

# Table of contents

Volume 679

2021

◀ Previous issue      Next issue ▶

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## Preface

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## Papers

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Converting husbandry waste into liquid organic fertilizer using probiotic consortiums  
(*Lactobacillus* sp., *Rhodopseudomonas* sp., *Actinomycetes* sp., *Streptomyces* sp.)

S Amrullah, M. Amin and M Ali

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A review: bioactive compounds of macroalgae and their application as functional beverages

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S G Widyaswari, Metusalach, Kasmianti and N Amir

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012003

Improvement quality of sugar cane bagasse as fish feed ingredient

L H Suryaningrum and R Samsudin

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012004

The Growth and Yields of Shallot (*Allium Wakegi* Araki) CV. lembah palu Growing under Hydroponic Substrate Systems

R Yusuf, S A Lasmini, M Sandi, A Rahim and I Wahyudi

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012005

Application of glycerol on bioplastic based carrageenan waste cellulose on biodegradability and mechanical properties bioplastic

S N Fauziyah, A S Mubarak and D Y Pujiastuti

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012006

Gill and skin pathology of hybrid grouper (*E. fuscoguttatus* x *E. lanceolatus*) infested *Zeylanicobdella arugamensis* worms in different infestations degree

M Nisa, G Mahasri and L Sulmartiwi

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012007

Growth performance of tambaqui (*Colossoma macropomum*) supplemented with honey prebiotic in stagnant peat ponds

H Silalahi, R Djauhari and S S Monalisa

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012008

The Concentration of polyethylen glycol (PeG) 400 on bioplastic cellulose based carrageenan waste on biodegradability and mechanical properties bioplastic

D S Maulana, A S Mubarak and D Y Pujiastuti

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012009

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## Fish oil extraction as a by-product of Tilapia (*Oreochromis* sp.) fish processing with dry rendering method

S H Suseno, A K Rizkon, A M Jacob, Kamini and D Listiana

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012010

## Optimization of Extraction Time on The Characteristic of Gelatin from Scales of Red Snapper (*Lutjanus* sp.)

I Safi'i, W Tjahjaningsih and E D Masithah

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012011

## Screening acetylcholinesterase inhibitors from marine-derived actinomycetes by simple chromatography

M Kamaruddin, I Marzuki, A Burhan and R Ahmad

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012012

## Assessment of Heavy Metal Lead (Pb) Contents in Canned Crab Products by Atomic Absorption Spectrophotometry (AAS)

M Agustina, Mulyono and W Tjahjaningsih

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012013

## The concentration of sorbitol on bioplastic cellulose based carrageenan waste on biodegradability and mechanical properties bioplastic

M D Arief, A S Mubarak and D Y Pujiastuti

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012014

## Identification photoprotective activity of marine seaweed: *Eucheuma* sp

R D Kasitowati, A Wahyudi, R Asmara, D Aliviyanti, F Iranawati, M A P Panjaitan, D C Pratiwi and S Arsad

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012015

## Analysis of the density and intensity of metallothionein in *Crassostrea cucullata* oyster hulls in the coastal fishing port of Mayangan Probolinggo, East Java using immunohistochemical techniques

W Isoni, N Maulida and M I Mubaroqi

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The effect of different bait on the catch of traps in the waters of the tip of Pangkah Gresik Regency, East Java

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012017

The Identification and Distribution Components of Polycyclic Aromatic Hydrocarbon Contaminants at the Port of Paotere, Makassar, South Sulawesi

I Marzuki, I Pratama, H E Ismail, I. Paserangi, M Kamaruddin, M. Chaerul and R Ahmad

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012018

Koi (*Cyprinus carpio*) Hatchery techniques: its performance in BBI Boyolali

M G Laksono, Sugianta and M B Santanumurti

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012019

Ectoparasite infestation and survival rate of pacific white shrimp (*Litopenaeus vannamei*) that immunized with crude protein *Zoothamnium penaei* in intensive ponds

G Mahasri, A T Mukti, M Nisa, G C Prakosa and W H Satyantini

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012020

Study of crude extract yield and gonad maturity level of sea cucumber *Phylloporus dobsoni* correlation

S Andriyono, E D Masithah and D Winarni

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012021

Techniques of additional *Kappaphycus alvarezii* on seaweed face mask production

N R Prima and S Andriyono

[+ Open abstract](#) [View article](#) [PDF](#)

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012022

Ice cream properties affected by carrageenan from seaweed deference type drying methods

I Irawan and Fitriyana

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The effect of washing on the making of surimi and kamaboko tilapia (*Oreochromis sp.*)

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The effect of storage on the making of surimi and kamaboko tilapia (*Oreochromis* sp.)

