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Effect of soaking time of blood cockle (*Anadara* sp.) shells powder with hydrochloric acid on the characteristics of nano calcium

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Abstract. Nanotechnology is an applied science at the atomic scale. Nanotechnology engages the construction of small structures and devices by manipulating each of the molecules and atoms that has unique and strong characteristics. The aim of this research is to know the effect of long soaking of blood cockle (*Anadara* sp.) shells powder in the hydrochloric acid on the characteristics of nano calcium, which include particle size analysis, analysis of the levels of minerals, yield, and analysis of proximate (moisture and ash). The research using RAL with 4 treatments (control, soaking 24 hour, soaking 48 hour, soaking 72 hour) with 5 replicates. Particle size, levels of minerals, and analysis of proximate (moisture and ash) analyzed by descriptive methods, while the yield data were analyzed with statistic. The best result is on 48 hour treatment. It can be seen from the size of particles, the levels of minerals, and yield.

1. Introduction

Shellfish production in Indonesia continues to increase from year to year. Shellfish production data from 2000 to 2010 has increased 5.18% annually. This commodity produces waste in the form of shells whose utilization is not optimum yet [1].

Shells of blood cockle (*Anadara* sp.) is a source of calcium. Research conducted by Rohanah *et al.* [2], showed that the calcium content found in shells (bivalves) was 39.38%. The main compound contained in blood shells is calcium carbonate (CaCO₃) [3]. The results of research conducted by Mohamed *et al.* [4], the results obtained that the blood shells (*Anadara granosa*) contain 98.99% of calcium carbonate, while the results of Bharatham *et al.* [5] is the calcium carbonate that content in blood shells (*Anadara granosa*) was 95.7% or 96%.

The existing of calcium carbonate has a micro size. Calcium which has a micro size releases calcium ions for a long time. Technology needs to be developed to accelerate the release of calcium ions. The technology that needs to be developed is nanotechnology, which is the formation of smaller calcium sizes. Nano calcium has a very small size which is 10⁻⁹ m. One of the uses of nano-sized calcium carbonate in the industrial field is to manufacture toothpaste. The size of nano calcium carbonate resembles that of calcium apatite in tooth enamel and can prevent caries in teeth [6].



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One of the processes of making nano calcium from blood shells is the extraction process. The extraction process is done by immersing the shell powder in a solution of HCl. The soaking process facilitates the calcium release reaction. Immersion carried out in acid can reduce phytate content, this content is known as chelating calcium [7]. Optimal soaking time will cause the opening of the pores of the shell to the maximum, resulting in a high yield [8]. Based on the description above, the effect of blood shells (*Anadara* sp) immersion with HCl was investigated on the extraction of nano calcium characteristics. The aim of this study was to determine the effect of the immersion time of blood cockle shells (*Anadara* sp.) with hydrochloric acid on the characteristics of nano calcium, which includes particle size analysis, mineral content analysis, yield, and proximate analysis (water and ash content).

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2. Materials and methods

2.1 Place and time of research

Research of nano calcium was held in March 2017 at the Education Laboratory, Faculty of Fisheries and Marine, Airlangga University. Testing of particle size and mineral content by using SEM-EDX has been carried out in the Laboratory for Energy and the Environment, Robotics Building, Institute of Technology Surabaya.

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2.2 Tools and materials

Equipment that used in nano calcium production is Beaker glass, furnace, thermometer, oven, hotplate, filter paper, whatman paper, pH paper, fume hood, gloves, masks, pipettes, magnetic stirrers, glass spatulas, vacuum, analytical scales, analytical tools proximate, and SEM-EDX. The material that used in nano calcium production is a blood cockle shell (*Anadara* sp.) that obtained from Kenjeran Beach, Surabaya. The materials are HCl, NaOH, and sterile aquadest.

2.3 Research preparation

The initial step carried out in this study was the preparation of blood shells carried out by washing the shells. Dried shell with the sun's heat. Crushed the dried shells with a 100 mesh grinder to turn into flour. Tested the shell flour with the proximate test (moisture and ash content) to determine its chemical composition [9].

