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Optimization of Extraction Process of Nanocalcium from Eggshell

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***Abstract*—Different methods of nano-calcium carbonate extraction and the optimization production process was studied. The extraction methods were done by using alkaline solution (NaOH 1 N), acid solution (HCl 1 N), and precipitation. In the alkaline and acid extrcation, the powder and the solutions were refluxed with the ratio of 1:7 (wt/v) for 1 hours at 100°C. Meanwhile, the precipitation method was done by mixing the eggshell powder with HCl 1 N with the ratio of 1:7 (wt/v) for 1.5 hours at 90°C, which was subsequently precipitated by NaOH 3 N. The obtained nanocalcium powder was characterized by AAS. The extraction using alkaline and acid solution yielded 84.66% and 85.99% of nanocalcium, while the precipitation method obtained nanocalcium less than 1%. The proximate test shows the content of eggshell powder consists of 0.19% water, 98.07% ash and 0.09% lipid. The extraction using NaOH was optimized by varying the extraction time from 1 to 4 hours. The highest yield was reached from the extraction for 1 hour.**

Keywords: nanocalcium, eggshell, extraction, precipitation

I. INTRODUCTION

The eggshell is one of the household wastes which the number is abundant. The mineral content of eggshell is dominated by calcium in the form of calcium carbonate ($CaCO_3$) [1]. Therefore, the eggshell would have potency to be used as raw material to produce calcium as food supplement.

Calcium is commonly consumed in the form of micro calcium (10^{-6}m). The absorption of calcium in the body related to the size of particle, as the size is smaller, the absorbtion improves [2]. Therefore, in order to enhance the absorbtion effectivity, the size of calcium is reduced to nano-size (10^{-9}m), so called nanocalcium.

This research was conducted by comparing different methods to synthesize nanomaterial, i.e. by top-down and bottom-up approachment. The top-down method is to make nano structure by reducing the coarse material by grinding process. In the experiment, the top-down method was carried out by extracting the calcium using alkaline and acid solvent. Both of methods had been done by Lakahena et al. by using the fishbone of *Nile Tilapia*. Initially, the raw material were crushed which was subsequently extracted by alkaline (NaOH) and acid solvent (HCl) [3].

The precipitation method is a bottom-up approachment technique. This method was done by dissolving an active substance in the solvent, which subsequently added by antisolvent. The mixture of solvent/antisolvent lead to a solution of being saturated and the nucleation occurs more rapidly, thus nanoparticle is formed [4]. According to Gulsun et.al. and Haskell, the advantages of precipitation method include the simple process, low cost, and the obtained particles could be smaller than 100 nm [5, 6]. However, there is a drawback of this method, i.e. the obtained nanoparticle need to be stabilized to prevent the forming of micro-crystals [4].

The aims of the research was to study the effect of different extraction methods towards the yield of nanocalcium and to optimize the extraction process. In addition, the content of nanocalcium was analyzed in order to know its purity.

II. METHODS

A. Raw Material Pretreatment

The main material was eggshell which collected from the household waste. The dried eggshell was crushed into powder with size of 150 mesh.

B. Extraction Process

The extraction process was carried out in a set of refluxed apparatus by using different methods, i.e. extraction using alkaline, acid solution, and precipitation. The extraction using alkaline and acid solution were done by mixing the eggshell powder with NaOH 1 N and HCl 1 N, respectively. The ratio of powder dan solution was 1:7 (wt/v) and the extraction conducted for 1 hour at 100°C.

The precipitation method was done by mixing the eggshell powder and acid solution (NaOH 1 N) with ratio of 1:7 (wt/v) and refluxed for 1.5 hour at 90°C. Then, the reflux product was precipitated by alkaline solution of NaOH 3 N. The proximate analysis of eggshell powder was done before and after extraction. In addition, the obtained powder was characterized by AAS. The optimization process was done for the best method by using the same procedure and varying extraction time from 1 to 4 hours.

III. RESULTS AND DISCUSSION

A. Chemical Compound of The Eggshell

The eggshell powder as raw material for producing nanocalcium had been analyzed its chemical compound. The result of the proximate analysis shown the powder content was water (1.48%), ash (95.09%), and lipid (0.345).

The ash content in the powder shows the mineral content (Ca, Mg, P, CO_3, and Mn) in the eggshell. The ash content of eggshell used in this experiment was in

accordance with the analysis done by Rumanof et.al. which reported the ash content of eggshell used in their experiment was 95.1% [7]. However, another analysis done by Setyaningrum et al. shows different result, that the ash content used in their research was 67.61%. The difference of ash content in the eggshell was confirmed due to the species and the environment [8].

