Exploration of composition, elements, and microstructure of body and shell on tropical mole crab (*Emerita emeritus*)

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Exploration of composition, elements, and microstructure of body and shell on tropical mole crab (Emerita emeritus)

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Abstract. Mole crab (Emeria emeritus) has a distinctive composition of its shell structure that can harden and soften due to mineral and composition properties in the body itself. There is no preliminary information about the content and structure of E. emeritus, therefore the writing of this article was to understand the body and shell composition, elements and microstructure of E. emeritus. The scope of this research was divided into two parts, i.e. body observation of E. emeritus showed that the greatest result were 40.03% of ash content, 8.74 g/100 g protein of glutamic acid, 6.42 g/100g protein of aspartic acid, 5.03 g/100 g fat of palmitic acid, and 3.23 g/100 g fat of omega-9. Another scope of its shell showed 55.24% of crystal purity phase was magnesium calcite in Lattice rhombohedral form on the highest peak angle at 29.76° and the lowest at 23.3°, 36.35°, 39.85°, 43.63°, 48.16°, and 49.11°. Besides, it was found ±37.32% of oxygen element, ±32.06% of carbon, ±15.09% of calcium, 8.74% of nitrogen, ±2.18% of magnesium, 0.36% of aluminum, 0.63% of phosphorus, and 0.47% of sodium. Functional groups in the absorption spectrum bands were followed by magnesium calcite at 1030 cm⁻¹, 874 cm⁻¹, and region of 700 cm⁻¹ to 400 cm⁻¹, while O-H amine groups at 3402 cm⁻¹, 1657 cm⁻¹ and 1412 cm⁻¹. In addition, low spectrum band demonstrated connection with Al, Mg, Si and Na in the region of 1155 cm⁻¹ to 928 cm⁻¹. In microstructure found four main layers consisting of epicuticle, exocuticle, endocuticle, and epidermis layer, which has common structure generally.

1. Introduction

Marine biota has a distinctive composition of its shell structure that can harden and soften due to mineral and composition properties in the body itself [1]. This uniqueness due to the variety of composition and mineralization contained in its shell. These impacted on mechanical properties for its shell strength [2-3]. Wegst and Ashby [4] explained the biological materials can be multifunctional as the result of combination biological, mechanical properties, and other functions. Thus, there are the differences in grouping of marine organic materials which categorize as metals, polymers, inorganic materials, composites, concentrated, and adhesives [5]. Biological material development has been evolving rapidly in the field of biomaterial, therefore the exploration of natural material on marine biota is highly important to be developed.

Mollusca and Crustacea have hard shell component as a body protection. Its main components are silica, chitin, calcium carbonate that make hard and rigid structure of their shell properties [6]. One of
small Crustacea found in Indonesian waters was mole crab. Mole crab has strong genetic relationship with shrimp, crab, and lobster [7]. Mole crab is one of the largest communities living in sandy beach area on spring until autumn season [8]. This biota widely found and has a fairly high distribution in the coastal areas of Java island, Sumatra, Sulawesi, and Papua (9-21).

In terms of the diversity of mole crabs in Indonesia, there are seven species of superfamily which have been found, i.e Emerita emeritus, Hippa adactyla, H. admirabilis, H. marmorata, H. ovalis, H. Kelaino and Albunea symmista [13, 14,16,18]. In the discovery was explained that only three species found in Java Island, there were E. emeritus, H. adactyla and A. symmista [15]. These species have high economic value, because it is used as a typical snack of the southern coastal area of Java Island. Mashar et al. [13] reported that the population percentage of these three species in Java Island were 70.5-75.3% of E. emeritus; 22.5-24.7% of H. adactyla and 2.2-4.8% of A. Symmysta. However, the commonly known and widespread species in the world is Emerita analoga.

