Morphological characteristics and chemical composition of Cuttlebone (*Sepia* sp.) at Muara Angke fishing port, Jakarta Indonesia

**K U Henggu***

Wira Wacana Christian University Sumba, Faculty of Science and Technology, Fishery Product Technology Study Programs. Street R. Suprapto, No.35 Waingapu, East Sumba, NTT 87113 Indonesia

*Corresponding Author : krisman@unkriswina.ac.id

**Abstract.** Processing of cuttlefish in Indonesia for export or household needs only uses the body, including the meat to the head, on the other hand, the cuttlebone is categorized as a by-product of processing waste. Therefore, estimates of cuttlebone resulting from this process are very abundant. This study aims to examine the physicochemical of cuttlebone (*Sepia* sp.) and their utilization. The results showed that morphologically, the cuttlebone was composed of 40% dorsal structure and 60% lamellar structure, and the dorsal structure consisted of phragmacone and proostracum. The cuttlebone is composed of a septum and pillars. Proximate content (dry basis) and heavy metal content of cuttlebone, namely ash content 89.61%, lipid 0.35%, protein 4.78%, carbohydrate 5.26%, metal content of cadmium 0.6 mg/kg while lead and negative mercury. Macro mineral content (calcium, magnesium, potassium, phosphorus) is 40.18%, micro minerals (manganese, iron, zinc, sodium) 0.73%. The absorption of cuttlebone functional groups identified elements of CaCO$_3$ aragonite, β chitin in lamellar and α chitin in the dorsal structure.

1. Introduction

Physiologically, the cuttlebone functions to protect the internal organs and acts as a storage medium for gas and liquid which functions to maintain cuttlefish at a certain depth [1]. Processing of cuttlefish at Muara Angke Jakarta's fishing port only uses the body parts that include the meat to the head, on the other hand, the cuttlebone is categorized as a by-product of processing waste. Cuttlebone comprises 15-20% of the total body proportion [2]. Therefore, the estimated waste of the cuttlebone produced at the Muara Angke fishing port in Jakarta is 1.29-1.52 tons/year.

Generally, the use of cuttlebone in Indonesia is a source of minerals in bird and turtle feed. Several community groups use cuttlebone as casting for carving gold and silver jewelry. Cuttlebone is known to have bioactive components (alkaloids, steroids, terpenoids, tannins, glucosides) which to be used as an anti-inflammatory source [3], biomaterial apatite [4], and a source of the amino acids serine and glycine [5]. The availability of cuttlebone waste which is quite abundant and the utilization is still low, it is necessary to make a validation effort. The validation is based on the organic and inorganic chemical composition and potential uses. Therefore, this study aims to determine the physicochemical characteristics of the cuttlebone (*Sepia* sp.).
2. Material and methods
This research was conducted at the Laboratory of Raw Material Preparation of the Department of Aquatic Products Technology, Faculty of Fisheries and Marine, Bogor Agricultural University. The cuttlebone (Sepia sp.) was obtained from the Muara Angke Jakarta Fishery Port. The cuttlebone was washed and dried then analyzed proximate [6], morphology [7], and functional groups [8]. Chitin extraction from the cuttlebone (Sepia sp.) was carried out based on the acid-base extraction method [9]. Data from the physicochemical analysis were tested non-parametrically in the form of the mean value (µ), standard deviation (∆x), and data presentation in descriptive form.

3. Result and Discussions
3.1. Cuttlebone
The cuttlebone is the internal part of the cuttlefish which is white and flat. The cuttlebone consists of two parts, namely the exoskeleton (dorsal) and exoskeleton (lamellar). The dorsal structure (Figure 1) has a thickness of ± 1 mm with hard mechanical properties, due to calcification of calcium carbonate (CaCO$_3$). The CaCO$_3$ calcification is thought to be due to a biological nucleation process that occurs in the environment. The presence of minerals such as calcium carbonate (CaCO$_3$) aragonite and calcite found in the living environment of organisms greatly affects the formation of inorganic compounds in the shells of organisms [10].

