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Proximate characteristics of nano calcium in Blood Cockle (*Anadara granosa Liin*) shell from four different locations

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Proximate characteristics of nano calcium in Blood Cockle *(Anadara granosa Liin)* shell from four different locations

S Widyastuti[1] and D Evawati[2]

[1] Department of Environmental Engineering, University of PGRI Adi Buana Surabaya Indonesia.
[2] Department Home Economics, University of PGRI Adi Buana Surabaya Indonesia.

Email: rafirudi@yahoo.co.id[1], diana_evawati@yahoo.com[2]

Abstract. The purpose of this study was to investigate the proximate characteristics found of nano-calcium in shells of blood cockle (*Anadara granosa* liin) in four different areas, namely Kenjeran, Surabaya; Gresik Regency; Sedati District, Sidoarjo Regency; and Bluru District, Sidoarjo Regency. The parameters used were yields, proximate analysis and mineral content of calcium (Ca) and lead (Pb). The results showed that the nano-calcium in blood cockle shell from Kenjeran, Surabaya, had the lowest yield value of 0.2448 grams. The highest proximate result of ash content was found in the shell of the cockle from Bluru, as much as 53.46%.

1. Introduction

The problem of calcium deficiency in human body is because the available calcium is in the size of micro calcium which is not optimally absorbed by the body. This may lead to calcium deficiency that results in a variety of symptoms on bone, teeth, blood, nerves, and body metabolism (Tongchan et al., 2009). Therefore, we need the technology of size reduction, which is in the form of nanotechnology. Nanotechnology can create calcium in a very small size (10-1000 nm). Nano calcium can be directly absorbed by the body perfectly, making it more efficient than the calcium currently consumed publicly. Minimizing the size of the calcium minerals into nano (10-9 m) with nano-blend technology will make the absorption directly by the cells more perfect (Suptijah 2009). Nano calcium is more stable so it can be used specifically on the cell layer, spreads via the lymphatic system, high bioavailability, and has low toxicity. The process in the body occurs slowly so that the dose becomes low and the frequency of consumption becomes rare, and can also be aimed at the body's immune system.

Valentina et al. (2014) revealed that the intake of calcium and vitamin D in Indonesian children was lower than the Nutrition Adequacy Rate (NAR). In addition, based on the results of osteoporosis risk data analysis by the Nutrition Research Center, the Ministry of Health, 2 out of 5 people of Indonesia have the risk of osteoporosis. This is also supported by the Indonesian White Paper issued by the Association of Osteoporosis Indonesia in 2007. Osteoporosis in women over 50 years reached 32.3%, while in men over 50 years reached 28.8% (Kemenkes 2009).

Utilization of invertebrate waste (crustaceans), as an animal-compatible source of calcium for calcium supplements using nano-blend technology, is a viable breakthrough. Sea-cockle shells can be converted into nano-sized calcium minerals that can be utilized in various fields, such as for fortification of calcium in food and in clean water handling efforts to be used as filter media.

One of the aquatic animals as a source of calcium to be studied was the shell of shellfish, the blood cockle (*Anadara granosa* Linn), which is one type of molluscs with high economic value and widely used by the community as a source of alternative food. The sources of calcium supplements are currently imported, while natural sources of calcium are found in marine animals, especially in hard-skinned shells, such as crabs and shrimp.

The production of this blood cockle shell has increased every year. However, this increase was not accompanied by the utilization of its waste which amounted to 88.90 tons in 2013 as compared to 2008 which was only 67.90 tons (Directorate General of Capture Fisheries, 2013; Fisheries and Marine Fisheries Statistics In Figures 2013).

Waste of shellfish contains a chemical pozzolanic compound, the lime (CaO) as much as 98.7. (Zuki Abu Bakar Zakaria, 2004). The content of lime in blood cockle is higher than that in barnacle or shrimp. Crustacean crust waste contains 30-40% protein, 30-50% calcium carbonate, and 20-30% chitine (Arbia et al., 2013).

