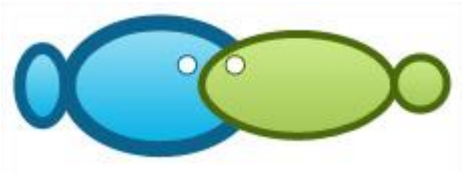

Effects of different farming locations on biocalcium characteristics of Mozambique tilapia (*Oreochromis mossambicus*) bones

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Abstract. Increased Mozambique tilapia (*Oreochromis mossambicus*) production is closely related to the increase in Mozambique tilapia processed products. This further increases waste, such as bones. With a calcium content ranging from 34 to 36%, fish bones have the potential to be processed into biocalcium. Meanwhile, bone mineral content is influenced by the ecology of fish habitat. This study aimed to determine the effect of the location (water conditions) of tilapia farming on the characteristics of biocalcium from Mozambique tilapia bones. Biocalcium was extracted from Mozambique tilapia bones (Sentani Lake, Rawa Pening Lake, and Wadaslintang Reservoir) using 1N NaOH solution. The results showed that differences in farming locations had a significant difference ($p<0.05$) on the yield, calcium content, and whiteness of biocalcium. Mozambique tilapia bone derived from farming in Sentani Lake produced biocalcium with the highest yield (30.26%) and whiteness value (87.83%), and the particles have a smaller size on the nanometer scale (16.9% measuring 341.8 nm and 83.1% measuring 95.40 nm) compared to biocalcium from Rawa Pening Lake and Wadaslintang Reservoir. Based on the FTIR test, it could be seen that biocalcium in all samples is available in the form of calcium phosphate and calcium carbonate. The difference in farming locations did not affect the morphology of the biocalcium, with all samples having square-shaped particles. Thus, the results of this study could be used as a reference in choosing quality raw materials based on the conditions of aquaculture.
Key Words: bone powder, nanocalcium, tilapia aquaculture, whiteness.

Introduction. Mozambique tilapia (*Oreochromis mossambicus*) has a relatively high protein content, 21 to 29% (Herawati et al 2019). This has caused an increase in production by 13.13% from 2012 to 2017, associated with an increase in market demand (Marine and Fisheries Ministry 2019). The growth in production has caused an overflow of byproducts, such as viscera, heads, skins and bones (Riyadi et al 2019). An abundant amount of fish bones could cause adverse environmental impacts if not utilized. Meanwhile, Hemung (2013) reported that bone waste produced from the body of tilapia amounts to 10-15%.

Utilization of fish bones is usually in the form of fish meal and feed with low economic value. Fish bones contain 60 to 70% minerals (Yin et al 2015), with calcium having the highest concentration, 34 to 36% (Benjakul et al 2017). Hence, fish bones have the potential to be processed into biocalcium. Biocalcium is generally used as an additive in the food and pharmaceutical industries (Darmanto et al 2017). Most calcium supplements are available in the form of calcium carbonate and calcium citrate. The most common sources of natural calcium are lime and dolomite (Putkham et al 2018). However, fish bones are a renewable and sustainable source of biocalcium.

Calcium from fish bones has high bioavailability (Malde et al 2010). Thus, it can be used as a source of biocalcium in functional food. However, in general, biocalcium from fish bones has micrometer (μm) size, so it is not optimally absorbed in the body. Nanotechnology helps in reducing particle size, so it can be easily absorbed by the body.

Therefore, nanometer-sized biocalcium (nm) has been developed. The size of the nanoparticle ranges from 1 to 1000 nm (Huang et al 2009).

Researchers have previously investigated the utilization of fish bones, including tilapia bone powder and its calcium bioavailability (Hemung 2013), calcium from yellowfin tuna (*Thunnus albacares*) bones and nutritional characteristics (Nemati et al 2017), biocalcium powder from skipjack tuna (*Katsuwonus pelamis*) bones (Benjakul et al 2017), and nanocalcium from skipjack tuna bones (Harmain et al 2018). Research on the application of calcium has also been carried out, namely on nanocalcium from fish bones to improve the gelation properties of surimi (Yin & Park 2014), nanocalcium from catfish (*Clarias batrachus*) bone in seaweed noodles (Halimah et al 2016), and biocalcium from skipjack tuna bone in wheat crackers (Benjakul & Karnjanapratum 2018). Jeong et al (2013) have also reported that nanocalcium did not cause toxicity, so it is safe for consumption. However, to our findings, none reported the effect of the condition of waters on the quality of the fish bones nanocalcium. Meanwhile, a previous study by Herawati et al (2019) on Mozambique tilapia cultivated in different waters, show that the fish had different meat qualities. Therefore, the aim of this research was to determine the effect of farming locations on the characteristics of biocalcium from Mozambique tilapia bones.

Material and Method

Materials. The materials used in this study were bones from Mozambique tilapia, which were farmed in Lake Sentani (Papua, Indonesia), Lake Rawa Pening (Central Java, Indonesia), and Wadaslintang Reservoir (Central Java, Indonesia). 30 fish of 300±0.15 g each were collected and transported to the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Diponegoro University by maintaining their cold chain. The fish bone waste were from the manufacture of surimi in previous studies (Kurniasih et al 2019). This study was conducted from July to October 2019.

