapentaenoic acid) (12.12%) and DHA (Docosahexaenoic acid) (17.17%).

TABLE 2

IDENTIFICATION OF CRAB AND FISH FATTY ACIDS

		Fatty acid	(%) crab	(%) fish
SFAs	myristic acid	C14:0	3.63	10.23
	palmitic acid	C16:0	20.18	17.97
	palmitoleic acid	C16:1	3.60	14.28
MUFAs	Stearic acid	C18:0	3.88	3.63
	Oleic acid	C18:1	28.03	18.45
PUFAs	Linoleic acid	C18:2	7.32	3.93
	ALA	C18:3	4.08	2.53
	EPA	C20:5	12.12	3.87
	DHA	C22:6	17.17	25.12

SFAs=Saturate fatty acids , MUFAs=Monosaturate fatty acid , PUFAs= Poly unsaturated fatty acids ,ALA= Alpha Linolenic acid, EPA= Eicosapentaenoic acid, DHA= Docosahexaenoic acid

The fatty acid profile exhibited greater percentages of oleic

acid (28.03%) and EPA (12.12%) when compared to existing data related to that of commercial fish oil.

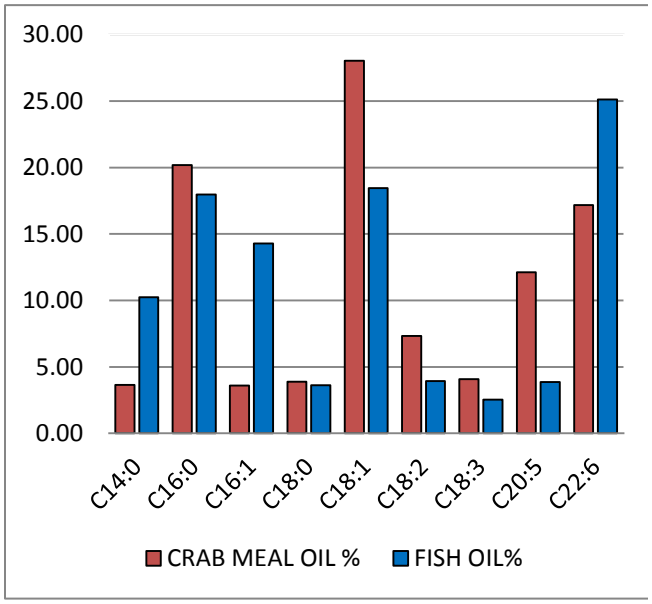

Fig. 3. *Fatty acid composition percentage of crab and fish oil.*

3.3 Water Soluble Proteins in Crab meat

Lane Marker carries the molecular marker. Lanes S1, S2 and S3 carry shark meat whereas; lanes T1, T2 and T3carry

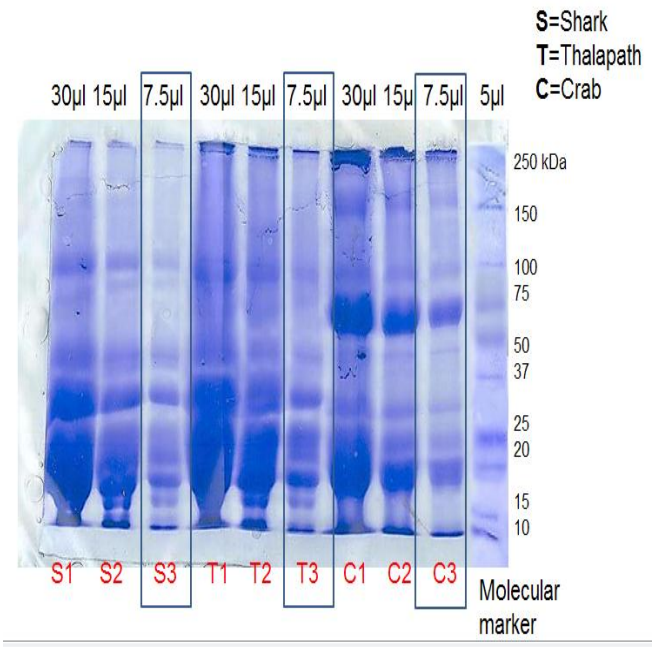

Fig. 4. *Electrophoretic patterns of three different flesh samples in three different volumes.*

