องค์ประกอบทางเคมีและการแยกส่วนโปรตีนกล้ามเนื้อปลาหมึกกระดอง

Chemical Compositions and Fractionations of Protein in Cuttlefish (*Sepia pharaonis*)Muscle

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บทคัดย่อ: จากการศึกษาองค์ประกอบของกล้ามเนื้อปลาหมึกกระดองส่วนหัวและส่วนลำตัวพบว่า กล้ามเนื้อทั้งสองส่วนประกอบด้วย โปรตีนร้อยละ 11.90-14.91 ไขมันร้อยละ 0.47-2.52 เถ้าร้อยละ 1.20-1.29 และคอลลาเจนร้อยละ 0.64-1.87 ส่วนหัวปลาหมึกกระดองประกอบด้วยโลหะสูงกว่าส่วนลำตัว โดยมีสังกะสีเป็นอิออนโลหะที่พบมากที่สุด กล้ามเนื้อปลาหมึกกระดองประกอบด้วยซาร์โคพลาสมิกร้อยละ 28.35-33.27 โปรตีนไมโอไฟบริลร้อยละ 53.11-58.53 โปรตีนที่ละลายได้ในด่างร้อยละ 3.15-5.32 และโปรตีนสโตรมาร้อยละ 4.79-13.11 และเมื่อวิเคราะห์องค์ประกอบของกล้ามเนื้อโดย SDS-PAGE พบว่าประกอบด้วยโปรตีนไมโอซินเส้นหนา (MHC) พาราไมโอซิน และ แอคติน เป็นองค์ประกอบหลัก โดยส่วนลำตัวมีพาราไมโอซินสูงกว่าเนื้อส่วนหัว แต่ส่วนหัวมี MHC สูงกว่าส่วนลำตัว ไขมันของปลาหมึกกระดองส่วนหัวและส่วนลำตัวประกอบด้วยกรดไขมันอิสระร้อยละ 0.98-1.71 ไดกลีเซอไรด์ร้อยละ 12.37-18.88 และฟอสโฟลิปิดร้อยละ 79.41-86.65

Abstract: Chemical compositions and fractionation of proteins in cuttlefish muscle were studied. Head and mantle contained 11.90-14.91 % protein, 0.47-0.52% fat, 1.20-1.29% ash and 0.64-1.87% collagen. Head portion contained higher content of microelement than mantle. Zinc was found as the predominant element in cuttlefish muscle. Cuttlefish muscle consisted of 28.35-33.72% sarcoplasmic protein, 53.11-58.53% myofibrillar protein, 3.15-5.32% alkali–soluble protein and 4.79-13.11% stroma protein. Composition analyzed by SDS-PAGE revealed that myosin heavy chain (MHC), paramyosin and actin were major proteins. Mantle contained greater content of paramyosin than head portion, but head portion had higher MHC content than mantle. Lipids from head and mantle contained 0.98-1.71% free fatty acid, 12.37-18.88% diglyceride and 79.41-86.65% phospholipid.

Introduction: Cephalopods constitute an important part of the marine resource for human consumption. They are commonly consumed in Mediterranean and Far East, particularly in Japan. Due to their abundance and rapid stock renewal, they are recognized as the most promising resource. Total world landing of squid increased from 1.7 million tons in 1990 to approximately 2.48 million tons in 1997 (Ruiz-Capillas *et al.* 2002). Frozen cephalopods are the important product of Thailand with the third export value in 2001. Thailand exported frozen cuttlefish and squid for 70,875,205 kilograms with a value of 11,298 million baht (Thai Customs Department, 2002)**.** Cuttlefish used for processing has been reported to be varied, resulting in the differences in quality of final products. However, information regarding the composition of cuttlefish is scarce. The objective of this study was to characterize and fractionate the muscle protein in cuttlefish head and mantle.

Materials and methods: Cuttlefish (*Sepia pharaonis*) with the size of 6-10 cuttlefish/kg was taken from a dock in Songkla. 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. The cuttlefish was separated into head and mantle portions. Mantle was cleaned, deskined and eviscerated. For the head including tentacles, the eyes were removed but skin was still remained. The cuttlefish samples were kept in ice during preparation. Cuttlefish muscle was determined for moisture, ash, crude fat and crude protein content according to the method of AOAC (1999). The total collagen content was estimated on the basis of hydroxyproline content according to the method of Woessner (1961) and 11.11 was used as a converting factor for calculating collagen content. Determination of element content (Fe, Cu, Co, Mn, Zn, Ag, Cd, Cs and Pb) was carried out using Atomic Absorption Spectrophotometry. Cuttlefish muscle was also subjected to fractionation according to the method of Hashimoto *et al*. (1979). Protein pattern in each fraction was determined in comparison with that of whole muscle using SDS-PAGE made of 4% stacking gel and 10% running gel according to the method of Laemmli (1970). Cuttlefish lipids were extracted as described by Bligh and Dyer (1959). The lipid compositions were determined by Thin Layer Chromatography / Flame Ionization Detection Analyzer (TLC-FID).

Results and Discussion:

1. Chemical compositions

The chemical compositions of both head and mantle of cuttlefish are shown in Table 1. Head portion contained lower protein content (11.90%) than mantle, which contained 14.91% protein. Fat content was 0.47-0.52 % and ash content was 1.20-1.29%. Head portion possessed higher collagen content than mantle. Since head and tentacle contained the skin, it might serve as the important source of collagen.