Research on the utilization of limestone in the shells of marine animals has been aimed at local flour production (Wardani, 2009), nano calcium manufacture from local barnacle (Pilisbryoconcha exilis) by Khoerunnisa (2011 in Vanessa Lekanena et al. 2014), nano calcium production from crab shell (Portunus sp.) by Iis Setyani Minarty (2012), nano calcium production from vannamei shrimp (Litopenaeus vannamei) by Suptijah (2012) or tilapia bone (Vanessa Lekanena et al 2014). Shell of shrimp and crabs should be processed into chitin since the dominant content is chitin. This is different from blood cockle shell that has no chitin content, but high calcium content (Wardani, 2009). Applications of nanotechnology in the food sector include enhanced flavor, color, texture and consistency of food products, enhanced absorption and bioavailability of nutrients and bioactive compounds (Greiner 2009).

The precipitation method is a bottom-up approach technique. In the precipitation method the active substance is dissolved into the solvent, then added with another solution which is not solvent (anti-solvent). This causes the solution to become saturated and rapid nucleation occurs to form nanoparticles (Kenth 2009). The advantages of this method are simple and low cost (Gulsun et al., 2009). The disadvantage of this method is that the nanoparticles formed must be stabilized to prevent the emergence of micro-sized crystals (Kenth 2009)

According to Haskell (2005), precipitation method is performed by controlling the solubility of the material in the solution through changes in pH, temperature, or solvent. The precipitates produced from very saturated conditions have many small particles. The advantage of this method is that it can produce particles smaller than 100 nm and using very low energy consumption.

Nano calcium is a highly efficient predigestive mineral in entering body cells because of its super-small size (nanometer) so it can be absorbed quickly and perfectly (Suptijah 2009). Gao et al. (2007) stated that mice given with nano calcium had a low calcium effluent in feces and urine compared to mice fed with micro calcium.

The purpose of this study was to study the chemical characteristics of nano calcium of blood cockle shells (*Anadara granosa* liin) at four different sites. Parameters observed were yields, chemical characteristics such as proximate content, calcium mineral content and heavy metal Pb.

2. Research Methods

The materials used in this research were blood cockle (*Anadara granosa* Linn) shell from TPI Pantai Kenjeran, Surabaya; TPI Sedati District, Sidoarjo Regency; Gresik; and TPI Bluru District, Sidoarjo Regency. The ingredients for nano calcium extraction were HCl and NaOH. The tools used in this research comprised extractor, glassware, oven, hotplate, and furnace. The research stages were as follows: the preparation of blood cockle (*Anadara granosa* liin) shell sample, the making of nano calcium powder with the treatment of long extraction on yield and calcium mineral content, heavy metal Pb, and nano calcium proximate test.

2.1. Preparation of blood cockle (Anadara granosa liin) shell
Preparation of the blood cockle (*Anadara granosa* liin) shell was done by washing the shell. The shell was then dried with the sun's heat. The dried shell was then destroyed by a 125 mesh hammer mill to make the shell powder.

2.1.1. The making of nano calcium powder. Two hundred grams of shell flour were then extracted with HCl solvent at 90°C with a treatment time of 2 hours of extraction. The extraction results were then filtered with filter paper to obtain liquid/filtrate. The obtained filtrate was precipitated with the addition of NaOH 4 N and then stirred and left until precipitation no longer formed. The precipitate obtained was then separated by decantation. The precipitate was then subjected to the process of neutralization using distilled water until pH 7. The next stage was the drying stage of sediment with oven and continued with combustion in the furnace at a temperature of 600°C so that nano calcium powder was formed. The powder was then calculated for the yield, chemical analysis, and composition of calcium as well as heavy metal Pb.

2.2. Procedure of nano calcium quality parameters analysis
2.2.1. Measurement of nano calcium yield. Yield is a ratio percentage of the monocalcium final weight content to the weight of blood cockle shell (*Anadara granosa*) before treatment.

2.2.2. Analysis of total nano calcium (Ca,) mineral with AAS (AOAC International, 2005) and heavy metals Pb (Reitz et al., 1987). The principle of total mineral testing is to know the value of metal absorbance by using Atomic Absorption Spectrophotometer (AAS) method. The sample was weighed as much as 2 grams, then put into 150 ml Erlenmeyer. Samples in Erlenmeyer was added with 5 ml of HNO3 65%. Prior to measurement, destruction was done to the samples that would be analyzed using concentrated acids until a clear solution was obtained. Metals that form complex mixtures can be analyzed. Sensitivity and detection limits are frequently used parameters in AAS. The sensitivity for lead metal is 0.19 mg/L and the detection limit is 0.01 mg/L.