![Figure 1. Cuttlebone (Sepia sp.)](image)

The dorsal cuttlebone is composed of two layers, namely the phragmacone and proostracum layers. The phragmacone layer is a calcified CaCO$_3$ compound, while the proostracum with brownish and transparent characteristics is thought to be a chitin layer which acts as the overall shell-forming matrix (Figure 2a). Proostracum is composed of organic components in the form of polysaccharides which are laminated by biologically inorganic components, for example, proteins, lipids, and carbohydrates secreted by organisms through the visceral, then form a chitin matrix and proceed to the mineral nucleation process biologically to form a shell [11]. The proportions of the cuttlebone in this study were 40% dorsal and 60% lamellar.

![Figure 2. Cuttlebone morphology.](image)
Cuttlebone lamellar has a mechanical structure that is brittle and soft, solid, white, and has pores. The lamellar pores of the cuttlebone are chambers bounded by pillars and septum (Figure 2b, 2c, 2d). The formation of pores in cuttlebone lamellar is due to the liquid secreted from the visceral, then stored and forms a siphuncle in the lamellar which functions to maintain the presence of cuttlefish at a certain depth in the water [2].

3.2. Proximate and heavy metal composition
The proximate composition of cuttlebone which includes moisture, ash, lipid, and protein content (Table 1) is dominated by ash 89.61%, carbohydrates 5.26%, protein 4.78%, and lipid 0.35%, respectively. The high ash content indicates the presence of inorganic components in the form of micro minerals, especially calcium carbonate (CaCO3) and other micro minerals.

| Proximate Composition (%) | Information  |
|---------------------------|--------------|
| Moisture                  | 3.54 ± 0.11  | wet basis   |
| ash                       | 89.61 ± 0.26 | dry basis   |
| lipid                     | 0.32 ± 0.19  | dry basis   |
| proteins                  | 4.78 ± 0.23  | dry basis   |
| Carbohydrate (by difference) | 5.29 ± 0.02 | dry basis   |
| Heavy metals              | SNI Standard |
| Lead (Pb)                 | negative     | 0.25        |
| Mercury (Hg)              | negative     | 0.03        |
| Cadmium (Cd)              | 0.6 ± 0.17   | 0.20        |

Organic compounds such as lipid, protein, carbohydrates, and chitin contained in cuttlebone have a relatively low composition compared to the ash content which is interpreted by high inorganic compounds. This shows that the organic composition only acts as a shell-forming matrix, while inorganic compounds act as fillers that can form mechanical properties. The organic composition of the shells of aquatic organisms is generally 30-40% and 60-70% inorganic [12]. The content of heavy metals in the cuttlebone (Table 1) shows that lead and mercury contain below the threshold set by the National Standardization Agency for Indonesia through the Indonesian National Standard (SNI) Number 7387: 2009 [13], however, the cadmium content exceeds the required threshold. The high content of cadmium in the cuttlebone indicates that the shells are not safe from heavy metal contamination, although the cadmium content in the shells is still accepted as a raw material in the synthesis of hydroxyapatite. According to the International Organization for Standardization No. 1375: 2015 [14], the content of cadmium in raw materials for biomaterial synthesis (hydroxyapatite) is maxed 5 mg/kg.

3.3. Macro and micro minerals composition
Macro and micro mineral content include calcium, phosphorus, potassium, magnesium, iron, zinc, manganese, and sodium (Table 3) shows that 40.18% are macro minerals while the micro minerals are 0.73%. This shows that the cuttlebone is mostly composed of macro minerals such as calcium, magnesium, phosphorus, and potassium.
Table 2. Composition of macro and micro minerals of cuttlebone (Sepia sp.)

| Minerals  | Composition (mg/100g) dry basis |
|-----------|---------------------------------|
| Macro     |                                 |
| - Calcium | 31.634 ± 0.04                   |
| - Magnesium | 86.1 ± 0.18                   |
| - Potassium | 39.6 ± 0.10                   |
| - Phosphor | 8.424 ± 0.06                   |
| Micro     |                                 |
| - Mangan  | 0.4 ± 0.05                      |
| - Iron    | 3.2 ± 0.02                      |
| - Zinc    | 2.1 ± 0.07                      |
| - Sodium  | 716 ± 0.12                      |

Mineral elements, especially those high enough in calcium cuttlebone, are used as a source of calcium in foodstuffs as well as calcium precursors in hydroxyapatite synthesis. Hydroxyapatite is an inorganic compound that is very important to be applied as a bio ceramic-based human bone scaffold because it has good biocompatibility, bioactivity, and osteoconductive properties. Other minerals such as potassium, magnesium, phosphorus, manganese, iron, zinc, and sodium are very beneficial for human health [15].