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**OPEN ACCESS** 012025

Molecular identification and prevalence of endoparasite worms in Silver pompano (*Trachinotus blochii*) in floating net cages of Mari-culture Center, Lampung

L N F Haryanto, S Subekti, H B Ardiyanti, M K Amiin, R E K Akbar, I Achmadi and M A Yudarana

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**OPEN ACCESS** 012026

Microbiology safety of green mussel, *Perna viridis* after treated with boiling and *sous vide*

A S Samsudin and N U Karim

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The utilization of chitosan from Comb-pen shell (*Atrina pectinata*) as an emulsion stabilizer in the production of hand body cream

Y Supratin, L Sulmartiwi and E Saputra

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The potential of chitosan from comb-pen (*Atrina pectinata*) shell waste on the characteristics of hand body cream

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Spawning technique of yellowfin tuna (*Thunnus albacares*) infloating nets cage

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Enlargement technique of humpback grouper (*Cromileptes altivelis*) with floating nets cage

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Microencapsulated fish oil powder by spray drying using combination of wall materials in Kasetsart University, Bangkok  
I Aprilia, D Y Pujiastuti and W Klaypradit  
[+](#) Open abstract [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012032  
Effect of deacetylation conditions on physicochemical properties of chitosan derived from shrimp shell and squid pen  
A R Basarah, D Y Pujiastuti and Yaowapha Waiprib  
[+](#) Open abstract [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012033  
Substitution of patin *Pangasius pangasius* flour in making sticks as an alternative of food high protein and source of calcium for autism patients  
V Amelia, S Subekti and L Sulmartiwi  
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- 
- OPEN ACCESS** 012034  
Antioxidant properties from seaweeds *Kappaphycus alvarezii*, *Euchema spinosum* and *Sargasum* sp. using different solvent  
A A Abdillah, M A Alamsjah and N E Nasution Sugijanto  
[+](#) Open abstract [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012035  
Effect of kappa-carrageenan on physicochemical properties of mantou (Chinese steamed bread)  
M H C Putra and A A Abdillah  
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The application of betacyanin microcapsules as natural food colorant on beverage model  
S R Nurbaya, W D R Putri, E S Murtini and A Khamidah  
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- 
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Microbial quality and diversity of *Caesio cunning* and *Scolopsis taenioptera* harvested by using trap and trawl fishing techniques  
M Suhaimi and N U Karim  
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Characteristics physicochemical of melanin from squid ink (*loligo sp.*) extracted by ethanol

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012039

Analysis of fluctuating asymmetry of black strain tilapia *oreochromis niloticus* and red strain tilapia *Oreochromis niloticus* in Kabat Fish Hatchery Center Banyuwangi, East Java

D Kurniawan and A H Fasya

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Utilization of fermented Seligi leaf flour *Phyllanthus buxifolius* toward the specific growth rate, daily growth rate and survival rate of siam catfish (*Pangasius pangasius*)

Y G Budiman, M Lamid, B S Rahardja and M Amin

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Improving crude protein and crude fat content of Seligi leaf (*Phyllanthus buxifolius*) flour through probiotic fermentation

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Addition of turmeric in feed on growth and survival rate of Nilasa red tilapia (*Oreochromis sp.*)

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012043

Characterization of semi-refined kappa-carrageenan from *Kappaphycus alvarezii* with different solvents in Tanjung Sumenep

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The substitution effect of bone fish flour milkfish (*Chanos chanos*) physical and chemical characteristics of cookies

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Substitution of commercial feed with maggot meal (*Hermetia illucens*) to the growth rate, feed conversion ratio and feed efficiency catfish (*Pangasius pangasius*)

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Correlation between water quality with prevalence of black tiger shrimp (*Penaeus monodon*) infested by ectoparasite protozoa in traditional ponds of Wonorejo, Surabaya

M A Luthfi, S Subekti and G Mahasri

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Study of nitrogen (N) and phosphorus (P) in the land of mangrove sediments in ecotourism area Wonorejo Surabaya and coastal area of Jenu Tuban

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Corellation of water quality to the prevalence of ectoparasite in milkfish (*Chanos chanos*) in Sedati District, Sidoarjo

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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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Addition of crude fish oil (CFO) in feed toward fat and energy retention of mud crab (*Scylla serrata*)

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Effectiveness of giving clove oil as an anaesthetic for survival rate and number of leucocytes in cantang grouper (*Epinephelus* sp.) in the closed transportation

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012053

Effect of the addition of crude fish oil (CFO) in feed to the content of EPA and DHA in mud crab (*Scylla serrata*)

