CHAPTER 2

CHEMICAL COMPOSITION AND THERMAL PROPERTY OF CUTTLEFISH (*SEPIA PHARAONIS*) MUSCLE

2.1 Abstract

The chemical composition and thermal property of cuttlefish (*Sepia pharaonis*) muscle were studied. The head and mantle contained 11.9-14.9% protein, 0.5% fat, 1.2-1.3% ash and 0.6-1.8% collagen. Lipids from the head and mantle contained phospholipid as the major component (78.6-87.8% of total lipid), with 10.6-19.5% diglyceride. Polyunsaturated fatty acids constituted 50.3-54.9% of fatty acids with a high content of DHA and EPA. The n-3 PUFA contents were greater than n-6 PUFA. The C16:0 and C18:0 were the most abundant saturated fatty acid in the head and mantle. The cuttlefish muscle consisted of myofibrillar proteins as the major protein (53.1-58.4%). Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed that myosin heavy chain (MHC), paramyosin and actin were the major proteins and varied with the portions. Differential scanning calorimetric (DSC) study revealed three transitions corresponding to the thermal denaturation of myosin and paramyosin, connective tissues and actin, at temperatures of 49.8-50.3, 59.8-60.3 and 74.7-78.8 °C, respectively. Zinc and iron were the dominant trace minerals in both portions.

2.2 Introduction

Cephalopods including cuttlefish, squid and octopus are the important marine resource since they are rich in taste and have few inedible parts (Sikorski and Kolodziejska, 1986). Additionally, the muscle contains low saturated fat (Navarro and Villanueva, 2003), high vitamin C content (Passi *et al*., 2002) and is a good source of minerals such as calcium, potassium, zinc, iron, phosphorus and copper (Bustamante *et al*., 2000; Ichihashi *et al*., 2001; Craing and Overnell, 2003). However, they consist of high sodium and cholesterol contents

(Okuzumi and Fujii, 2000). Cephalopods are not only consumed as fresh, but also manufactured into processed food in huge quantity such as dried (Kugino *et al*., 1993), frozen (Paredi and Crupkin, 1997; Ueng and Chow, 1998), and chilled products (Hurtado *et al*., 2001), etc.

The structure of mantle, the major edible portion of squid, is considerably different from that of muscles of fish, bird and mammalian. It has many unique differences in protein composition, resulting in the different musculature (Sikorski and Kolodziejska, 1986) and biochemical properties of proteins (Paredi and Crupkin, 1997). The thick filament of squid is formed from a composite core to protein called paramyosin, around with the myosin is coiled in structure that is unique to squid (Sikorski, 1994). Generally, squid has a longer and thicker filament than those of vertebrates (Kantha *et al*., 1990). The tough texture is caused by the peculiar structure of squid tissue. The mantle is composed of several layers of muscle fibers and connective tissue sheets, sandwiched at various angles to each other (Otwell and Hamann, 1979). Collagen, which is present in considerably large amounts up to about 11% of total protein in the muscle of *Illex argentinus*, plays a significant role in the textural changes due to cooking (Kolodziejska *et al*., 1987).

Cuttlefish has been increasingly consumed and exported. Mostly, cuttlefish is processed as frozen product. *Sepia pharaonis* and *Sepia aculeata* are two main species commonly used for cuttlefish processing in Thailand. However, the former is more abundant than the latter. The variation in quality of cuttlefish, especially with different fishing ground, is one of problems facing in cuttlefish industry. So far, a little information regarding the chemical composition of cuttlefish has been reported. The objective of this study was to determine the chemical compositions and thermal properties of cuttlefish head and mantle.

2.3 Materials and Methods

Chemicals

Chloroform, methanol, sulfuric acid, nitric acid, isopropanol, chloramines T, ρ-dimethylamino-benzaldehyde were purchased from Merck (Darmstadt, Germany). Sodium dodecylsulfate (SDS) and □-mercaptoethanol (□ME) were obtained from Sigma (St. Louis,

MO, USA). Acrylamide, N,N,N',N'-tetramethylethylenediamide (TEMED) and bis-acrylamide were obtained from Fluka (Buchs, Switzerland).