Production of biocalcium. The production of biocalcium referred to Benjakul et al (2017), with modifications. After the bones were washed, they were boiled for 2 h. The fish bones were dried at 80ºC, then extracted with 1 N NaOH at a ratio of 1:3 (w/v) for 1 h in a waterbath (WiseBath, Korea) at 100ºC (in triplicate). The fish bones were neutralized, filtered using nylon screen, then dried at 50ºC for 12 h. To produce biocalcium, milling process was carried out using a grinder and screened with a 100 mesh screen.

Biocalcium yield. Yield is the ratio of biocalcium final weight to the weight before undergoing treatment (AOAC 2005). The yield was calculated using the following equation:

Yield (%) = (Final weight/Initial weight) x 100

Calcium content. The determination of calcium content was carried out with permanganometric titration after the method of Widiany (2019) with modifications. The sample (5 g) was burned in a furnace. HNO_3 1:3 (w/v) was added to the cooled ash until all ash dissolved. Filtering was done using filter paper. The filtrate (10 mL) was collected and 5 drops of Mr-BCG indicator were added (until the color turned red). NH_4OH was added until the color turned blue and HNO_3 was added until the color turned back to red. Oxalic acid (2.5%) was added into the mixture of the sample and then heated. The samples were filtered using filter paper. The residue collected was moved using distilled water in H_2SO_4 and then heated. The sample was titrated using a standard solution of $KMnO_4$ 0.1 N until the color changed to purple. Calcium content was calculated the following formula, knowing that 1 mL $KMnO^4$ 0.1 N shows 0.002 g calcium:

Calcium content (%) = (Titration volume x dilution factor x 0.002)/Sample weight x 100

Whiteness. The whiteness measurement was determined based on knowing the value of lightness (L*); green-redness (a*), where -a* indicates green and +a* indicates red; and blue-yellowness (b*), where -b* indicates blue and +b* indicates yellow using chromameter (Minolta CR-200, Osaka, Japan). The whiteness value was determined based on the next equation:

$$\text{Whiteness} = 100 - [(100-L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$$

Particle size distribution. Particle size distribution of biocalcium (nm) was measured using Zetasizer Nano (particle size analyzer). Particle measurement was carried out using the Dynamic Light Scattering (DLS) method. The biocalcium powder was first dispersed in aquadest.

Fourier-Transform Infrared Spectroscopy (FTIR). The functional group of biocalcium was identified by FTIR. Spectra resolution of 1 cm^{-1} was determined by the spectrum of the 100 series FTIR spectrometer (Shimadzu FTIR 8400, Japan) and the spectra profiles were performed with a region of 4000 to 400 cm^{-1}.

Morphology observation. The morphology of biocalcium was observed by scanning electron microscopy (SEM) (Jeol JSM 6510LA, Japan), with 5000x magnification at 20 kV. Platinum was used to coat biocalcium for making it conductive before being visualized.

Statistical analysis. Data on yield, calcium levels, and whiteness in triplicate were carried out with Analysis of Variance (ANOVA) and Duncan's Multiple Range Test to see significantly different treatments ($p<0.05$). Statistical analysis was performed using SPSS version 16.

Results and Discussion

Yield. Yield is one of the important parameters in biocalcium properties. The efficiency and effectiveness in producing biocalcium will affect the yield. With a greater the yield, the process and treatments applied are more efficient. The results showed that different farming locations had a significant difference in the yield of Mozambique tilapia bone biocalcium ($p<0.05$). Mozambique tilapia bones from Rawa Pening Lake produced lower levels of biocalcium in the meal than Mozambique tilapia bones from fish farmed in Sentani Lake and Wadaslintang Reservoir. Based on the research of Logesh et al (2012), the yield of bone powder positively correlates with the amount of calcium and phosphorus in the fish bone. High calcium and phosphorus content produce higher yield. Moses et al (2018) reported that habitat could affect the quantity and quality of tilapia.

This study used a strong base solvent (NaOH) treatment. The biocalcium yield in this study ranged from 20.65 to 30.62% (Table 1). These results are lower than the yield of bone powder from skipjack tuna bones soaked with HCl for 24 h, which was 40% (Harmain et al 2018). However, it has a higher value than the yield of bone powder from haddock (*Melanogrammus aeglefinus*) bones, soaked with NaOH and ethanol, which was 23.7% (Huo et al 2010). Thus, the environment of the farming location, method of production, and species could influence the yield of biocalcium from fish bone.

Table 1

Characteristics of Mozambique tilapia (*Oreochromis mossambicus)* bone nanocalcium from 3 different farming locations

Location	*Yield (%)*	*Calcium content (%)*	*Whiteness*
Sentani Lake	30.26 ± 0.33^{a}	28.88 ± 0.1^{a}	87.83 ± 0.27^{a}
Rawa Pening Lake	20.65 ± 0.26^{b}	29.29 ± 2.49^{ab}	81.34 ± 0.65^{b}
Wadaslintang Reservoir	30.62 ± 0.55^{a}	30 ± 0.61^{b}	86.64 ± 0.25^{c}

Note: data is presented as mean±SD; different superscripts indicate significant differences ($p<0.05$).