2.4 Process of nano calcium

Shell flour produced from the destruction process is then performed soaking shells using 1N HCL for 0, 24, 48, 72 hours with a ratio of 1: 8. The shell flour soaked, then extracted by using a temperature of 90 ° C for 1 hour.

Results of subsequent extraction filtering with whatman paper in order to obtain fluid / filtrate. The filtrate obtained precipitation process is carried out with the addition of 3 N NaOH and stirring. The next process is allowed to stand until precipitation is not formed again.

The precipitate resulting from this process are separated by decantation. The neutralization process is then performed on the sediment using distilled water until the pH is 7. The drying process is performed after the deposition with a neutral pH oven with a temperature of 105°C, and followed by burning in a furnace at 600°C for 4 hours to remove organic materials. The last stage of nano calcium powder pulverized by using a mortar and analysis [10]. Characterization is done on nanokalsium generated in this study. Analysis was conducted on the calculation of particle size analysis, analysis of mineral content, yield and proximate analysis (moisture and ash).

2.5 Characterization of nano calcium

2.5.1 Procedures particle size analysis and mineral content

The particle size and nano calcium mineral content with different immersion times were analyzed using SEM-EDX. How it works SEM-EDX is a powder sample that has been placed on a specimen

holder inserted into the chamber specimen, then put in a SEM-EDX device and the tool is ready for operation.

The sample in the SEM-EDX device will interact with electron beam flow. Interactions that occur will then be detected and changed into an image by SEM analysis and also in graphical form by EDX analysis [11]. The picture that has appeared is measured particle diameter to get the particle size.

2.5.2 The yield calculation

The yield is a percentage of the comparison of the final weighting level of the nanocalcium to the shell weight of the blood shell before undergoing treatment [12]. The number of yields can be calculated using the equation:

$$\text{Yield} = a \text{ (gram)} / b \text{ (gram)} \times 100\%$$

Information:

a = Weight of the process

b = Weight of initial ingredients

2.5.3 Calculation of water content

Moisture content was analyzed using a moisture analyzer. The first step is to make sure the moisture analyzer is in a normal state and an electric current is available. The moisture balance position is regulated by adjusting the support screw so that the position is exactly horizontal by looking at the position of the point on the water pass located in the center of the circle. Next, the instrument is turned on and adjusted to 0.5-1 g with a temperature of 105 ° C, then enter the sample to test the water content. The results of the moisture content will be listed on the PC screen directly. Record the results on the PC moisture analyzer screen.

2.5.4 Calculation of ash content (AOAC, 2005)

Calculation of ash content is done by weighing a sample of 2 grams, then placed in a porcelain cup and blended in a furnace with a temperature of 600°C for 2 hours. The next process is to weigh the samples that have been planted. Calculation of ash content is done using the formula: $K.A = (\text{final sample} + \text{cup}) - \text{cup} \times 100\% / (\text{initial sample} + \text{cup}) - \text{cup}$.

2.6 Data analysis

Data of water and ash content, particle size, mineral content were analyzed by descriptive method. The yield data obtained from the nano calcium test results with different immersion times were analyzed using statistical methods. Data were analyzed with ANOVA (Analysis of Variant). The yield data showed that the results were not significantly different so that the Duncan test was not carried out. Data analysis was performed with SPSS 16.0.

3. Result and discussion

Based on the results on the effect of immersion time of blood cockle shells (*Anadara* sp.) with hydrochloric acid on the characteristics of nano calcium obtained the following results:

Testing of water and ash content is carried out on the raw material of a cockle shell. The results of water and ash content analysis of raw materials in blood shells (*Anadara* sp.) can be seen in Table 1.

Table 1. Analysis of water and ash content of blood shells (*Anadara* sp.)

Shells (<i>Anadara</i> sp.)		
Repetition	Water Content (%)	Ash levels (%)
1	0.85	95.77
2	0.93	95.95
3	0.89	95.85
Mean ± SD	0.89 ± 0.035	95.86 ± 0.090

³ The results of the analysis of the percentage of water content of raw materials in blood shells (*Anadara* sp.) showed low results, that is < 1%. While the results of ash content showed high results, that is > 95% (Table 1.). Water is an important component in a material. Water can affect the appearance and there is a certain amount in a material [13]. The results of measurements of water content showed low results on the raw material of shells. This is thought to be caused by the process of drying the shell before it is destroyed.