Sample preparation

Cuttlefish (*Sepia pharaonis*), in the size range of 8-10 cuttlefish/kg, caught in the Gulf of Thailand in June 2003 and offloaded 24 h later, were taken from a dock in Songkhla, Thailand. The samples were placed in ice with a cuttlefish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1 h. Cuttlefish (1 kg) were randomly taken, pooled and used as the composite samples. Three different composite samples from different batches caught at different times in June were used in this study. The edible portions of the cuttlefish were separated into mantle and head portions. The mantle was cleaned, deskinned and eviscerated. For the head including tentacles, the eyes were removed but skin was left on. The head portion is generally consumed with the skin attached. Each portion was powderized by blending the samples with liquid nitrogen. The samples were kept in ice until analysis.

Proximate analysis and determination of collagen content

Cuttlefish muscle was determined for moisture, ash, fat and protein contents according to the method of AOAC (1999). The total collagen content was measured on the basis of hydroxyproline content according to the method of Bergman and Loxley (1963). Samples were hydrolyzed in 6 N HCl at 110 $^{\circ}$C for 24 h prior to determination and 11.11 was used as a converting factor for calculating collagen content (Mizuta *et al*., 2003).

Determination of lipid composition and fatty acid profile

Cuttlefish lipids were extracted as described by Bligh and Dyer (1959). The fatty acid compositions were determined as fatty acid methyl esters (FAME) using a gas chromatography, GC-14A (Shimadzu Co., Tokyo, Japan) equipped with fused silica capillary

column Carbowax-30 M (30 m, 0.25 mm ID) and flame ionization detector (FID). Helium was used as the carrier gas at a flow rate of 30 cm/sec. The initial temperature of the column was set at 170 °C and increased to 225 °C with a rate of 10 °C/min and then held at 220 °C for an additional 20 min. The detector temperature was set at 270 °C, while the temperature at the injection port was maintained at 250 °C. Retention time of FAME standards was used to identify chromatographic peaks. Peak area was quantitated and expressed as % of total lipid (AOAC, 1999).

The lipid compositions were determined by thin layer chromatography/flame ionization detection analyzer (TLC-FID). Scanned quartz rods (silica gel powder coated Chromarod S III) were dipped in 3% boric acid solution for 5 min, dried and rescanned with the TLC-FID analyzer. The sample solution (1 μL) were spotted on the rod and the separation were performed in the mixtures of benzene: chloroform: acetic acid (52: 20: 0.7) for approximately 35 min. Then, the rods were dried in an oven (105 °C) for 5 min before analyzing with the flame ionization detector. The analytical condition was H_2 flow rate of 160 mL/min, air flow rate of 2000 mL/min and scanning speed of 30 sec/scan. Retention time of lipid composition standards was used to identify chromatographic peaks. Peak area was quantitated and expressed as % of total lipid.

Fractionation of muscle protein

Cuttlefish muscle was subjected to fractionation according to the method of Hashimoto *et al*. (1979). Each fraction contains different compositions, e.g. non-protein nitrogen, sarcoplasmic protein, myofibrillar protein, alkali-soluble protein and stroma. Nitrogen content in each fraction was measured by Kjeldahl method (AOAC, 1999). Protein patterns of whole muscle and each fraction were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) made of 4% stacking gel and 10% running gel according to the method of Laemmli (1970).

Thermal transition measurement

Thermal transition of muscle from cuttlefish head and mantle was measured using the differential scanning calorimetry (DSC) (Perkin-Elmer, Model DSCM, USA). The samples (15-20 mg wet weight) were placed in the DSC hermetic pans, assuring a good contact between the sample and the pan bottom. An empty hermetic pan was used as a reference. The samples were scanned at 10 °C/min over the range of 10 to 100 °C. T_{max} was measured and denaturation enthalpies (ΔH) were estimated by measuring the area under the DSC transition curve.

Determination of trace mineral content

Iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), cadmium (Cd) and lead (Pb) contents were determined by the inductively coupled plasma optical emission spectrophotometer, ICP-OES (Perkin Elmer, Model 4300 DV, USA) according to the method of AOAC (1999). Cuttlefish head and mantle (4 g) were homogenized with 4 mL of concentrated nitric acid. The homogenate was heated with hot plate until digestion was completed. The digested samples were transferred to a volume metric flask and the volume was made up to 10 mL with deionized water. The solution was subjected to ICP-OES analysis. Flow rates of argon to plasma, auxiliary and nebulizer were kept at 15, 0.2, and 0.8 L/min, respectively. Sample flow rate was set at 1.5 mL/min. The wavelengths for analysis of Fe, Cu, Mn, Zn, Cd and Pb were 238.2, 327.4, 257.6, 206.2, 228.8 and 220.4 nm, respectively. The concentration of mineral was calculated and expressed as mg/kg sample.

