Mineral contents and their solubility on calcium carbonat calcite nanocrystals from cockle shell powder (Anadara granosa Linn)

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Abstract. Prepared and characterized calcium carbonate calcite nanocrystals improves solubility. Calcium carbonate calcite nanocrystals were synthesized using precipitation method from the waste of blood clam cockle shells (Anadara granosa Linn). This study was conducted to analyze mineral composition of nanocrystals calcium carbonate calcite cockle (Anadara granosa) shell for calcium fortification of food applications and to evaluate the solubilities of Calcium and Phosphorus. The sample of nanocrystals from cockle shells was evaluated to determine the content of 11 macro- and micro-elements. These elements are Calcium (Ca), Magnesium (Mg), Sodium (Na), Phosphorus (P), Potassium (K), Ferrum (Fe), Copper (Cu), Nickel (Ni), Zinc (Zn), Boron (B) and Silica (Si)). Cockleshell powders were found to contain toxic elements below detectable levels. The solubilities of Calcium and Phosphorus were p<0.05.

1. Introduction
The mineral content in food is only one of the initial parameters to assess the quality of the food since its bioavailability is more important. Among many nutritional components in food, minerals play an important role in maintaining the viability of organisms in a healthy and normal way. Bioavailability is defined as the proportion of a nutritional component that can be used to run and maintain normal body metabolism [1,21]. The minerals are bioavailable if they are in dissolved form. However, not all dissolved minerals are bioavailable, so the form of dissolved minerals is needed to facilitate the absorption of these minerals in the body [2,9-10]. Therefore, the first step to study mineral bioavailability is to identify the mineral content of the foodstuff and its solubility.

The first objective of this study was to find out the macro and micro mineral content of nanocrystals calcium carbonate powder from the shells of blood clams (Anadara granosa Linn). The second objective of this study was to study its effect on mineral solubility. From the results of this study, we will obtain information about the conditions of the making of nanocrystals calcium carbonate that produce the highest mineral solubility so that it can be used as a reference in food processing, especially for food fortification.
2. Methods

2.1. Materials and instruments
The material used in this study were blood clam shells (*Anadara granosa* Linn) derived from Fish Auction Center, Kenjeran Beach, Surabaya. Instruments for nanocrystal calcium carbonate extraction were HCl, NaOH. The tools used in this study included extractor, glassware, oven, hotplate, and furnace.

2.2. Synthesis of calcium carbonate powder
The synthesis of calcium carbonate powders was prepared from cockle shells according to the procedure described by Islam et al. [3-4]. Cockle shells were obtained from the market. Samples of the cockle shells were dried in an oven at 50°C for seven days, and the shells were then crushed and blended into a fine powder, which was sieved through a 200-um laboratory stainless steel sieve (Endecott, London, England).

2.3. Nano calcium production procedure
Shell flour was immersed in HCl in a concentration of 1.5 N for 48 hours. Shells that have been soaked in HCl were then extracted at a temperature of 90°C. The extraction result was then subjected to filtering to obtain fluid/filtrate. Calcium crystal formation was conducted by precipitation through gradual addition of NaOH solution dropwise to the filtrate until the formation of precipitation saturated calcium hydroxide (Ca(OH)2). Furthermore, the process of crystal separation and crystal neutralization was done by using distilled water. Crystals obtained was then put into the oven at 105°C for 1 hour for precipitate weight became stable, then the crystals were burned using an electric stove to eliminate organic content. Furthermore, the crystals were made incandescent in a furnace at 600°C for 6 hours to form calcium oxide (CaO), then the crystal extraction was smoothed with mortar. The obtained nano calcium was then analyzed chemically and physically.

2.4. Characterization of calcium carbonate nanocrystals
The following instruments were employed for the observation and characterization of the calcium carbonate nanocrystals. The morphology and particle size of the nanocrystals were analyzed by using a scanning electron microscope (SEM, JOEL 7600F) that was operated at a voltage of 20 kV and equipped with an energydispersive X-ray spectroscopy (EDX) unit. The crystal powder was examined by X-ray powder diffraction (XRD) using a Rigaku X-ray diffractometer equipped with a Cu Ka (λ = 0.15406 nm) radiation source, and samples were scanned at a rate of 40/min. Pellets of the calcium carbonate nanocrystals were calibrated in a weight proportion of 1 wt % in Ker and the analyses were performed with a Fourier-transform infrared spectrometer (FTIR, model 100, Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT, USA) in the range of 400–4000 cm⁻¹.