The amount of distribution and diversity of mole crab in Indonesia has not been followed by research on the utilization aspect, both nutrient content and biological material aspect. The utilization of mole crab just developed into traditional processed food, such as rempeyek or fried yutuk. The research of nutrient content of mole crab (E. emeritus) has been observed since 2003 that showed 17.22-21.56% of fat content and 7.75-14.48% of omega-3 (EPA and DHA) [22]. Mursyidin [23] added that mole crab contains 12.94% of omega-6, 11.11% of linoleic acid and 1.83% of arachidonic acid. Kardaya et al. [24] utilized mole crab as a cholesterol-lowering food and proved by decreasing 47-53% in Mus musculus BALB/C. In addition, the chemical content of mole crab include 9.1% of moisture content, 32.5% of crude protein, 10.2% of fat, 4.9% of crude fiber, 26.4% of ash, 9.3% of calcium, and 1.6% of phosphorus. However, no one explored the information of detail contents on its shell.

Recent studies have shown that mole crab is dominated by 35-38% of protein content and 31-35% of ash content [25]. The results are not slightly different from another crab in general which is 34-37% protein and 24-38% of ash content [26-28]. The high content of ash was estimated as a result of shell composition which is 50% covering the whole body [29]. Roer and Dillaman [30] described that mineral content, chemical properties, and morphological matrix of the shell were an important aspect to observe. Considering the amount of mole crab E. emeritus expansion (70.5-75.3%), and the absence of information on mineral content, chemical properties, and morphological matrix, thus this article writing is fundamental to do.

2. Material and Methods

2.1. Material

The main used ingredient was Emerita emeritus species of mole crab (obtained from the coast of Widara Payung, Central Java, Indonesia) and other materials including ionized water and acetone solution (CH$_3$H$_2$O, CAS No. 647-641 Merck 98%).

2.2. Method

E. emeritus was completely cleansed then analyzed of its proximate, amino acid profile, and fatty acid. Furthermore, the shell and other components separated. Shell cleansing used ionized water and immersion used acetone (ratio 1:2) for 24 hours to remove impurities [31]. Shells were observed for its morphological structure on the top, bottom and transverse section using Scanning Electron Microscope (SEM). Hereinafter, evaluated the elements composition was carried out using Energy Dispersive X-Ray (EDX). Analysis of crystal purity was carried out using X-Ray Diffraction (XRD) and functional groups was carried out using Fourier Transform Infrared Spectroscopy (FTIR).
3. Result and Discussion

3.1. Proximate

The evaluation of chemical content used to determine the proportion of moisture, ash, fat, protein, and carbohydrate content. Proximate analysis results presented in Table 1. The result showed the highest ash and moisture content respectively, 40% and 34%. Santoso et al. [25] also demonstrated the highest proximate level in mole crab were 74.90% of moisture content and 35.63% of ash content. It showed that ash content was slightly different from literature. The highly ash content of *E. emeritus* assumed from shell composition that 50% covering the body.

Table 1. Proximate, amino acids, and fatty acids composition profile of *E. emeritus*