Statistical analysis

Completely randomized design (CRD) was used in this study. The data obtained from the study was subjected to independent sample T test. The statistical analysis was performed by SPSS 11.0.

2.4 Results and Discussion

The proximate analysis and collagen content

Mantle was the main edible portion of cuttlefish constituting about 38.2% and head portion was 25.6% of total body. The chemical compositions of both head and mantle of cuttlefish are shown in Table 3. Head portion contained lower protein content and higher moisture than mantle portion. Fat content in both portions was 0.5 % and ash content was 1.2-1.3%. The result was in agreement with the 4th Amended Japanese Standard Food Content Tables (Okuzumi and Fujii, 2000), the edible part of squid contains 81.8% water, 15.6% crude protein, 1.0% crude fat and 1.5% crude ash. The lipid content of raw squid was about 1.0-2.0% (Okuzumi and Fujii, 2000). But it was different from Suyama and Kobayashi (1980) who reported that *Sepia pharaonis* contained 76.4% moisture, 20.2% protein, 1.4 % lipid and 1.9% ash. The chemical compositions of cephalopod are dependent on species, growth stage, habitat, season and anatomical region of cephalopod (Kreuzer, 1984). The head portion possessed higher collagen content than the mantle. Since head and tentacle had the skin on, skin might serve as the important source of collagen. Mizuta *et al*. (1994) reported that the approximate collagen content of squid (*Todarodes pacificus*) in mantle and skin was 1.0 and 3.4% per wet tissue, 4.6 and 21.9% per dry tissue, and 5.4 and 28.4 % per crude protein. The function of each of the anatomical regions of the live organisms can have a considerable effect on the amount of collagen. The arms perform a grasping function and therefore have to withstand more strain than the mantle (Moral *et al*., 2002).

Table 3. Chemical compositions of cuttlefish muscle (g/100g, wet basis)

Compositions	Head	Mantle
Moisture	84.42±0.13^{a}	82.78±0.05^{b}
Protein	11.90±0.14^{b}	14.91±0.61^{a}
Fat	0.52±0.01^{a}	0.47±0.01^{a}
Ash	1.29±0.02^{a}	1.20±0.24^{a}
Collagen	1.87±0.21^{a}	0.64±0.22^{b}

Values are given as mean ± SD from triplicate determinations. Different superscripts in the same row indicate significant differences ($p<0.05$). Converting factor for collagen calculation is 11.11.

Lipid composition and fatty acid profile of cuttlefish

Phospholipid was the major component (78.6-87.8%) of lipids in both head and mantle of the cuttlefish, followed by diglyceride and trace amounts of triglyceride and free fatty acid (Table 4). The result was in agreement with Takama *et al.* (1999) and Kreuzer (1984). Unlike that of fish, squid lipid tends to be low in triglycerides and high in phospholipids (Okuzumi and Fujii, 2000). Therefore, most lipids in cuttlefish might be membrane lipids with high phospholipid content.

Table 4. Lipid compositions in cuttlefish muscle (% of total lipid)

Lipid compositions	Head	Mantle
Free fatty acid	1.42 ± 0.28^{a}	0.64 ± 0.02^{b}
Triglyceride	0.44 ± 0.10^{b}	0.90 ± 0.02^{a}
Diglyceride	19.50 ± 1.66^{a}	10.63 ± 2.07^{b}
Phospholipid	78.64 ± 1.91^{b}	87.83 ± 2.18^{a}

Values are given as mean ± SD from triplicate determinations. Different superscripts in the same row indicate significant differences ($p<0.05$).