2. Trace element content

Trace element content of cuttlefish head and mantle was different. In general, head portion had higher content of all elements analyzed than mantle (Table 2). Among all elements tested, Zn was the dominant element in both portions. Cu and Fe were also found at the high level. Those elements might contribute to oxidation acceleration in cuttlefish muscle during handling, processing as well as storage. Cd and Pb were also higher in head portion, compared to the mantle. The contamination of elements from inhabitat might result in the accumulated elements in cuttlefish muscle, which may be associated with the quality changes.

3. Protein fractionation

Protein in cuttlefish plays an essential role in both nutritional value and sensory properties. Cuttlefish muscles contained several classes of proteins based on solubility. Protein and non-protein nitrogenous component of cuttlefish are shown in Table 3. Myofibrillar proteins constituted as the major protein in both head and mantle. Sarcoplasmic protein was found as the second predominant protein in cuttlefish muscle. It was noted that stroma was higher in head portion, compared to the mantle. From SDS-PAGE, myosin heavy chain (MHC), paramyosin and actin were shown to be the important components in cuttlefish muscle. Paramyosin with MW of 106 KDa is a protein commonly found in mollusk. Higher content of paramyosin was found in mantle, compared to head portion. This differences in protein compositions may result in different protein properties and characteristic of both portions from cuttlefish.

4. Lipid compositions

Lipids from head and mantle contained high content of phospholipid (Table 4) and diglyceride was found as the second predominant constituent. Therefore, most lipids in cuttlefish might be membrane lipids with high phospholipid content.

Conclusion: Compositions of cuttlefish muscle were different between head and mantle portion. Head contained higher content of fat, elements and collagen than mantle. However mantle comprised higher content of protein, especially myofibrillar protein than head. Both portions of cuttlefish contained high content of phospholipid.

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Keywords: cuttlefish, nitrogenous protein, microelement, chemical composition

Table 1 Chemical compositions of cuttlefish muscle (g/100g wet basis)*

Chemical compositions	Head	Mantle
Moisture	84.45±0.13	83.60±0.05
Protein	11.90±0.14	14.91±0.61
Fat	0.52±0.01	0.52±0.01
Ash	1.29±0.02	1.20±0.24
Collagen	1.87±0.21	0.64±0.2

*Mean ±SD from triplicate determinations

Table 2 Element content of cuttlefish muscle (mg/kg wet basis)*

Type of element	Head	Mantle
Fe	4.87±0.68	4.46±0.22
Cu	6.70±0.09	3.51±0.22
Mn	0.1 4±0.01	0.18±0.04
Zn	16.79±0.54	15.08±0.68
Cd	1.14±0.18	0.59±0.19
Pb	5.20±0.14	1.44±0.08

*Mean ±SD from triplicate determinations

Table 3 Protein and non-protein nitrogen components in cuttlefish muscle (mgN/g wet basis)*

Protein Fractions	Head	Mantle
Non-protein N	1.44±0.14	2.17±0.07
Sarcoplasmic	4.83±0.11 (28.35)**	7.13±0.22 (33.72)
Myofibrilla	9.04±0.35 (53.11)	12.33±0.97 (58.35)
Alkali-soluble	0.9±0.02 (5.32)	0.67±0.08 (3.15)
Stroma	2.23±0.07 (13.11)	1.01±0.04 (4.79)

*Mean ±SD from triplicate determinations
**Numbers in parenthesis represent percentage distribution

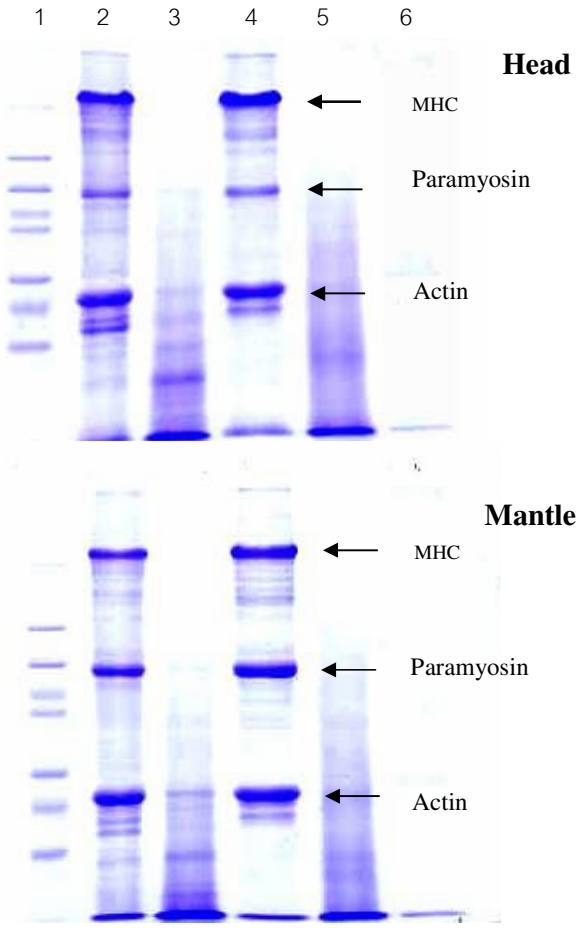

Figure 1 SDS PAGE pattern of muscle protein fraction of cuttlefish (10% gel), lane 1= marker, lane 2=whole muscle, lane 3 = sarcoplasmic protein, lane 4= myofibrillar protein, lane 5= alkali soluble protein, lane 6 = stroma

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

Lipid composition	Head	Mantle
Free fatty acid	1.71±0.14	0.98±0.33
Diglyceride	18.88±0.69	12.37±0.90
Phospholipid	79.41±0.78	
86.65±0.15		

*Mean ±SD from triplicate determinations