| Proximate (%) | Error (±) | Amino Acid (g/100g protein) | Error (±) | Fatty Acid (g/100g fat) | Error (±) |
|---------------|-----------|-----------------------------|-----------|-------------------------|-----------|
| Water         | 33.84     | 0.03                        |           | Non-Essential           | Saturated |
| Ash           | 40.03     | 0.11                        | Aspartic Acid | 6.42 | 0.07 | Lauric acid | 0.21 | 0.01 |
| Fat           | 0.15      | 0.01                        | Glutamic Acid | 8.74 | 0.07 | Myristic acid | 0.89 | 0.04 |
| Protein       | 11.96     | 0.02                        | Serine     | 4.08 | 0.04 | Pentadecanoic acid | 0.07 | 0.01 |
| Carbohydrate  | 13.82     | 1.99                        | Glycine    | 4.33 | 0.06 | Palmitic acid | 5.03 | 0.18 |
| Total         | 99.81     | 2.16                        | Alanine    | 4.64 | 0.05 | Heptadecanoic acid | 0.12 | 0.00 |
| L-Tyrosine    | 2.83      | 0.03                        |            |           | Stearic acid | 1.62 | 0.08 |
| Total         | 31.05     | 0.31                        | Arginine   | 2.97 | 0.06 | Acyl behenate | 0.07 | 0.01 |
| Histidine     | 1.41      | 0.04                        |            |           | Lignoceric acid | 0.03 | 0.00 |
| Arginine      | 2.97      | 0.06                        |            |           |                |         |       |
| Total         | 4.38      | 0.10                        |             |           | Palmitoleic acid | 1.68 | 0.06 |
| Essential     |           |                             | Essential   |          | Oleic acid | 2.72 | 0.12 |
| Threonine     | 3.53      | 0.05                        |            |           | Oleic acid | 0.51 | 0.06 |
| Leucine       | 3.49      | 0.02                        |            |           | Total         | 4.91 | 0.23 |
| Lysine        | 4.07      | 0.06                        |             |           | Linoleic acid | 0.46 | 0.03 |
| Methionine    | 0.95      | 0.00                        |             |           | Linolenic acid | 0.05 | 0.01 |
| Valine        | 3.46      | 0.00                        |             |           | Linolenic acid | 0.04 | 0.00 |
| Phenylalanine | 3.23      | 0.04                        |             |           | Eicosapentaenoic acid | 0.94 | 0.01 |
| Isoleucine    | 2.21      | 0.01                        |             |           | DHA           | 0.97 | 0.06 |
| Total         | 20.94     | 0.19                        |             |           | EPA           | 0.94 | 5.84 |
|               |           |                             | Polyunsaturated |        | Arachidonic acid | 0.29 | 0.01 |
|               |           |                             |             |           | Omega-3      | 1.96 | 0.08 |
|               |           |                             |             |           | Omega-6      | 0.8  | 0.04 |
|               |           |                             |             |           | Omega-9      | 3.23 | 0.18 |
|               |           |                             |             |           | Docosadienoic acid | 0.04 | 0.00 |
|               |           |                             |             |           | Docosahexaenoic acid | 0.97 | 0.06 |
| Total         | 10.69     | 6.31                        |             |           | Total         | 10.69 | 6.31 |
3.2. Amino Acid Profile
Amino acid are protein which was formed by bonding between amino and carboxyl groups [32]. Amino acids are divided into two parts, i.e non-essential amino acids (produced by the body) and essential amino acids (which must be obtained from food) [33]. The result of amino acid analysis of *E. emeritus* presented in Table 1. Amino acids were detected as much as 15 types of amino acid, there were 6 non-essential amino acids, 2 semi-essential amino acids, and 7 essential amino acids. Non-essential amino acids have the largest component rather than the essential and semi-essential amino acids, which were presented in aspartic acid and glutamic acid. This result resembles Santoso et al. [25] that showed the largest component of non-essential amino acids were aspartic acid and glutamic acid. Glutamic acid contains monosodium glutamate that generally utilized as flavour [34] and aspartic acid were commonly used as glucogenic and pyrimidine precursors [32].

3.3. Method Fatty acid profile
Fatty acids are organic acids that having linear hydrocarbons chain, hydroxyl groups (-COOH) and methyl groups (CH3) [35]. Fatty acids serve as energy sources for cells or to modify phospholipids into cells or membranes [36]. The result of fatty acid profile analysis on *E. emeritus* displayed in Table 1. Fatty acids were detected as much as 23 types, there were 8 saturated fatty acids, 3 monounsaturated fatty acids, and 12 unsaturated fatty acids. The highest fatty acid profile was 10.69g/100g of fat in unsaturated fatty acids, with the biggest compositions found in omega-9 and omega-6, respectively. 3.23g/100g of fat and 1.96g/100g of fat. Omega-9 fatty acids have 30.22% percentage and omega-3 fatty acids have 18.33% of total unsaturated fatty acids. In 8.04g/100g of fat of saturated fatty acids have the highest composition on palmitic acid 5.03g/100g of fat (62.56% of total saturated fatty acids). Monounsaturated fatty acids (4.91g/100g of fat) have the highest composition on oleic acid type 2.72g/100g of fat (55.4% of total monounsaturated fatty acids). Santoso et al. [25] demonstrated the result of amino acid profile of *E. emeritus* including EPA (3.27g/100g of fat) and DHA (1.32g/100g of fat), monounsaturated fatty acid was 18.31g/100g of fat of palmitoleic acid and saturated fatty acid was 21.65g/100g of fat of palmitic acid. Ozugul and Ozugul [37] explained that palmitic acid represents 53-65% of total saturated fatty acids, oftenly found in plants, animals, and microorganisms [38]. Whereas, oleic acid regularly found in 35-40% of chicken meat and frequently used in margarine with 47% composition [39]. These level of polyunsaturated fatty acids can be caused by seasonal change, geographical location, and environmental salinity [40].