2.5. Total mineral analysis
Samples that will undergo mineral testing were subjected earlier to wet ashing process referring to the methods of Reitz et al. [8]. A total of 1 g of sample was fed into Erlenmeyer 150 ml. A total of 5 ml of HNO3 was added to the Erlenmeyer flask and left for 1 hour and then heated over the hotplate for ±4 hours, and cooled. A total of 0.4 ml of concentrated H2SO3 was added, then reheated. After the color changed from brown to clear yellow, the sample was added with a mixture of HClO4 and HNO3 of 3 ml, reheated for ±15 minutes.

Furthermore, the sample was added with 2 ml of distilled water and 0.6 ml of concentrated HCl, then heated back until dissolved and cooled. After dissolving, the sample was then diluted to 100 ml in the flask. The content of Mg, Ca, K, Na, Zn, Cu, Fe was measured using Atomic Absorption Spectrophotometer (AAS) brand Shimadzu type AA 680 flame emission. The analysis of phosphor was carried out by the molybdate-vanadate method [5]. Samples of wet ashing results were treated with nitric acid to convert all metaphosphates and pyrophosphates into orthophosphates. Then, the sample was
treated with molybdate acid and vanadate acid so that the orthophosphate present in the sample will react with the reactants, forming a yellow vanady molybdophosphate acid complex.

2.6. Analysis of dissolved minerals
Preparation of samples to obtain dissolved mineral fraction was done based on research that has been done by Suzuki et al. (2000) and Santoso (2003) with modifications. A total of 10 g of sample was added with water or 1% NaCl or 0.5% acetic acid each of 40 ml and homogenized.

The soluble fraction was generated by using a mixer at a speed of 5000 -10000 rpm for 2 minutes. Then the sample was heated using a hotplate at a temperature of 100 degrees C for 20 minutes, centrifuged at 10,000 rpm, 2 degrees C for 10 minutes. The results of the centrifuges were then filtered using Whatman filter paper 42. The filtration result was then measured using AAS. The percentage of dissolved minerals was calculated by comparing the number of dissolved minerals with total minerals.

3. Results and discussion

3.1. Characterization of calcium carbonate nanocrystals
Chemical analysis using XRF has been conducted to estimate the mineral composition in the cockle shell. The finding demonstrates that cockle shell is made up of calcium, which is the same outcome with another study such as that of Awang-Hazmi et al. [10] who also performed chemical analysis on cockle shell in Malaysia. Table 2 indicates the comparison of chemical analysis obtained in this study and the one conducted by Awang-Hazmi et al. [10].

The analysis of crystal structure using XRD illustrates that raw cockle shell is made up of calcite and CaCO3. It is one type of crystal form of calcium carbonate other than calcite and vaterite. Calcite is the most stable polymorphism of calcium carbonate. Aragonite has higher density and hardness which make it very suitable material in plastic, paper, glass fiber and other industry [3]. The analysis also indicates the presence of lime, CaO, in the calcined cockle shell. Thus, these findings suggest that thermal decomposition of cockle shell converts CaCO3 contained into CaO.

Calcium carbonate has three anhydrous crystalline phases: calcite, which is thermodynamically stable under ambient conditions; aragonite, which is a high-pressure polymorph that is less stable than calcite; and vaterite, which is the least stable among the three polymorphs and has the ability to transform into one of the other two polymorphs [4]. Among these three polymorphs, calcite is thermodynamically stable [3, 11]. XRD result is in samples number 3 and number 6 can be seen in Fig.1 respectively.