The snippet was mounted above the hot plate until all samples dissolved. The sample was added with 0.4 ml H2SO4, then heated over hot plate until the solution reduced (more concentrated). The sample was allowed to cool and then added with 2-3 drops of mixed solution HClO4: HNO3 (2: 1), then re-mounted above the hot plate until the color changed from brown to dark yellow. The sample was cooled, then put into a 100 ml flask. When precipitate was present, the sample was filtered with glass wool.

A number of standard stock solutions of each mineral were diluted by using distilled water until their concentration was within the desired metal working range. The standard solutions, blanks, and samples were fed into the Atomic Absorption Spectrophotometer (AAS) with wavelengths of each mineral type, then we measured the absorbance or peak standard, blanks and samples at the corresponding wavelength and parameters for each mineral.

2.2.3. Water content analysis (AOAC 2005). Water content analysis was done by using oven method. The principle is to evaporate the free (H2O) water molecules present in the sample. Then, the sample is weighed until the constant weight is obtained with the assumption that all water contained in the sample has been evaporated. The difference in weight before and after drying is the amount of water vaporized.

2.2.4. Ash content analysis (AOAC 2005). Analysis of ash content was done using oven method. The principle is the burning or spoiling of organic materials that are decomposed into water (H2O) and carbon dioxide (CO2) but inorganic substances are not burned. This inorganic substance is called ash. The ash content analysis procedure is as follows: the flask to be used is first heated in the oven for 30 minutes at a temperature of 100-105 º C, then cooled in a desiccator to remove moisture and be weighed.

2.2.5 Fat content analysis (AOAC 2005). Fat content analysis was done by Sokhlet method. The principle is that the fat contained in the sample is extracted using a non-polar fat solvent. The fatty acid analysis procedure is as follows: a fat flask that will be used is heated in the oven for 30 minutes at a temperature of 100-105ºC, then cooled in a desiccator to remove the moisture and be weighed.

2.2.6. Analysis of protein content (AOAC 2005). Analysis of protein content was done by Kjeldahl method. The principle is the oxidation of carbonaceous materials and the conversion of nitrogen into ammonia by sulphuric acid. Furthermore, ammonia reacts with excess acid to form ammonium sulphate. Ammonium sulphate formed is dissociated and the solution is made alkaline with NaOH. The evaporated ammonia will be bound with boric acid. The amount of nitrogen contained in the solution is determined by titration using an acidic acid solution. The protein content is expressed in units of g/100 g of sample (%).

2.2.7. Carbohydrate levels (Winarno, 1997). Determination of carbohydrate content used by difference with formula as follows:
% carbohydrate = 100% - (water content + protein content + ash content + fat content)%

2.3. Chemical characteristics of blood cockle (Anadara granosa liin) shell monocalcium
Chemical analysis to determine chemical characteristics of the monocalcium of blood cockle shell (*Anadara granosa* liin) includes yields, proximate content (water content, ash, protein, fat, by difference carbohydrates), and minerals (calcium and lead). The results of the test of calcium (Ca) and lead (Pb) of the monocalcium of *Anadara granosa* liin blood cockle shell produced can be seen in Table 1.

Table 1. The results of calcium (Ca) and lead (Pb) test

No.	Notes	Site of sampling			
		Bluru Sidoarjo	Gisik Cemandi Sidoarjo	Gresik	Kenjeran. Surabaya
1.	Yields	5.292 gram	0.25 gram	0.7258 gram	0.2448 gram
2.	Ca level (%)	21.25 %	53.40 %	18.25 %	57.30 %
3.	Pb level (ppm)	1	0.0009 %	0.0001 %	0.0001 %

2.4. Proximate analysis results

Table 2. Chemical characteristics of nanocalcium of blood cockle (*Anadara granosa* liin) shell