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012054

The effectiveness combination of maggot (*Hermetia illucens*) flour with commercial feed on growth rate, feed conversion ratio, and feed efficiency of tilapia (*Oreochromis niloticus*)

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Depuration of heavy metals Pb and Cd content in blood cockles (*Anadara antiquata*) with different filters

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012056

Substitution of commercial feed with fermented banana peel flour (*Musaceaea* sp.) and fish meal to feed consumption level, specific growth rate, feed efficiency, fat retention, and energy retention in siam catfish (*Pangasius hypophthalmus*)

A Aisyah, A S Gustiningrum, Agustono and M A Al-Arif

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012057

The effect *Moringa oleifera* leaf extract and *Lactobacillus acidophilus* supplementation on crude protein and crude fat retention in Tambaqui, *Colossoma macropomum*

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Molecular identification and prevalence of ectoparasite worms in barramundi (*Lates calcarifer*) in Lampung Waters

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Morphological profile of L2 *Anisakis typica* on Indian Mackerel (*Rastrelliger kanagurta*) from Sedati Fish Auction, Sidoarjo-East Java, Indonesia using Scanning Electron Microscope (SEM)

N Suryani, S Subekti, S Koesdarto and M K Amiin

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012060

Occurance of Anisakis of mackarel tuna (*Euthynnus affinis*) from Sendangbiru fishing auction place, East Java, Indonesia

R Bobsaid, P D W Sari and S Subekti

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012061

The study of virus collation with the polymerase chain reaction (PCR) method in export fishery commodities

L Kurniawati and K T Pursetyo

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012062

Effect of Culture Combination on Growth and Carrageenan Content of *Kappaphycus alvarezii* Green and Red Variety

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012063

Nitrate and phosphate dynamics of phytoplankton abundance in Kanceng River, Sepuluh, Bangkalan, East Java, Indonesia

D D Nindarwi, S H Samara and M B Santanumurti

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012064

Assessment of Seasonal Waters Quality Based on Abundance, Diversity, and Domination of Phytoplankton in Bajulmati Reservoir

E W Pertiwi, E D Masithah and Suciyono

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012065

Effect of sublethal dose organophosphate pesticides on embryogenesis and hatching rate of silver rasbora eggs (*Rasbora argyrotaenia*)

A B Prastika, L Sulmartiwi and L Lutfiyah

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The correlation between temperature and intensity of *Zeylanicobdella arugamensis* on cantang grouper (*E. fuscoguttatus* × *E. lanceolatus*) from traditional ponds in the Kampung Kerapu Lamongan East Java Indonesia

D D Afifah, G Mahasri and W H Satyantini

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012067

Utilization of *Nitrosomonas* sp and *Nitrobacter* sp probiotic towards total suspended solid and ammonia level in Nile tilapia culturing using aquaponic system

K H Dwiardani, Prayogo and B S Rahardja

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012068

The administration of *Caulerpa racemosa* extract on total bacteria and survival rates of white shrimp (*Litopenaeus vannamei*) after infected by *Vibrio parahaemolyticus*

A F Pratiwi, W H Satyantini, G Mahasri, L Sulmartiwi, A T Mukti and Sudarno

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012069

Examination of Taura Syndrome Virus (TSV) in white shrimp (*Litopenaeus vannamei*) and tiger prawn (*Penaeus monodon*) with Polymerase Chain Reaction (PCR) method

A N Fadilah and A H Fasya

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012070

Potential utilization of kombucha as a feed supplement in diets on growth performance and feed efficiency of catfish (*Clarias* sp.)

H U Ramadhan, Prayogo, H Kenconoajati, B S Rahardja, M H Azhar and D S Budi

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Application of edible film from chitosan as biodegradable packaging

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012072

Substitution of commercial feed with fermented banana peel flour (*Musaceaea* sp.) and fish meal to crude protein, energy, crude lipid and organic matter of meat in siamese catfish (*Pangasius hypophthalmus*)

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The effect of garlic (*Allium sativum*) and turmeric (*Curcuma longa*) extract addition in commercial feed on feeding rate, feed efficiency and feed conversion ratio of gouramy fish (*Osphronemus gouramy*)

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012074

The Variability in Population Structure of Gastropods in Sedati Waters, Sidoarjo Regency, East Java

S Fadliyah, L A Sari, K T Pursetyo, A Zein, M H Idris and Y Cahyoko

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The effect of pH and incubation time on crude protease enzymes activity of *Bacillus mycoides* from anchovy isolates (*Stolephorus* sp.)