The fatty acid profile of head and mantle of cuttlefish is shown in Table 5. PUFAs were found as the major fatty acids with the range of 50.3-54.9%. The contents of n-3 PUFA were 3.4 times greater than those of n-6 PUFA. C22:6 n-3(DHA) and 20:5 n-3(EPA) were the dominant PUFAs in lipid from both portions. DHA and EPA were found at the level of 31.6

and 8.3% in the lipid from head portion and 28.3 and 7.6 % in the lipid from mantle portion. DHA/EPA ratio in head portion (4.10) was higher than that in mantle portion (3.72). DHA and EPA are the most characteristic acid for cephalopods (Culkin and Moris, 1970; Danstan *et al*., 1988; Navarro and Villanueva, 2000). Navarro and Villanueva (2000) found that cephalopods in their early stages of growth show high requirement for PUFA. DHA constituted 20-30% of total fatty acid in lipids of cuttlefish, squid and octopus hatching. Arachidonic acid (C20:4 n-6) ranged from 1.1 to 1.9% (Passi *et al*., 2002). Gibson (1983) found that arachidonic acid constituted 9.8% of total fatty acid in an octopus from Southern Australia. Among the saturated fatty acids, C16:0 and C18:0 were the most abundant fatty acids in the lipid extracted from head and mantle of cuttlefish.

Composition of muscle proteins

Protein and non-protein nitrogenous components in both head and mantle portions of cuttlefish are shown in Table 6. Myofibrillar protein constituted as the major protein in both portions. Sarcoplasmic protein was found as the second predominant protein in the cuttlefish muscle. Stroma was higher in the head portion, compared to the mantle portion. From the result, the contents of non-protein nitrogenous components (NPN) in the muscle of head and mantle were 1.4 and 2.2 mgN/g, respectively. NPN is used as quality parameters for cephalopod, along with sensory aspect, such as smell and taste (Iida *et al*., 1992). NPN included different compounds such as free amino acid, imidazole, dipeptide nucleotides, trimethylamine oxide (TMA-O), trimethylamine (TMA), urea and products of postmortem changes (Sikorski, 1994; Foegeding *et al*., 1996). Most of these substances are indicative of changes since they are the substrates for contaminant bacterial growth in cephalopod muscle (Ruiz-Capillas *et al*., 2002). Okuzumi and Fujii (2000) reported that squid contains the highest content of a majority myofibrillar protein (77-85%), followed by sarcoplasmic protein (12 to 20%) and stroma protein (2-3%). The type of protein and their functional status are the two factors influencing the texture of cephalopod muscle (Moral *et al*., 2002).

Table 5. Fatty acid profile of cuttlefish lipid (% of total lipid)

Fatty acids	Head	Mantle
C14:0	1.1	1.2
C15:0	0.6	0.7
C16:0	17.7	20.3
C16:1 n-7	0.7	0.8
C17:0	1.5	1.7
C17:1	0.3	0.3
C18:0	9.6	11.0
C18:1 n-9	3.6	4.3
C18:1 n-7	1.6	1.7
C18:2 n-6	0.3	0.6
C18:3 n-3	0.1	0.1
C18:3 n-6	0.4	0.4
C20:0	0.2	0.2
C20:1 n-7	0.2	0.1
C20:1 n-9	3.4	3.6
C20:2 n-6	0.5	0.5
C20:3 n-3	0.3	0.3
C20:4 n-6	7.7	7.2
C20:5 n-3(EPA)	8.3	7.6
C22:4 n-6	1.5	1.4
C22:5 n-6,n-3	4.2	3.9
C22:6 n:3(DHA)	31.6	28.3
C24:1	0.4	0.2
Unidentified peak	4.2	3.6
Total	95.8	96.4

Saturated	30.7	35.1

Table 5. Fatty acid profile of cuttlefish lipid (% of total lipid) (continued)

Fatty acids	Head	Mantle
Monounsaturated	10.2	11.0
Polyunsaturated	54.9	50.3
n-3	42.3	38.1
n-6	12.6	12.2
n-3/n-6	3.4	3.1
DHA/EPA	3.8	3.7

Table 6. Protein and non-protein nitrogen components in cuttlefish muscle (mgN/g, wet basis)

Protein fractions	Head		Mantle	
Non-protein nitrogen (NPN)	1.44±0.10[b]		2.17±0.07[a]	
Sarcoplasmic	4.83±0.11[b]		7.13±0.22[a]	
	(28.35)	(33.71)		
Myofibrillar	9.04±0.35[b]		12.33±0.97[a]	
	(53.11)	(58.35)		
Alkali-soluble	0.90±0.02[a]		0.66±0.08[b]	
	(5.42)		(3.15)	
Stroma	2.23±0.07[a]		1.01±0.04[b]	
	(13.12)		(4.79)	

Values are given as mean ± SD from triplicate determinations. Different superscripts in the same raw indicate significant differences ($p<0.05$). Numbers in parenthesis represent percentage distribution.