3.4. Micro characteristic using SEM
The microstructure observation of *E. emeritus* shell using SEM on the top, bottom, and transverse section were demonstrated in Figure 1 (b-d), while the full morphology presented in Figure 1a [41,42]. The microstructure observation at the top of the shell showed curve patterns and resembled a labyrinth cliff (Figure 1e) [43]. The curve patterns presented on the surface of the shell were expected to assist burrowing process into the substrate (sands) while defending and avoiding from predators [44,45,46,20].

The bottom of the shell section displayed a flat surface structure and curve patterns form. These considered to contribute molting process (shell alteration) and the process of shell attachment to the meat. While in the transverse section, appeared as if a composition resembling epicuticle, exocuticle, and epidermis. The endocuticle consist of principle layer restricted by membrane and epidermis layer. This results were similar to microstructure analysis result on crabs that performed by Roer and Dillaman [30], therefore in microstructure can be concluded that the shell structure of *E. emeritus* resembled the crab shell structure generally.

3.5. Elements analysis using EDX
The element analysis aimed to discover the primary content of *E. emeritus* shell. The observed sections including the top, bottom, and transverse of the shell presented in Figure 2. The results showed significant differences, also found consistent elements presented in all sections, such as oxygen, carbon, and calcium. In the top section of the shell, Al, Mg, N, Ca, C, and O were found with the highest components in O (28.20%), C (26.45%), and Ca (17.97%). Meanwhile in the bottom section,
O (41.75%), C (55.92%), and Ca (2.33%) were discovered. However, in transverse section was found O, C, Ca, Mg, P, and Na, with the highest components were O (42.01%), C (13.80%), Ca (24.98%), and Mg (3.00%).

Several studies have revealed that the element content found in crab shells were carbon, calcium, magnesium, potassium, and iron [47,48,49]. If observed in microstructure (Figure 1d), from epicuticle layer to epidermis later would be found eight elements contained in it. Specifically, the percentage magnesium element decreased into the epidermis layer, and it was proved by the results of analysis in the bottom section of shell that only found in elements of O, C, and Ca. From these results can be estimated that elements on *E. emeritus* shell had calcium carbonate (CaCO$_3$) or magnesium calcite (MgCa(CO$_3$)$_2$) compounds. In addition, the N element (8.74%) was also considered to indicate the presence of chitin compounds.

![Figure 1](image.png)

**Figure 1.** The Visualization results of (a) Mole crab (*E. analoga*) morphology structure (Knox 1963; canaturalis.com 2015), shell microstructure on (b) top surface, (c) bottom and (d) transverse. (e) Illustration scheme on top surface resembles labyrinth cliff (Arakaki *et al.* 2015).
3.6. Crystal phase analysis using XRD

The analysis of *E. emeritus* shell using XRD aimed to determine the pattern of phases formed. The results of pattern is presented in Figure 3 that has been adapted to the Joint Committee on Powder Diffraction Standards (JCPDS No. 01-086-2336). The results demonstrated the *E. emeritus* shell has crystalline phase pattern of magnesium calcite compound (MgCa(CO$_3$)$_2$) with the Lattice rhombohedral form. The highest peaks were found at angle of 23.3°, 36.35°, 39.85°, 43.63°, 48.16°, and 49.11° that indicated MgCa(CO$_3$)$_2$ phase pattern with 55.24% degree of purity. The formed phase pattern supported the estimation results of EDX analysis (Figure 2) that showed Mg and Ca elements.

| Element       | Top surface [wt.%] | Error in wt.% | Bottom surface [wt.%] | Error in wt.% | Transverse surface [wt.%] | Error in wt.% |
|---------------|--------------------|---------------|------------------------|---------------|---------------------------|---------------|
| Oxygen (O)    | 28.20              | 3.68          | 41.75                  | 5.12          | 42.01                     | 5.21          |
| Carbon (C)    | 26.45              | 3.23          | 55.92                  | 6.40          | 13.80                     | 1.88          |
| Calcium (Ca)  | 17.97              | 0.56          | 2.33                   | 0.10          | 24.98                     | 0.77          |
| Nitrogen (N)  | 8.74               | 0.56          |                        |               |                           |               |
| Magnesium (Mg)| 1.35               | 0.10          |                        |               |                           |               |
| Aluminium (Al)| 0.36               | 0.05          |                        |               |                           |               |
| Phosphorus (P)| -                  | -             |                        |               |                           |               |
| Sodium (Na)   | -                  | -             |                        |               |                           |               |