3.4. Functional groups

The functional group is an interpretation of the components of the cuttlebone through the transmission absorption produced by FTIR. Identification of functional groups is based on changes in molecular vibrations due to electrostatic modification of valence in alkanes, alkenes, arenes, amines, carboxyl and hydroxyl bonds. The results of functional group analysis (Figure 3) show that the cuttlebone is composed of two main compounds, namely inorganic form of calcium carbonate (CaCO$_3$) aragonite and organic is β chitin.

Cuttlebone was identified based on the vibrations generated by the carboxyl (O-C) bonds from the carbonate ion at wave numbers of 1,600-600 cm$^{-1}$ (Figure 3 b). The absorption of this functional group is divided into four main types of vibration, namely stretching symmetric ($v_1$) at a wavenumber of 1,083 cm$^{-1}$, bending out-of-plane vibration ($v_2$) 871-700 cm$^{-1}$, asymmetric stretching vibration ($v_3$) 1,507 cm$^{-1}$ and in-plane split bending vibration ($v_4$) at wave number 713 cm$^{-1}$. The absorption of CaCO$_3$ aragonite phase transmission (Figure 3b) identified at wave number 713-700 cm$^{-1}$ is characterized by overlapping vibrations due to the asymmetrical bonding of carbon ions to two hydrogen molecules (C=O). The aragonite phase is generally identified in the 713 cm$^{-1}$ wave which is characterized by overlapping vibrations due to ionic bonds in the hydrogen molecule [16].

The cuttlebone organic compounds were identified at a wavenumber of 3,466-2,521 cm$^{-1}$ which is characterized by three forms of vibration, namely stretching vibrations at a wave number of 3,466 cm$^{-1}$, stretching symmetric vibrations of 2,985 cm$^{-1}$ and asymmetric stretching vibrations of 2,521 cm$^{-1}$. The vibration is thought to be chitin. Chitin is a natural copolymer composed of several N-acetyl-D-glucosamine units repeatedly on each β-(1-4) glycosidic chain which is characterized by the binding of glucose and nitrogen molecules to each monomer. Chitin obtained from cuttlefish shells (Figure 3a) is characterized by the vibrations identified in the amide absorption region I, II, III, and other vibrations that are not classified in the three amide absorption regions.
The absorption spectrum of cuttlebone (*Sepia* sp.). (a) β chitin, (b) calcium carbonate (CaCO₃).