SDS-PAGE patterns of different fractions from head and mantle portions of cuttlefish are shown in Figure 9. Proteins with low molecular weight were found in the sarcoplasmic fraction. For myofibrillar protein fraction, three major protein bands, corresponding to myosin heavy chain (MHC), actin and paramyosin, were identified. The paramyosin band in myofibrillar protein fraction of mantle portion was more intense than that found in head portion. The molecular weight of paramyosin from different species ranges from 95-125 KDa (Sikorski, 1994). Paramyosin constituted 14% of myofibril fraction of the striated muscles of squid (Sano *et al*., 1986). For alkali fraction, smear bands with low molecular weight were found. The differences in protein compositions may result in different properties and characteristics between both portions. Myofibrillar proteins from the bivalves *Aulacomya ater ater* (Molina) consist of MHC, paramyosin, actin, troponin and myosin light chain with MW of 200, 110, 42, 37 and 17 KDa (Paredi *et al*., 1998).

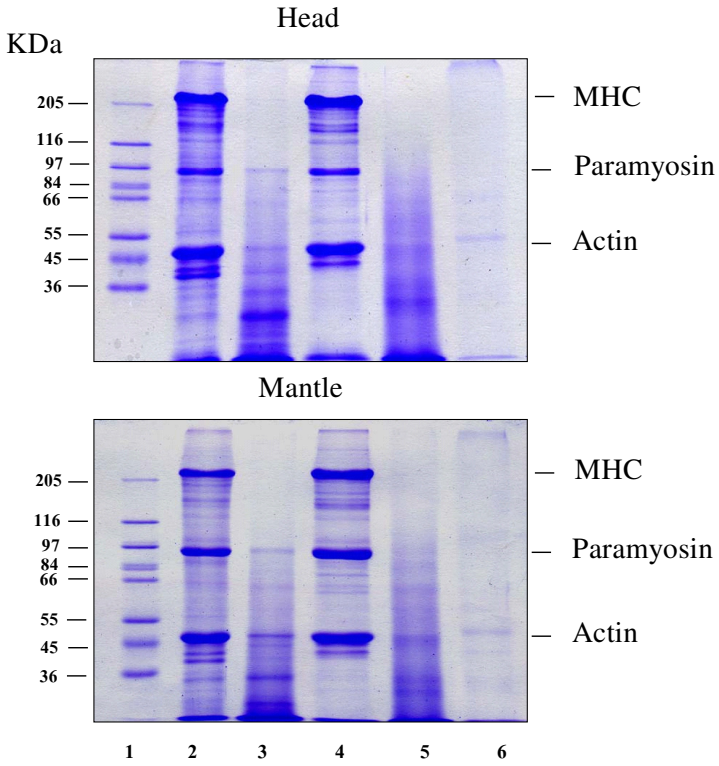

Figure 9. SDS-PAGE pattern of cuttlefish whole muscle and fractions, lane 1: high molecular-weight standard, lane 2: whole muscle, lane 3: sarcoplasmic protein fraction, lane 4: myofibrillar protein fraction, lane 5: alkali soluble protein fraction and lane 6: stroma fraction.

Trace mineral content in cuttlefish

The trace mineral contents of the cuttlefish head and mantle were different as shown in Table 7. Among all minerals tested, Zn was the dominant trace mineral in both portions. Cu and Fe were also found at the high content, but much higher content of Fe was found in head portion. Those minerals might contribute to oxidation of cuttlefish muscle during handling, processing as well as storage. Higher content of Cu was observed in mantle portion, compared with head portion. Pb was very low in both portions. Cu and Zn are essential trace metals required by a wide variety of metal-dependant enzymes. Cephalopods use the soluble Cu containing protein heamocyanin as a respiratory pigment (Decleir *et al*., 1978). However, Cd and Pb are the toxic mineral for consumption (Ichihashi *et al*., 2001). Zn has been found in several cephalopods at different concentrations: 12.8 ppm in squid (Gripe *et al*., 2000), 14.1 ppm in cuttlefish and 20.6 ppm in octopus (Miramand and Bentley, 1992). The major sources of minerals to marine organisms are seawater and feed (Ichihashi *et al*., 2001). The contamination of minerals from polluted environment might result in the accumulated minerals in cuttlefish muscle, which may be associated with the quality changes.