Ash is the residue from combustion of an organic material. The ash content and composition depends on the type of material being analyzed. Ash content in blood shells was 95.86%. Ash content in blood shells is higher than gravestone shells. The level of gravestone ash in the Khoerunnisa [10] was 55.31% and in the Wardhani [14] was 93.34%. The high ash content is due to the fact that the blood clam shells have a high mineral content. Ash content is influenced by mineral components in the material [16]. Blood shells contain high calcium carbonate so as to produce high ash levels. According to Mohamed *et al.* [4], the content of calcium carbonate in blood shells was 98.99%.

After measuring the water and ash content in the shell's raw material, the process of immersion of shell powder in HCl is carried out. According to Khoerunnisa [10], the immersion reaction of the shells in HCl solution is $\text{CaCO}_3 + 2\text{HCl} \rightarrow \text{CaCl}_2 (\text{soluble}) + \text{H}_2\text{CO}_3$. The initial process of mixing blood shells with HCl, formed a lot of foam and air bubbles that lasted about ± 10 minutes. This is in accordance with Minarty [12] states that many foam and air bubbles are caused by the formation of CO_2 and H_2O gases on the surface of the solution.

The process of soaking with HCl will cause a reaction to release calcium. Then the extraction process is carried out. The extraction results are then filtered to produce clear filtrate. The content in the filtrate is calcium carbonate (CaCO_3) which is then carried out the precipitation process. This is in accordance with Khoerunnisa [10] which states that calcium in the crab shell in the form of calcium carbonate (CaCO_3) is carried out in the precipitation process using NaOH. The precipitation method is a mixture of acid-base which produces crystalline solids and water [15]. The reaction that occurs during the precipitation process is $\text{CaCl}_2 + 2\text{NaOH} \rightarrow \text{Ca}(\text{OH})_2 + 2\text{NaCl}$.

The precipitation process will produce deposits in the form of calcium hydroxide and NaCl solution. The salt solution (NaCl) formed is separated by decantation and neutralized by using aquadest, so that the remaining ($\text{Ca}(\text{OH})_2$). The next process was dried in oven using a temperature of 105°C for 24 hours, and ashing using a temperature of 600°C for 4 hours. Ignition process will produce calcium oxide (CaO). The final product produced is nano calcium oxide powder.

Nano calcium that has been made the yield calculation. The yield is the percentage of raw material that can be utilized. The results of the calculation of nano calcium can be seen in Table 2.

Table 2. Calculation of nano calcium

Treatment	Yield (%)
Without Soaking	0.96 A \pm 0.242
Soaking 24 hours	1,078 a \pm 0.315
Soaking 48 hours	A 1.402 \pm 0.448
Soaking 72 hours	1,172 a \pm 0.307

Description: The immersion time no significant effect on the yield of nano calcium. Notation indicated by different superscript letters in the same column shows the comparison between treatments did not have significant differences ($P > 0.05$).

Based on data analysis results, it is known that the immersion time with HCl has no real effect on the yield of nano calcium. The yield produced in this study continued to increase until the 48-hour, shell immersion powder was treated and decreased at 72 hours. Increasing the extraction time will cause an increase in the mass of the dissolved substance to the optimal time, if more than the optimal time the yield does not increase.

The highest yield was found in the soaking time treatment of 48 hours, that is 1.402%. This is lower than the research conducted by Suptijah *et al.* [8], which explains that the yield with an optimum

soaking time of 48 hours produces the highest yield of 13.92%. The yield of nano calcium is influenced by the length of time immersion of shell powder in HCl. This is because the longer immersion time will produce a higher yield until an equilibrium concentration occurs in a solution called the saturation point [8]. Khoerunnisa [10] stated that the length of time for HCl extraction had an effect on the yield of nanocalcium from local gravestone shells (*Pilsbryconcha exilis*).