**Figure 2.** Results of elements analysis at the top, bottom, and transverse surface of the *E. emeritus* shell

**Figure 3.** Structure of Lattice and crystal phase pattern of *E. emeritus* shell
3.7. Crystal phase analysis using XRD
Dai et al. [49] reported from his research related to crab shell (China) that showed the highest peak angle at 29.5° for the calcite phase. Arulvel et al. [50] added the explanation of crab shell waste (India) that demonstrate a sharp peak angle at 26-30°, which was indicated a strong interaction between magnesium and calcium carbonate ion bonds. The XRD measurement illustrated that 86.75% of the total E. emeritus shell was magnesium calcite as the primary mineral component.

Strong bonds between magnesium and calcite have a strong influence on chemical coating techniques because they can increase bond strength in solids [50]. Moreover, it also found other elements such as nitrogen, sodium, and aluminum at the peak of around region of 2theta 9.05°, 19.84°, and 7.12°. The presence of sharp and strong peak angle indicated the magnesium calcite crystal structure in biopolymer matrix were formed. According to results in Figure 1-3, E. emeritus shells have composite structure consisting of organic or inorganic materials.

3.8. Functional Group Analysis using FTIR
FTIR analysis was performed to determine the functional groups that presented in E. emeritus shell. The FTIR spectrum absorption peaks illustrated in Figure 4. The results showed spectrum absorption peaks at 3402 cm\(^{-1}\), 1657 cm\(^{-1}\) and 1412 cm\(^{-1}\) indicating the presence of OH bonds and NH bonds from amine groups, it can be an O-H amine group indicating the presence of chitin compounds. The appearance of CaCO\(_3\) at peaks of 1030 cm\(^{-1}\) and 874 cm\(^{-1}\) indicated calcium carbonate compound. Previous study revealed that calcium carbonate presented at peaks of 1080 cm\(^{-1}\), 873 cm\(^{-1}\), 861.93 cm\(^{-1}\), 876 cm\(^{-1}\), and 870 cm\(^{-1}\) [50,51].

The low absorption values at the spectrum peaks were considered as a result of ion interaction between magnesium and carbonate. As the XRD data obtained, the impurity of magnesium calcite compound due to the interaction with magnesium ions that shown at the 2361 cm\(^{-1}\) absorption binding the carboxyl CO groups. Low absorption peaks presented in the region of 700 cm\(^{-1}\) to 400 cm\(^{-1}\) indicated calcium bonds, which were also considered to bind to phosphorus ions (calcium phosphate compound). Besides, low spectrum vibrations demonstrated the presence of bonding interaction with Al, Mg, Si, and Na at the range of 1155 cm\(^{-1}\) to 928 cm\(^{-1}\).

![Figure 4](image_url)

**Figure 4.** Results of functional groups spectrum absorption using FTIR of E. emeritus shell
4. Conclusion
The exploration of composition, elements, and microstructures of body and shell of tropical mole crabs (*Emerita emeritus*) has been successfully performed. The body of *E. emeritus* has the highest composition on ash content, non-essential amino acids (aspartic and glutamic acids), saturated fatty acids (palmitic acid), monounsaturated fatty acids (oleic acid) and polyunsaturated fatty acids (Omega-9 and Omega-3). In *E. emeritus* shell microstructure illustrated four main layers consisted of epicuticle, exocuticle, endocuticle, and epidermis layers, resembled crabs layers structure generally. In addition, the shell contained elements of O, C, Ca, N, Mg, Al, P, and Na, which were dominated by magnesium calcite compounds and were proved by the results of XRD analysis. In the FTIR analysis, the absorption spectrum bands were found which indicated the magnesium calcite and amine compounds.

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