The lipid content of the eggshell used in this experiment was similar with the analysis result done by Nasution [9]. The lipid content in the eggshell was derived from the cuticle layer. The cuticle layer contains 90% of protein and a small number of carbohydrate and lipid [10]. The lipid content can also be derived from the membrane layer of the eggs.

B. The Effect of Different Extraction Methods

The three different methods of nanocalcium extraction was conducted according to the procedure described earlier. The set parameters are presented in Table 1.

TABLE I. PARAMETERS SET IN THE EXTRACTION PROCESS

Parameters	Extraction methods		
	Extraction using alkaline solution	Extraction using acid solution	Precipitation
Temperature	100°C	100°C	90°C
Solvents	NaOH 1N	HCl 1N	HCl 1N
Antisolvents	-	-	NaOH 3N
Time	1 hour	1 hour	1.5 hour
Initial soaking	-	24 hours	24 hours

The extraction using NaOH causes protein in the eggshell dissolves, thus the calcium content is easier to be extracted. In this process, the extraction temperature set 100°C in order to improve the rate of hydrolysis. During the alkaline extraction, by heating while stirring the egshell powder led to forming of foams. The foams was an indication of the lipid reduction due to occurance of saponification reaction between NaOH and the lipid content [11]. Reaction of eggshell powder and alkaline solution occured according to equation 1.

$$CaCO_3 + NaOH \rightarrow Ca(OH)_2 + Na(CO_3) \quad (1)$$

The second method used in the experiment was the extraction using acid solvent (HCl). In this method, the acid softens the eggshell, thus the mineral content could be dissolved. As reported by Suptijah et al., based on their research using shrimps shell, the initial soaking of the shell powder using NaOH led the shell pores maximally open, thus the formed room provides the acces for HCl, therefore the mineral was easily extracted [2]. The calcium released from the eggshell by HCl follows equation 2.

$$CaCO_3 + HCl \rightarrow CaCl_2 + H_2CO_3 \quad (2)$$

In the beginning of mixing process of eggshell and HCl 1 N led to foaming due to the formation of CO_2 and H_2O in the solution.

The other method done in the experiment was precipitation. This method was conducted by dissolving calcium in the eggshell using HCl which subsequently added by NaOH. The mixing of acid and basic causes the solution saturated and resulted in the precipitation of calcium with nano size. The precipitation method could be done by dissolving an active substance in the solvent which subsequently added by antisolvent [4]. The mixture of solvent/antisolvent led to saturation of solution, thus nucleation occured rapidly which followed by formation of nanoparticle.

In the precipitation method, calcium carbonate ($CaCO_3$) contained in the eggshell was reacted by HCl to form $CaCl_2$. The formed calcium chloride ($CaCl_2$) was precipitated by NaOH resulted in formation of calcium hydroxide ($Ca(OH)_2$) and salt [12]. The reaction follows equation 3 and 4.

$$CaCl_2 + 2NaOH \rightarrow Ca(OH)_2 + 2NaCl \quad (3)$$

The salt (NaCl) was removed during netralization process. The remaining product of $Ca(OH)_2$ converted to calcium oxide (CaO) at 600°C. Since the H_2O evaporated at this temperature, the final product was nano-calcium oxide.

Yield of the producted nanocalcium from the experiment is presented in Table 2.

TABLE II. YIELD OF NANOCALCIUM FROM THE EXPERIMRNT

Methods	Yield (%)
Extraction using NaOH	84.66
Extraction using HCl	85.99
Precipitation	0.89

The highest yield of nanocalcium was reached from the extraction using HCl, eventhough the alkaline extraction did not show much different. It was predicted that alkaline solution dissolved not only the calcium, but also the protein and lipids from the eggshell. According to the presented data, the precipitation method resulted in nanocalcium less than 1% which was predicted due to the temperature used to extraction was lower than 100°C.

The analysis of nanocalcium using AAS (Atomic Absorption Spectroscopy) shows the calcium content in the product as can be seen in Figure 1.