Analysis of water and ash content in nano calcium products is done after the yield calculation process is complete. Water and ash content testing was carried out on nano calcium products with different soaking time treatments. The results of the analysis of water content and nano calcium ash can be seen in Table 3.

Table 3. Analysis of moisture and nano calcium ash

Nano Calcium	Water content (%)	Ash levels (%)
	Mean \pm SD	Mean \pm SD
Without soaking	1.11 \pm 0.155	84.79 \pm 0.351
Soaking 24 hours	1.48 \pm 0.275	82.71 \pm 0.337
Soaking 48 hours	1.84 \pm 0.210	91.24 \pm 0.719
Soaking 72 hours	2.16 \pm 0.136	83.00 \pm 0.661

The results of the analysis of the water content percentage in nano calcium continues to increase. Water content in nano calcium showed the lowest results, that is 1.11% in the treatment without immersion and the highest was 2.16% in the 72 hour immersion treatment (Table 3.). This is because there is precipitation process in nano calcium making process. The precipitation process will produce Ca (OH) 2 and NaCl. H2O content that causes nano calcium water levels to increase.

The results of the ash content show the opposite, that is the percentage decreases of ash content in nano calcium. The lowest ash content is at the 24-hour immersion and the highest at 48 hour soaking. The value of lower ash content is possible because the mineral content of nano calcium is decreases because the making process of nano calcium has undergone a graying process for 4 hours. This is in accordance with Suhartini [17] which states that the furnace process can reduce mineral content due to high temperatures. High ash content values indicate the calcium content contained in nano calcium [10].

Particle size analysis was performed on nano calcium products using SEM magnification 30.00x. Particle size is obtained by measuring the smallest diameter of calcium nano particles. The Smallest Particle Size Results Using SEM can be seen in Table 4. Nano calcium particles with a magnification of 30,000x can be seen in Figure 1. (A) without Immersion, (B) 24 hour immersion, (C) 48 hour immersion, (D) 72 hour immersion.

Table 4. Range of smallest particle size by using SEM

Treatment	Particle size
Without Soaking	338.7 to 809.1 nm
Soaking 24 hours	813.9 nm
Soaking 48 hours	167.3 to 203.1 nm
Soaking 72 hours	354 to 448.7 nm

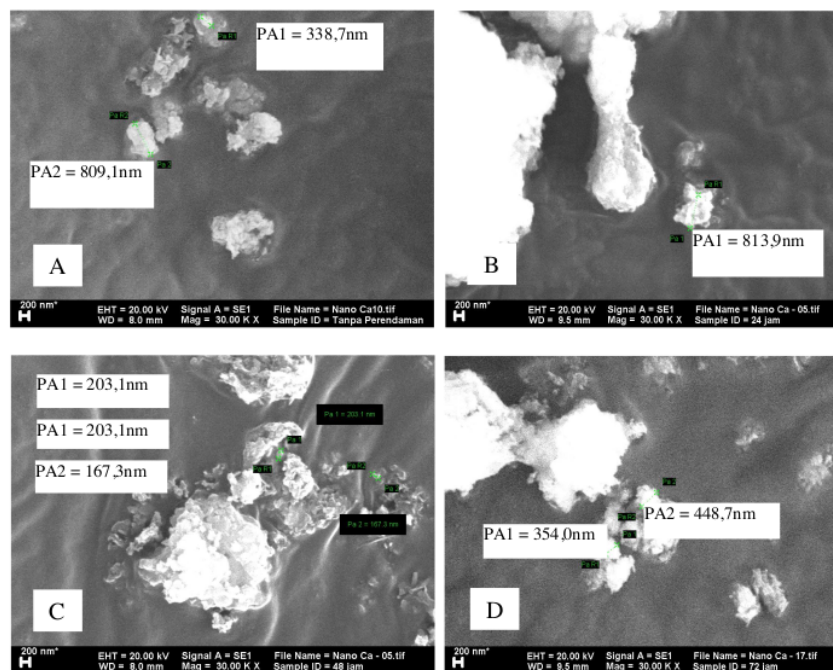


Figure 1. Nano calcium particle without soaking (A), Nano calcium particle soaking for 24 hours (B), Nano calcium particle soaking for 48 hours (C), and Nano calcium particle soaking for 72 hours (D).