The transmission absorption of chitin amide I was identified at the wavenumber 1,666 cm⁻¹ which is a C = O vibration due to carbon bonding in the oxygen group with the NH₂ group. Amide II at the wavenumber of 1,555 cm⁻¹ has a bending vibration form which is influenced by the C-H bond and the C-N stretch on the carbonyl group which has a carbon-to-hydrogen double bond (NHCOCH₃). Amide III at 1,319 cm⁻¹ is a bending split in-plane vibration of the carbon bond on the amide group (CO-NH₂). Another bending vibration shown at wave number 1,378 cm⁻¹ is a vibration caused by the C-H and C-H₃ bonds on NHCOCH₃. The stretching split in-plane vibrations identified in waves 3,582 and 3,105 cm⁻¹ are bonds between hydroxyl groups (OH⁻) and amines (N-H). Aliphatic bending vibrations were identified when the transmission wave resistance was 2,922 cm⁻¹ caused by flapping the CH₃ group. This vibration is a characteristic of the alkyl group in chitin which is hydrophobic. The carbon asymmetric vibration (C-O-C) identified at the transmission wave number 1,153 cm⁻¹ is a cyclic N-acetyl-D-glucosamine bond in the chitin structure. The bending vibration in the 901 cm⁻¹ waves is caused by the anomic carbon (C-H) bonding of the cyclic glucopyranose group which forms a β-(1-4) glycosidic chain with the characteristics of the anomeric center configuration being equatorially opposite to the methyl glucosamine group. The vibration identified in the 901 cm⁻¹ waves is the main characteristic of the β-chitin structure. Research related to the main characteristic of the β-chitin structure is generally identified in the transmission wave number 800-950 cm⁻¹, with the frequency of the C-H bonding axial position on α-chitin and equatorial for β-chitin [17]. Chitin identified in cuttlefish shells has the potential for development as adsorbent of heavy metal waste and dyes, preservatives, anti-fungal, cosmetics, pharmaceuticals, flocculants, anti-cancer, and anti-bacterial properties.
4. Conclusion
The cuttlebone of the *Sepia* sp. species obtained at the Muara Angke Fishing Port, Jakarta, is morphologically composed of lamellar and dorsal structures. The dorsal structure consists of phragmacone and proostracum layers while the lamellar is composed of a septum and pillars. Cuttlebone is dominated by calcium carbonate (CaCO$_3$) aragonite which is interpreted by high ash content while the organic compound is in the form of $\beta$ chitin. Macro mineral compounds, especially calcium carbonate and $\beta$ chitin contained in cuttlebone, have the potential to be used as food additives, biomaterials, and advanced materials.

5. Reference
[1] Pabic C, Rousseau M, Bonnaud-Ponticelli L, and von Boletzky S 2016 *Vie et Milieu*. **66**, 1
[2] Lee D 2016 *J. Fish. Aquat. Sci.* **19**, 1
[3] Amir N, Ananda D, Elvianti N, and Ardi A 2019 *JIPTEKS Perikanan*. **6**, 12
[4] Henggu K U, Ibrahim B, and Suptijah P 2019 *J. Pengolah. Has. Perikan. Indones*. **22**, 1-13
[5] Xiao S, Zheng X, Wang Z, and Wang R 2005 *Res. Bull*. **66**, 235-241
[6] [AOAC] Association of Official Analytical Chemist 2005 *Official Method of Analysis of The Association of Official Analytical of Chemist* (Arlington: AOAC, Inc)
[7] Raith M M, Raase P, and Reinhardt J 2012 *Guide to Thin Section Microscopy* (Bonn: Universitas of Bonn) p 23-27
[8] Walters M A, Leung Y C, Blumenthal N C, Konsker K A, and LeGeros R Z 1990 *J. Inorg. Biochem*. **39**, 193-200
[9] Suptijah P 2004 *J. Pengolah. Has. Perikan. Indones*. **7**, 1
[10] Marin F, Le Roy N, and Marie B 2012 *Front. Biosci*. **4**, 99-125
[11] Doguzhaeva L A and Summesberger H 2012 *Neues Jahrb. Geol. Paläontol*. **266**, 31-38
[12] Meyers M A, Chen P Y, Lin A Y M, and Seki Y 2008 *Prog. Mater. Sci*. **53**, 191-206
[13] [BSN] Badan Standardisasi Nasional 2009 *Batas Maksimum Cemaran Logam Berat Dalam Pangan: SNI 7387:2009* (Jakarta: Dewan Standardisasi Nasional)
[14] [ISO] International Organization for Standardization 2015 *ISO 1375:2015 Implan for surgery, calcium phosphates. Part 3 bone substitutes based on hydroxyapatite and beta tricalcium phosphate* (Russian: National Standard of Russian Federation)
[15] Soetan K O, Olaya C O, Oyewole O E 2010 *African J. Food, Agric. Nutr.* **4**, 200
[16] Vagenas N V, Gatsouli A, and Kontoyannis C G 2003 *Talanta*. **59**, 831-836
[17] Cardenas G, Cabrera G, Taboada E, and Miranda S P 2004 *J. Appl. Polym. Sci*. **93**, 1876-1885

Acknowledgments
Thanks to Wira Wacana Christian University Sumba for providing research funding to the author.