Characterization of nano calcium powder from blood cockle (Anadara sp.) shell produced by using different hydrochloric acid concentration

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Abstract

— Blood cockles one of shellfish which can potentially be developed as a source of protein and minerals. The shell provides a source of calcium, as the structure is similar to that of bone. This research aims to characterize nano calcium powder from blood cockle (Anadara sp.) shell produced by using different hydrochloric acid (HCl) concentrations. Four concentrations vis. 0.5 N, 1.0 N, 1.5 N and 2.0 N were designed by using a completely randomized design. This research revealed that different the concentration affected the particle size and mineral content of the nano calcium powder, where concentration of 1 N was the most appropriate concentration used. The yield generated was 0.82% with the calcium content of 62.89%, the particle size of 454.4nm and high uniformity.

1. Introduction

Blood cockle is one of clam variety which has great potency to be developed as a source of protein and minerals required to sustain Indonesia’s human consumption. The shell of this species contains calcium carbonate of 66.70% [1] and 95-99% of the entire mass of the cockle [2]. Calcium carbonate has various polymorph characteristics of which specify further utilizations, such as active compound in fertilizer [3], biodiesel production catalyst [4], bone tissue regeneration [5] and bio-absorption removing heavy metal ions [6].

Kenjeran is one of the sub-districts in Surabaya where produces blood cockle abundantly. Prior observation revealed that fishermen capture blood cockle from the Kenjeran sea, proceeded it by removing the muscle from the shell and then sold in the traditional market. They waste the shells along the coast of Kenjeran sea and so far the coastal area contains lots of useless shells which can kill the natural view of Kenjeran sea.

Prior studies reveal that blood cockle shells contain aragonite polymorph calcium with high purity and quality that has similar structure to the bone tissue. Hence, this fact shows the high potential use of calcium from blood cockle shell for biomedical purposes [7]-[11].

Calcium with micro size is the most consumed calcium by most people, although it could not be optimally absorbed by the human body. This causes calcium deficiency throughout the human population. Reference [12] shows the size reduction of calcium compound is required to enhance the digestibility of the calcium because calcium with nano size is capable of filling spaces between cells, which generally can only be filled by compounds with colloidal particle [13], [14] and the affinity of the calcium could be boosted because the size is reduced and furthermore the surface area increases [15].
Nano calcium powder requires demineralization by using hydrochloric acid, which releases other compounds associated with calcium to enhance the solubility. Minerals could be withdrawn by acidic solutions such as hydrochloric acid, sulfuric acid and lactic acid [16]. The recent application of nano calcium was for developing nutraceuticals, artificial teeth, toothpaste and mouthwash. This research aims to characterize nano calcium powder from blood cockle (*Anadara* sp.) shell produced by using different hydrochloric acid (HCl) concentration.

2. Material and methods

2.1. Materials

Shells with the diameter of 4cm were obtained from Sentra Pasar Ikan Bulak Pantai Kenjeran Surabaya, HCl (Merck, Germany), NaOH (Merck, Germany) and distillate water. All used chemicals were analytical grade.

2.2. Shell powder preparation

Shell powder (moisture content: 0.65%; ash content: 94.8%) was used in this study. Shells were washed with tap water and then sun-dried. The dried shells were then grinded and sieved through a filter with the number of 100 mesh.

2.3. Production of nano calcium powder

The scenario used in this study was reference [17] with minor modification. Approximately 100g of the shell was soaked in HCl for 24h with the ratio of shells to HCl solution was 1:8. The mixture was then heated at the temperature of 90°C for 1h, then filtered through filter paper .The filtrate was collected for further processes.

The crystallization was induced via a precipitation method by using NaOH solution 3N dropped gradually until the sediments formed. The sediment was then separated from the solution and neutralized by using distilled water. The neutralized sediment was then oven dried at the temperature of 105°C until persistent weight and then burned in a furnace.

2.4. Moisture content and ash content determination

This research employed a referenced method of AOAC (2007) to determine moisture content and ash content determination

2.5. Microstructure determination

The microstructure and composite homogeneity of the obtained samples were investigated using a Scanning Electron Microscopy (SEM) with Energy-dispersive X-ray spectroscopy (ZEISS EVO® MA 10, Quantax Energy Dispersive X-ray). Energy dispersive X-ray analysis measurements were performed under standard conditions

2.6. Experimental design

Four different HCl concentrations namely 0.5 N, 1.0 N, 1.5 N and 2.0 N was designed by using a completely randomized design. The treatment of 0.5 N, 1.0 N, 1.5 N and 2.0 N was represented by P1, P2, P3, and P4 respectively. The experiment was run in triplicate.

2.7. Data analysis

The yield data determined was analyzed using Analysis of Variance (ANOVA), followed by Duncan Test.