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Particle size and size distribution are the most important characteristics of nanoparticle systems. Particle size analysis shows non-uniform particle size ranging from 167.3 to 813.8 nm in all treatments. According to Mohanraj and Chen [18], nanoparticles are defined as particles measuring in the range of 10-1000 nm. The smallest particle size is found in nano calcium products with 48 hours of immersion, which is 167.3-203.1 nm (Figure 1C). The largest particle size is in nano calcium products with a soaking time of 24 hours, which is 813.8 nm (Figure 1B). The results obtained in the study are greater than the size of nano particles according to Muller and Keck [19], which is the size of nanoparticles ranging between 200-400 nm.

The results calculation show the immersion time can affect the size of nano calcium particles. This is presumably because the optimum immersion time causes more calcium particles to be released and the frequency of collisions will be greater. The greater frequency of collisions will result in smaller particle sizes. A saturated solution produces a larger particle size. This is presumably because the frequency of collisions between particles is reduced. Chemical reactions take place due to collisions between molecules with one another in the reaction. The reaction rate will be directly proportional to the number of collisions of molecules per second, or directly proportional to the frequency of molecular collisions when viewed in terms of the theory of collisions from chemical kinetics [20]. According to Firdaus [21], stirrer rotation speed used in the process of making nanoparticles can cause differences in size.

Nano calcium contains several mineral compositions. Mineral content in nano calcium is measured using EDX. The mineral content contained in nano calcium can be found in Table 5.

Table 5. Mineral Levels Contained in Nano Calcium

Mineral	Mineral Content (%)			
	Without submersion	Submersion 24 hours	Submersion 48 hours	Soaking 72 hours
ca	32.42	43.73	75.79	65.59
mg	13.94	10.92	10.85	9.36
P	24.20	22.49	6.59	11.29
Zn	15.00	14.56	3.74	7.22
Cl	9.64	6.26	1.87	4.59
Fe	1.61	0.95	0.95	1.38
K	2.07	0.82	0.16	0.37
Na	1.14	0.06	0.05	0.20

Particle size is influenced by mineral content in nano calcium. The most macro mineral contained in nano calcium is calcium (Ca). This is because the blood cockle shells contain high amounts of CaCO₃. The highest level of calcium was found in nano calcium with 48 hours immersion treatment time which was 75.79% (Table 5.). Calcium levels in this study differ from studies conducted by Suptijah [8], which is obtained as much as 85.49% calcium results on nano calcium vannamei shrimp branches with immersion for 48 hours.

Magnesium (Mg) is contained in high amounts in nano calcium products after calcium content. Magnesium is often found in mollusk shells with a magnesium content of more than 1%. Magnesium along with calcium is found in prismatic layers in the form of calcite and aragonite crystals [10]. Sodium (Na), phosphorus (P), and potassium (K) are also contained in nano calcium. This is thought to originate from the aquatic environment. Aquatic environments contain sodium, phosphorus, and potassium in the form of ions [22]. The mineral ions are diffused into the shell. The chloride (Cl) contained in nano calcium comes from the solvent used, which is HCl. The mineral content of micro iron (Fe) and zinc (Zn) in nano calcium shows a small amount. Iron content ranges from 0.95-1.61%. This content is much higher than the research of Suptijah [10] which produces iron content of 0.48-0.71%.

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4. Conclusion

The conclusion of this study is that nano calcium with various immersion times shows different characteristics. The smallest particle size in all treatments showed a yield range of 167.3-813.8 nm. The highest particle size, mineral content, and yield yielded on nano calcium with 48 hours soaking time. Soaking time for 48 hours is optimum on nano calcium. Proximate results (water and ash content) in raw materials and nano calcium products show different results. The value of water content in nano calcium products is higher than shell raw materials. The value of ash content shows the opposite results. Suggestion in this research is the need to process rapid stirring in the production of nano calcium to obtain smaller particle size and bioavailability tests so that it can be applied in various fields.