![Figure 1](image-url)
The phase of the cockle shell CaCO₃ powder was further confirmed by (Shimadzu IR Affinity Spectrophotometer) machine. An FTIR spectrum was generated by the absorption of electromagnetic radiation in the frequency range of 400 – 4000 cm⁻¹. The obtained spectrum is shown in the Fig. 2. The spectrum showed vibrational bands at 1456.391 cm⁻¹, 876.72 cm⁻¹, 712.7281 cm⁻¹, and 409.891 cm⁻¹, indicates plane bending. FT-IR analysis confirmed that the CaCO₃ nano powder obtained from cockle shell had the characteristic peak of carbonate group [20]. A sharp peak at 876.72 cm⁻¹ confirmed that the CaCO₃ nano powder obtained from cockle shell was calcite. FT-IR analysis also demonstrated that the powder consisted of CaCO₃.

![Figure 2. Fourier transforms infrared analysis of samples (a) number 3 and (b) number 6.](image1)

The morphological characteristics of the calcium carbonate nanocrystals presented in Fig 3(a) and 3(b) are the typical SEM micrographs of the samples under study.

![Figure 3. SEM results of samples (a) number 3 and (b) number 6.](image2)
3.2. Total mineral analysis

The result of analysis of mineral content and yield with AAS are as follows:

Table 1. Results of mineral and yield analysis

| Sample no. | Duration of extracting process (hour) | Mineral composition (%) |
|------------|--------------------------------------|-------------------------|
|            |                                      | Ca          | Mn          | Zn          | Fe          | Na          | K           | Pb          | Mg          |
| 1          | 1                                    | 13.2        | 0.0366      | 0.0113      | 0.5072      | 7.1548      | 0.0331      | 0.0009      | 3.012       |
| 2          | 1.5                                  | 15.2        | 0.0437      | 0.0096      | 1.2431      | 2.7814      | 0.1199      | 0.0009      | 3.122       |
| 3          | 2                                    | 13.0        | 0.0693      | 0.0104      | 1.3313      | 0.8235      | 0.0391      | 0.0009      | 3.156       |
| 4          | 1                                    | 14.5        | 0.0305      | 0.0063      | 0.7488      | 1.9182      | 0.0370      | 0.0009      | 3           |
| 5          | 1.5                                  | 15.5        | 0.0341      | 0.0182      | 0.7642      | 0.9098      | 0.0324      | 0.0009      | 3.024       |
| 6          | 2                                    | 48.5        | 0.0266      | 0.0105      | 0.6111      | 0.5907      | 0.0187      | 0.0009      | 3.031       |
| 7          | 1                                    | 54.5        | 0.0273      | 0.0058      | 0.6591      | 8.3825      | 0.0344      | 0.0010      | 3.111       |
| 8          | 1.5                                  | 50.5        | 0.0295      | 0.0043      | 0.2474      | 2.0492      | 0.0334      | 0.0009      | 3.123       |
| 9          | 2                                    | 47.7        | 0.0210      | 0.0113      | 0.4472      | 1.9342      | 0.0472      | 0.0009      | 3.142       |
| 10         | 1                                    | 48.5        | 0.0499      | 0.0118      | 1.2792      | 2.8433      | 0.0334      | 0.0009      | 3.522       |
| 11         | 1.5                                  | 41.1        | 0.0426      | 0.0122      | 0.3123      | 2.7835      | 0.0472      | 0.0009      | 3.528       |
| 12         | 2                                    | 62.9        | 0.2519      | 0.0873      | 5.2750      | 3.1125      | 0.1303      | 0.0009      | 3.653       |
| 13         | 1                                    | 38.9        | 0.0896      | 0.0208      | 1.6924      | 1.8142      | 0.0720      | 0.0009      | 3.433       |
| 14         | 1.5                                  | 36.4        | 0.115       | 0.0183      | 5.9855      | 1.3633      | 0.0389      | 0.0009      | 3.562       |
| 15         | 2                                    | 51.5        | 0.602       | 0.0976      | 7.0732      | 3.1300      | 0.0972      | 0.0009      | 3.524       |
| 16         | 1                                    | 15.1        | 0.139       | 0.0286      | 4.0274      | 0.4326      | 0.0262      | 0.0009      | 3.345       |
| 17         | 1.5                                  | 43.4        | 0.545       | 0.2108      | 8.2788      | 4.4500      | 0.1749      | 0.0009      | 3.721       |
| 18         | 2                                    | 37.4        | 0.770       | 0.2610      | 10.7067     | 5.8900      | 0.2770      | 0.0010      | 3.655       |