Table 7. Trace mineral content of cuttlefish muscle (mg/kg, wet basis)

Trace minerals	Head	Mantle
Fe	15.49 ± 1.15^{a}	2.07 ± 0.11^{b}
Cu	1.94 ± 0.07^{b}	2.54 ± 0.04^{a}
Mn	0.61 ± 0.04^{a}	0.29 ± 0.02^{b}
Cd	0.20 ± 0.01^{b}	0.42 ± 0.05^{a}
Pb	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
Zn	7.65 ± 0.46^{b}	10.36 ± 0.76^{a}

Values are given as mean ± SD from triplicate determinations. Different superscripts in the same row indicate significant differences ($p<0.05$).

Thermal transition of cuttlefish muscle

DSC thermograms of the cuttlefish muscle from both head and mantle portions are shown in Table 8. Three endothermic transitions with T_{max} values of 49.8, 59.8 and 74.7 °C were found in head portion. DSC thermograms of mantle portion also showed three endothermic transitions with T_{max} values of 50.3, 60.3 and 78.8 °C. DSC offers a direct method to study the thermal transition of muscle proteins *in situ* (Pulter *et al*., 1985). Similar to Paredi *et al*. (1996), three major endothermic transitions with T_{max} of 47.9, 56.8 and 9.3 °C were observed in whole muscles of male squid (*Illex argentinus*). Therefore, the first, second and third transitions were attributed to the denaturation of myosin and paramyosin, connective tissues, and actin respectively. Due to removal of skin, mantle meat exhibited smaller peak for second transition, compared with head muscle. From the result, similar T_{max} of the first and second peak was observed between head and mantle muscles. However T_{max} of actin in head portion was lower than mantle portion. Our results were in agreement with Rodger *et al*. (1984) and Paredi *et al*. (1990) who reported the similar T_{max} in other mollusk species. T_{max} values of cuttlefish muscle protein transitions were lower than those of mammalian muscle. Mammalian whole muscles showed three transitions with T_{max} values of 57-60, 62-67 and 74-80 °C, respectively (Wright *et al*., 1977; Wagner and Anon, 1986).

Enthalpies of the first and third transitions of mantle portion were 2.6 and 2.6 folds higher than those of head portion. Higher enthalpy indicated that higher energy was needed to denature the myosin/paramyosin in mantle than in head portion. This was accompanied with the higher T_{max} of both peaks observed in the mantle portion. However, enthalpy of the second peak in head muscle was higher than that found in mantle muscle. Much lower denaturation enthalpy for the second peak compared with head portion suggested that connective tissue in

mantle were susceptible to thermal denaturation than those of head portion. From the result, it was suggested that thermal stability of individual portion varied depending on cuttlefish portions.

Table 8. Peak transition temperature (T_{max}) and denaturation enthalpies (□H) of cuttlefish muscle protein

	Head		Mantle	
Peak	Tmax (°C)	□H (J/g)	Tmax (°C)	□H (J/g)
Peak1	49.78±0.58[a]	0.24±0.03[b]	50.33±0.44[a]	0.63±0.03[a]
Peak2	59.82±0.31[a]	0.23±0.20[a]	60.28±0.19[a]	0.06±0.01[b]
Peak3	74.72±0.25[b]	0.29±0.10[b]	78.83±1.00[a]	0.75±0.40[a]

Values are given as mean ±SD from triplicate determinations. Different superscripts in the same row under the same parameter indicate significant differences ($p<0.05$).

2.5 Conclusion

The cuttlefish contained high protein and mineral content but low lipid content. Phospholipid was the major cuttlefish lipids, which were rich in PUFA. MHC, paramyosin and actin were the major proteins in cuttlefish and varied with portions. Both head and mantle portions of cuttlefish comprised different trace minerals at varying concentrations.