proteins of Thalapath flesh. Lanes C1, C2 and C3 contain crab meat samples, respectively. Moreover, peptide-mapping was also carried out for the water-soluble protein fraction of all three types of samples.SDS-PAGE gels (Figure 4.4) indicated that the crab meat consisted of proteins in the 190 kDa range, 50-100 kDa range, and 15-50kDa range.

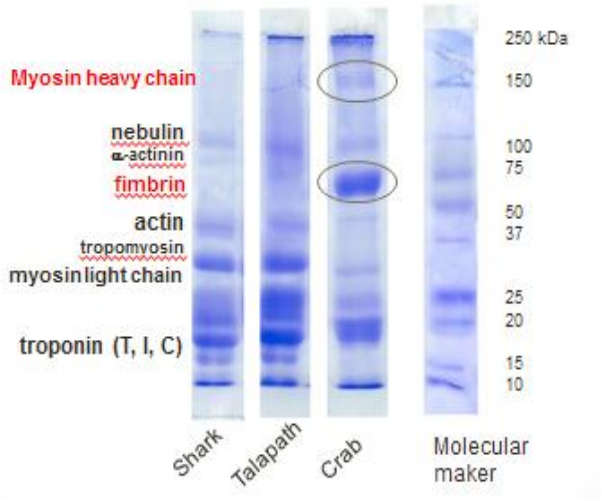

Fig. 5. *Crab meat sample shows high concentration of myosin heavy chain and fimbrin.*

The water-soluble fraction analysis revealed species-specific patterns on SDS-PAGE demonstrating greater amounts of myosin heavy chain and fimbrinin shark and thalapath flesh. Fish flesh samples showing more actin and tropomyosin peptide. The water soluble protein band at 210kDa, was identified possibly as the myosin heavy chain peptide, with fimbrinproteins in the 68kDa range.The banding in the 36 to 45kDarange was a fish sample containing both actin and tropomyosin. The band was identified possibly as a tropomyosin, 37 kDa,was found in both fish samples. Interestingly, the myosin heavy chain was identifiedas the major protein component, interfering with crab proteins.

4 Conclusion

According to the results obtained in this study, crab muscles contain as much as 75.7±0.069% of the total protein, and are a low-fat and high-protein food with relatively well-balanced essential amino acid compositions. The most widely available dietary source of EPA and DHA is crabs, such as *P.pelagicus*. It can then be inferred that the crab's meats (*P.pelagicus*) most suitable as a supplement of protein and mineral matter so as to balance human nutrition. Hence the consumption of crabs (*P.pelagicus*) would help as a good source of nutrients in human nutrition. Many fish, particularly at the higher end of the food chain, contain dangerous amounts of mercury and are not recommended as frequently eaten foods. However, crab-meat do not pose this threat as they are not top predators in the food chain. Furthermore, pregnant women and lactating women are advised not to consume large amounts of fish due to the mercury content but crab meal appears good for health of pregnant women, with regard to the mercury content. Fish oil and crab oil has been shown to lower blood pressure; those individuals who usually have low blood pressure should not take the crab and fish oil. Out of the three types of samples, crab meat consists of myosin heavy chain protein band. The protein profile, particularly the water-soluble fraction showed species-specific differences in relation to the peptide composition. Thus, meat of *P. pelagicus* could possibly be

differentiated by peptide mapping which shows thick bands for the myosin heavy chain (MHC) and fimbrin. With these results on *P. pelagicus;* it can be supplement of protein and Fatty acid composition so as to augment the human nutritional requirements. The species could also be used in the formulation of well-balanced animals feed.

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