5. References

- [1] Kementrian Kelautan dan Perikanan 2011 *Statistik Perikanan Tangkap Indonesia 2010* (Jakarta: Direktorat Jendral Perikanan)
- [2] Rohanah S, Anton, Kosasih Y, and Aristaking W 2009 *Pemanfaatan Tepung Limbah Kulit Kerang sebagai Bahan Paduan Semen Portland* (Bogor: Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor)
- [3] Lesbani A, Kurniawati R, and Mohadi R 2013 *Cakra Kimia* **1**, 1-7
- [4] Mohamed M, Rashidi N A, Yusup S, Teong L K, Rashid U, and Ali R M 2012 *J Clean Prod* **37**, 394-397

- [5] Bharatham H, Zakaria Md Z A B, Perimal E K., Yusof L M, and Hamid M 2014 *Sains Malay* **43**(7), 1023-1029
- [6] Dianti F, Triaminingsih S, and Irawan B 2014 *Pengaruh Pasta Gigi Siwak dan Pasta Gigi Nano Kalsium Karbonat terhadap Kekerasan Email yang Terdemineralisasi* (Jakarta: Universitas Indonesia) 17 p
- [7] Yagoub A E G A, Mohhamed M A, and Baker A A A 2008 *Pakistan J Nutr* **7**(1), 50-56
- [8] Suptijah P, Jacob A M, and Deviyanti N 2012 *J Akuatika* **3**(1), 1-11
- [9] [AOAC] Association of Official Analytical Chemist 2005 *Official Method of Analysis of The Association of Official Analytical of Chemist* (Arlington: The Association of Official Analytical Chemist, Inc)
- [10] Khoerunnisa. 2011. *Isolasi dan Karakterisasi Nano Kalsium dari Cangkang Kijing Lokal (Pilsbryconcha exilis) dengan Metode Presipitasi* (Bogor: Institut Pertanian Bogor)
- [11] Prasetyo, Y 2011 *Scanning Electron Microscope dan Optical Emission Spectroscopy* <http://yudiprasetyo53.wordpress.com/2011/11/07/scanningelectron-microscope-sem-dan-optical-emission-spectroscopy-oes/> diakses 20 Desember 2016
- [12] Minarty I S 2012 *Aplikasi Nanokalsium dari Cangkang Rajungan (Portunus sp.) pada Effervecent* (Bogor: Institut Pertanian Bogor) 50 p
- [13] Winarno F G 2008 *Kimia Pangan dan Gizi* (Bogor: MBrio Press)
- [14] Wardhani Y K 2009 *Karakteristik Fisik dan Kimia Tepung Cangkang Kijing Local (Pilsbryconcha exilis)* (Bogor: Institut Pertanian Bogor)
- [15] Chasanah F 2016 *Optimasi Produksi Glukosamin Hidroklorida dari Karapas Udang dengan Kombinasi Konsentrasi HCl dan Waktu Pemanasan* (Surabaya: Universitas Airlangga)
- [16] Purwasasmita B S and Gultom R S 2008 *J Life Phys Sci* **10**(2), 155-167
- [17] Suhartini N 2012 *Penentuan Kadar Abu* www.google.co.id, 9 March 2017
- [18] Mohanraj V J and Chen Y 2006 *J Pharm Res* **5**(1), 561-573
- [19] Muller R H, and Keck C M 2004. *J Biotechnol* **113**, 151-170
- [20] Chang R 2005 *Kimia Dasar: Konsep-Konsep Inti Jilid 2* (Jakarta: Erlangga)
- [21] Firdaus R A 2016 *Pengaruh Penambahan Polysorbate 80 terhadap Ukuran Nanopartikel Kitosan Karapas Udang Vaname (Litopanaeus vannamei)* (Surabaya: Universitas Airlangga) 66 p
- [22] Darmono 1995 *Logam dalam Sistem Biologi Makhluk Hidup* (Jakarta: UI-Press)

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