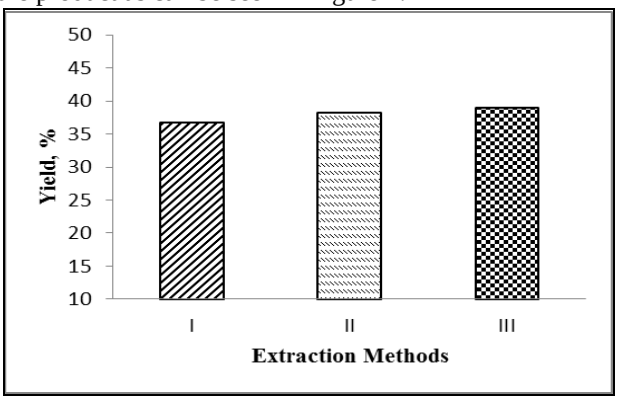

Figure 1. Calcium content of Nanocalcium from Extraction using Different Methods: I. Acid Extraction, II. Alkaline Extraction, and III. Precipitation

The precipitation method resulted in the highest calcium content which is in accordance with the result of Lakahena et. al. research using the fishbone of *Nile Tilapia*. They reported that extraction using both of acid and alkaline solvents could enhance the calcium content in the product [3]. In addition, the longer extraction time led to increasing the extraction yield. However, if the

extraction set too long, the yield decreased since the solution become saturated.

According to the obtained data, the extraction method using alkaline solvent was the best method which produced nanocalcium more than 50% and the purity was 38.24%. The chemical compound of nanocalcium powder was subsequently tested based on proximate method as presented in Table 3.

TABLE III. CHEMICAL COMPOUND OF NANOCALCIUM AND EGGSHELL POWDER

Chemical compound	Nanocalcium powder	Eggshell powder
Water content (%)	0.19	1.48
Ash content (%)	98.07	95.09
Lipid content (%)	0.09	0.34

From Table 3 can be known that after the extraction process, the water and lipid content of the eggshell powder lower than before the treatment. However, the ash content increased which was an indication of mineral content of calcium.

C. The Optimization of Extraction Time

Optimization of the process was done by varying the extraction time from 1 to 4 hours. Since the best method from this experiment was the alkaline extraction, the optimization was carried out based on this method. The resulted nanocalcium content is presented in Figure 2, while the yield is shown in Table 4.

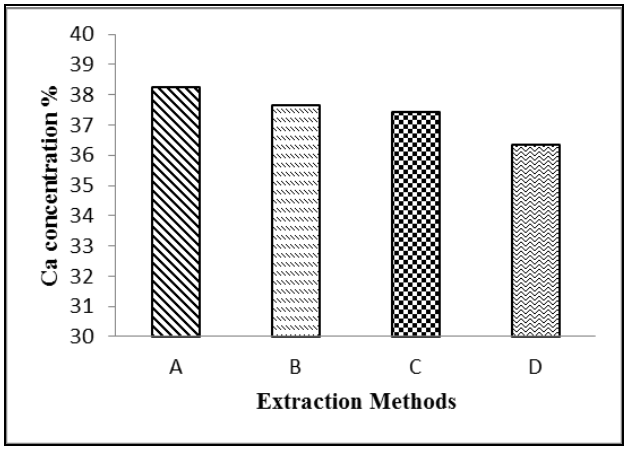

Figure 2. Calcium Content of Nanocalcium Powder from Extraction Process for (A) 1 hour, (B) 2 hours, (C) 3 hours, and (D) 4 hours.

From the obtained data can be seen that by setting the extraction time longer, the Ca content decreased. However, reduction of the Ca concentration was not significant.

TABLE IV. THE YIELD OF THE EXTRACTED NANOCALCIUM

Extraction Time (hour)	Yield (%)
1	84.66
2	84.64
3	84.63
4	84.61

In addition, the proximate analysis of the resulted nanocalcium from the extraction process for 1 to 4 hours can be seen in Table 5.

TABLE V. CHEMICAL COMPOUND OF NANOCALCIUM PRODUCED FROM EXTRACTION IN DIFFERENT TIME

Chemical compound	Extraction time (Hours)			
	1	2	3	4
Water content (%)	0.1904	0.1901	0.1893	0.1902
Ash content (%)	98.07	98.01	98.03	98.02
Lipid content (%)	0.09	0.092	0.083	0.08

From the resulted data can be seen that optimization of the nanocalcium extraction process using alkaline solvent was done for 1 hour which yielded the most nanocalcium with the highest Ca content of all.

IV. CONCLUSIONS

The extraction of nanocalcium from eggshell using alkaline (NaOH) and acid (HCl) methods yielded more than 80%, while the precipitation method resulted less than 1% of nanocalcium powder. The best method of extraction which resulted in the highest Ca concentration of about 38.25% was the alkaline process, although the data was not much different with the Ca content from acid extraction. The obtained data from the optimization of alkaline extraction shows the highest yield with the most nanocalcium content of this experiment could be produced by alkaline extraction for 1 hour. The proximate analysis shown the water, ash, and lipid content of nanocalcium were about 0.19%, 98%, and 0.09%, respectively.

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