3. Result and discussion

This study showed that the different HCl concentration affected the yield of nano calcium powder because of the more concentrated HCl, the more capacity of HCl in withdrawing minerals contained in clam shell [17], [19]. This research was in contrast with the prior study carried out by [20] where the concentration used was 1N to 3N. Although the used concentration is higher than used concentration than present study, the yield is not in line because many organic substances in cockle shell were also eluted by HCl with high concentration (Table 1).
Table 1. Yield obtained from a different HCl concentration in nano calcium powder

| Treatment | Yield (g) | Moisture content (%w/w) | Ash content (%w/w) |
|-----------|-----------|-------------------------|--------------------|
| P1        | 0.70 ± 0.181* | 1.0                    | 90.38              |
| P2        | 0.82 ± 0.278* | 1.0                    | 88.90              |
| P3        | 0.76 ± 0.195* | 0.99                   | 88.87              |
| P4        | 0.85 ± 0.317* | 0.99                   | 81.55              |

According to Table 2, the composition of mineral in the nano calcium powder was different from the reference. Reference [7] states that the distinctive mineral contents within samples is caused by the environmental condition of the blood cockle habitats. Blood cockle shells contain carbonate layer in between skin and body [21]. Reference [22] shows the composition of minerals is affected by the habitat of cockles (geographical and physicochemical characteristics), age, species, and size. Magnesium plays important role in determining the physiological activities of living organisms which is connected with calcium and phosphorus [14]. Natrium is one of the abundant minerals in cockle because of the salt properties in their habitat, it diffuses away into the shells [14].

Table 2. Mineral composition of nano calcium powder

| Mineral Composition (%) | P1 | P2 | P3 | P4 | Reference [18] shows |
|-------------------------|----|----|----|----|---------------------|
| Calcium (Ca)            | 51.85 | 62.89 | 56.70 | 73.93 | 62.90 |
| Magnesium (Mg)          | 23.58 | 14.15 | 24.72 | 13.92 | 3.65 |
| Sodium (Na)             | 14.48 | 13.70 | 10.20 | 5.20 | 3.11 |
| Phosphorus (P)          | 7.00 | 6.66 | 6.55 | 5.17 | n/a |
| Potassium (K)           | 1.25 | 1.17 | 0.86 | 1.00 | 0.13 |
| Ferric (Fe)             | 0.74 | 0.91 | 0.83 | 0.75 | 5.28 |
| Manganese (Mn)          | 0.61 | 0.41 | 0.10 | 0.03 | 0.25 |
| Zinc (Zn)               | 0.49 | 0.11 | 0.05 | 0.00 | 0.09 |

The moisture content of nano calcium produced in this study was 0.99%- 1%, this amount increased to the raw material (0.65%). Reference [23] shows the upward trend of moisture content is caused by the method of nano calcium production itself where the used water during neutralization allows water to diffuse into the material. The longer time consumed during soaking, the more moisture content of nano calcium powder produced. The ash content of nano calcium powder reduced, compared to the raw material. Reference [17] shows the demineralization process causes the minerals to hold off from shells and the calcination occurs for approximately 5h. The neutralization, raising to pH of 7 affects the ash content of the nano calcium powder because some minerals are dissolved by water used [24].

Particle size is the most crucial parameter of nano calcium powder, the microstructure analysis (Fig 1) showed that particle size with poor uniformity was obtained by all experiments in this study which the size was 309.5-942.9 nm. However, the use of HCl at the concentration of 1N tent to produce higher uniformity than other treatments (454nm – 900nm). Particle size with a bigger size is generated by highly concentrated solution used during production and indicates that the distance between particles is short [25]. This research was in line with prior studies, revealing that nano calcium powder has the particle size between 10nm and 1000nm [26], [27]. Theoretically, the formation of calcium with nano size is caused by intensive stirring over production where stirring could generate calcium with particular nano size and size distribution [28].

According to SEM-EDX analysis shown in Table 3, calcium was the most macro mineral amounted to 51% to 73% of all contained minerals in nano calcium powder. During the production the pores of shells powder were enlarged because of HCl immersion, furthermore, the minerals could be increasingly withdrawn by the used solution [12].
Prior studies have investigated element content of the nano calcium powder such as CaC [7]; [10]. They suggest to use studied nano calcium powder as bone tissue engineering because the structure of CaC is close to bone tissue. Therefore, this present study suggested to perform further study on the element composition of nano calcium powder and made recommendation for an appropriate use.

4. Conclusion
This study concluded that the different concentration of used HCl affected particle size and mineral content of the nano calcium powder. HCl with the concentration of 1N was the most appropriate concentration in this study, resulting yield, calcium content, and particle size of 0.82%, 62.89% and 454.4nm respectively with higher uniformity than other treatments in this study.

5. References
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