According to Acevedo et al. [12], molluscan shells comprise 95% calcium carbonate and 5% organic matrix. The precipitation method is an acid-base mixture that produces crystalline solids and water [14]. In this study, precipitation method was done by dissolving the shellfish calcium shell component into an acid solvent (HCl) because calcium dissolves in an acidic atmosphere. Then the NaOH solution was added to the calcium-containing HCl solution. The acid-base mixing causes the solution to become saturated and produces smooth, nano-sized calcium deposits. According to Kenth [13], precipitation method is done by dissolving the active substance into the solvent, then added with another solution that is not solvent (anti-solvent). This causes the solution to become saturated and rapid nucleation occurs to form nanocrystals.

Yield is the most important parameter to identify the economic value and effectiveness of a product or material. The calculation of yield value is based on the percentage of comparison between the final weight and the initial weight of the process. The higher the yield, the higher the economic value of the product. Similarly, the smaller the value of product yield, the lower the economic value or the value of its effectiveness [15]. Based on the analysis of coefficient of variant, the data diversity was low (homogeneous) (CV <20). The result of Kolmogorov Smirnov test showed that the yield data usually spread (P-value> 0.05).

The result of variance analysis showed that the length of extraction treatment had no significant effect (P-value = 0.05) on the yield of nano calcium powder produced. Duncan test showed that the treatment of 1.5-hour extraction was significantly different (P-value = 0.05) from 1 hour extraction time, but not substantially different from 2 hours extraction time. This is because the longer the extraction time, the more the yield produced, resulting in the equilibrium of concentration in the solution, which is called the saturation point. One hour extraction time produced of 50.02% yield. The yield increased to 8.53% when the extraction time increased (1.5 hr), as the extraction time increases, the more mineral
components were extracted from the shell. However, when the extraction time was raised again to 2 hours, the results did not differ significantly from the 1.5-hour yield. This was because the solution had experienced saturation point so that the yield had not increased.

Khoerunnisa [16] mentioned that the longer the extraction time, the higher the yield produced because the chance of contact between the material and the solvent is higher. However, if the extraction time is too long, the yield will decrease as the solution may have reached the saturation point. According to Brojer et al. [17] the increase in extraction time will cause an increase in the mass of the dissolved substance to the optimum time. When the optimum time is passed, the yield does not increase.

The mineral is part of the body and plays an important role in the maintenance of body functions, both at the level of cells, tissues, organs, as well as body functions as a whole. Minerals are classified into macro minerals and micro minerals. Macrominerals are minerals that the body needs in amounts greater than 100 mg a day, while the micromineral is required less than 100 mg a day [18]. The macromineral composition of these nano calcium powders are Ca, Mg, Na, P, and K, whereas the microminerals contained are Fe, Zn, and Mn. Based on the coefficient of variance analysis, the data diversity was low (homogeneous) (CV <20). The result of Kolmogorov Smirnov test showed that the mineral data of nano calcium powder usually spread (P-value> 0.05). The results showed the mineral content of nano calcium powder at 1-hour treatment.

3.3. Mineral solubility
Ca and P minerals respectively represent selected macro and micro minerals to be studied for their solubility properties about nano calcium production processes under various conditions. The solubility of Ca and P are respectively presented in Table 2.

| NaOH concentration | % Calcium | % Phosphor |
|--------------------|-----------|------------|
| 4 N                | 39.83     | 0.0050     |
| 3 N                | 33.07     | 0.0005     |
| 2 N                | 38.66     | 0.0040     |

Minerals will generally be bioavailable in dissolved form. The condition of dissolved minerals is needed to facilitate the absorption of minerals in the body. The solubility of minerals is influenced by several factors such as the degree of acidity, interaction with other components, and its own mineral form primarily due to processing [6-7].

The selection of blood clam shells as a source of calcium and phosphorus in this study is in line with the concept of zero waste product and an environment friendly process. Also, calcium may become an alternative source of milk since milk is expensive and not affordable to all.

4. Conclusion
The results of the mineral content analysis with AAS indicate that shells are safe for consumption and have sufficient mineral content for mineral body fulfillment. The statistical analysis showed that the time of extraction did not affect the calcium level, but the effect comes from the concentration of NaOH, especially the concentration of 4 N.

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