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The influence of garlic (*Allium sativum*) and turmeric (*Curcuma longa*) extract as attractant in commercial feed on feeding rate and fat retention in gouramy (*Osphronemus gouramy*)

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Beardless barb *Cyclocheilichthys apogon* (Valenciennes, 1842) (*Cypriniformes*, *Cyprinidae*): Distribution extension and first record from South Bali

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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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A Dinulislam, Sulistiono, D T F Lumbanbatu et al.
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R F Saputra, E D Masithah and P D Wulansari



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

D S Herlina<sup>1</sup>, L Sulmartiwi<sup>2\*</sup>, E D Masithah<sup>2</sup>

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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 (CaCO<sub>3</sub>) [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<sup>-9</sup> 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].



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).

## 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.

### 2.2 Tools and materials

Equipment that used in nano calcium production is Beaker glass, furnace, thermometer, oven, hotplate, filter paper, whattman 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 whattman 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.)

Repetition	Shells ( <i>Anadara</i> sp.)	
	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

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  $\pm 10$  minutes. This is in accordance with Minarty [12] states that many foam and air bubbles are caused by the formation of  $\text{CO}_2$  and  $\text{H}_2\text{O}$  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 ( $\text{CaCO}_3$ ) 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 ( $\text{CaCO}_3$ ) 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  $105^\circ\text{C}$  for 24 hours, and ashing using a temperature of  $600^\circ\text{C}$  for 4 hours. Ignition process will produce calcium oxide ( $\text{CaO}$ ). 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

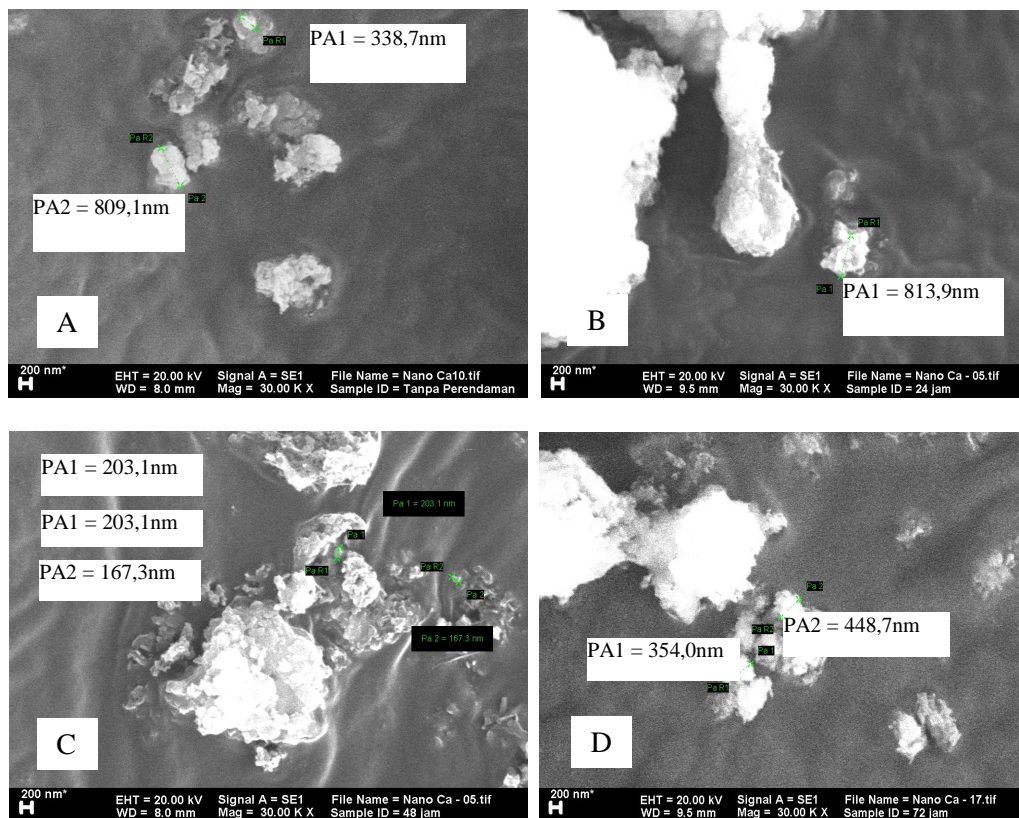
The results of the analysis of the water content percentage in nano calcium continues to increase. Water content in nano calcium showed the lowest results, that is 1.11% in the treatment without immersion and the highest was 2.16% in the 72 hour immersion treatment (Table 3.). This is because there is 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).

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  $\text{CaCO}_3$ . 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%.

#### 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.

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