Parameters	Bluru Sidoarjo	Gisik Cemandi Sidoarjo	Gresik	Kenjeran. Surabaya
Water level (%)	0.29	0.66	1.24	0.31
Ash level (%)	53.46	10.55	0.45	5.55
Protein level (%)	2.93	3.01	0.32	0.67
Fat level	1.12	4.02	0.23	0.46
Carbohydrate by di fference (%)	0.36	0.40	0.33	0.56

3. Result and Discussion

The moisture content of the shellfish's nano calcium is different from that obtained in Permana's study (2006) showing the water content of green shellfish (*Perna viridis* L.) powder of 0.85%. The study by Wardhani (2009) shows the water content of local shell graves of >90 mm was 1.19%. The low water

level was because nano calcium shellfish shell samples had been dried with sunlight. This was also due to the characteristics of shellfish that has a solid texture and composed of lime. The results showed that the highest chemical composition of shellfish nanoc alcium was ash content of 53.46%. High ash level shows that high mineral content is contained in the cockle's nano calcium shells. This is supported by the results of research by Wardhani (2009) who showed that local shell ash content was very high at 93.34%. The results are quite different from the results of research conducted by Permana (2006) which shows that the ash content of green shellfish (*Perna viridis* L.) was 77.13%.

The ash content values were different because the shell samples used were from different species. The high level of ash in the results of this study is caused by shells containing inorganic materials such as calcium carbonate. According to Acevedo et al. (2010), mollusc shells comprise 95% calcium carbonate and 5% organic matrix. John et al. (1972) mentions that the calcium content in the gravestone shell is composed of calcium carbonate formed from calcite and aragonite layers.

Rough protein analysis of barnacle shell showed low protein values. These results are in accordance with research conducted by John et al. (1972) showing that the protein content of the Pinctada maxima nacre layer is 2-3%. A study by Wardhani (2009) showed local shell protein content of 1.85%. Proteins in barnacle shell are thought to originate from the periostracum.

The periostracum layer contains fifteen to seventeen amino acids. The outer layer of the ligaments consists of lamella composed of fibrous proteins (Gregoire 1972). Suzuki et al. (2004) showed that prismatic coating on Pinctada fucata contains amino acid glycine and tyrosine.

The fat content produced from shellfish was not much different from the result of fat content analysis by Wardhani (2009) who examined the physical and chemical characteristics of local shellfish powder, which was 0.66%. The fat content in the bivalva shell is thought to originate from periostracum lining.

Periostracum layer contains fat, amino acids, and proteins (Gregoire 1972). According to Delong and Thorp (2009), the outer layers of the periostracum contain an organic matrix. Lee et al. (2007) states that the periostracum contains mucopolysaccharides, fats, and proteins, and serves to prevent corrosion of the outermost layer of the shell.

3.1. Pb content

Some calcium supplement products are shown to contain lead which may cause neurological disorders (Scelfo and Flegal 2000). The result of Pb analysis on nanocalcium powder is 1 ppm. The results are still below the threshold set by the National Academy of Sciences, Food Chemicals Codex and United States Pharmacopoeia (USP) ie 3 ppm, except that in Gisik Cemandi area of 9 ppm.

Research conducted by the University of Florida, Gainesville, reported that eight of the 22 tested calcium products proved to contain heavy metal Pb (University of Florida News 2000). Pb has toxic properties, which may resemble or compete with calcium. Plumbum (Pb) has an affinity for bone and replaces calcium work in bone mineral matrix (Needleman 2004). Plumbum has a strong bond with the transport protein used by calcium, but the affinity of binding of Pb is at least twice that of calcium. The transport mechanism of the gastrointestinal tract will lead to a competitive interaction between calcium and plumbun (Gulson et al., 2001).

4. Conclusion

We have successfully isolated the calcium from the solid waste of cockle *Anadara granosa* liin shells. The formation of calcium with smaller size (nanoparticles) was successfully performed by precipitation method. The best yield of nano calcium powder was obtained at Bluru Sidoarjo District. The content of lead was still within safe limits, except that in Gisik Cemandi. The high ash content in this study results was because the shellfish contains inorganic materials, such as calcium carbonate.

It is suggested to do mineral solubility test to identify the percentage of mineral solubility, especially nano calcium. A precipitation with a difference in NaOH concentration is necessary to determine the best formation of nano calcium deposits and the ease of neutralization process.

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