Calcium content. The calcium content in the Mozambique tilapia bones was between 28.88 and 30% (Table 1), being lower than the calcium content of sardine (*Sardinella longiceps*) bone powder, which was 26.39 to 32.73%. However, these results were higher compared to the concentration from bone powder of ribbon fish (*Trichiurus savala*), which was 19.33 to 27.81% (Logesh et al 2012). The difference in calcium content in fish bones was affected by the species (Huda et al 2010), the method of producing calcium powder and the type of solvent used (Logesh et al 2012), solvent concentration and boiling time (Nemati et al 2017).

Based on Table 1, it can be seen that the location of Mozambique tilapia farming affected the calcium content of fish bones. Mozambique tilapia from Wadaslintang Reservoir has the highest calcium content ($p<0.05$) in bones. Mozambique tilapia bones from Sentani Lake produced a similar yield with those from Wadaslintang Reservoir, but have a lower calcium content. This was due to the higher collagen content in Mozambique tilapia bones from Sentani Lake compared to those from Wadaslintang Reservoir. Thus, it could be said that calcium in bones could not be extracted because of the collagen matrix (Yin et al 2015).

Based on our previous research (Herawati et al 2019), different aquaculture systems from Indonesian waters can affect the proximate content, amino acid, and fatty acid profiles of Mozambique tilapia. The results showed that Mozambique tilapia cultivated in Sentani Lake presented higher values than Mozambique tilapia cultivated in Rawa Pening Lake and Wadaslintang Reservoir. The temperature of the waters of Sentani Lake, Rawa Pening Lake, and Wadaslintang Reservoir are 28, 27, and 24°C, respectively. The pH of the waters of Sentani Lake, Rawa Pening Lake, and Wadaslintang Reservoir are 7.2, 7.4, and 7.7, while dissolved oxygen is 3.3, 3, and 3.1 mg L^{-1}, respectively. The quality of aquaculture can support the growth of phytoplankton and algae, which can be used as natural feed. The optimum temperature for Mozambique tilapia growth is 25 to 30°C. Nemati et al (2017) stated that calcium content in bone powder is closely related to fish species, feed, amount of marrow in the bones, fat, tendons, and cartilage. Moreover, mineral content in fish bones is influenced by the ecology of captured fish, depending on season, availability of natural food, salinity, and temperature, among others (Talib et al 2014).

Although the yield of Mozambique tilapia bone calcium from Sentani Lake was higher, it is similar with the calcium content of Mozambique tilapia bone from Rawa Pening Lake. It assumed that Mozambique tilapia bone powder from Sentani Lake has higher phosphorus, protein, and fat contents than Mozambique tilapia bone meal from Rawa Pening Lake. Logesh et al (2012) reported that calcium and phosphorus are the main elements found in fish bones. Besides, fish bones also have proteins and lipids that could not be completely lost during calcium extraction or bone powdering process.

Whiteness. The results showed that different farming locations showed a significant difference in the whiteness value of biocalcium ($p<0.05$). Mozambique tilapia from Sentani Lake produced the highest whiteness value, while that from Rawa Pening Lake produced the lowest value (Table 1). This showed that the habitat conditions not only affect the nutritional content of fish meat (Herawati et al 2019), but also the characteristics and composition of Mozambique tilapia bones. The whiteness value was influenced by the amount of calcium contained, because calcium is white. Higher phosphorus content could reduce the value of whiteness (Harmain et al 2018). The low value of whiteness might be caused by the high content of protein and fat in the bones, which could not be completely degraded during the nanocalcium producing process (Talib & Zailani 2017).

The whiteness value results of Mozambique tilapia bone calcium from this study ranged from 81.34 to 87.83%. This value was higher than the results of Talib et al (2014), where yellowfin tuna bone powder extracted using water and acetic acid, which was 46.45 and 46.33%, respectively. The low whiteness value was due to the inability of acetic acid to degrade the lipid in the fish. Lipid will cause the color of bone powder to turn brownish due to the drying process. Benjakul et al (2017) added that some fatty acids found in fish bones could undergo oxidation during the drying process, resulting in

carbonyl compounds. Furthermore, free amino acids, peptides, and proteins in fish bones can form Maillard's reactions with carbonyl compounds, causing the bone powder to turn yellowish.

Particle size distribution. Particle size distribution of the biocalcium from Sentani Lake was 341.8 nm at 16.9% and 95.40 nm at 83.1%. Mozambique tilapia bone biocalcium from Rawa Pening Lake was 390.6 nm at 29.6% and 103 nm at 70.4%, while the biocalcium of Mozambique tilapia bone from Wadaslintang Reservoir was 1390 nm at 100% (Figure 1). These results indicate that biocalcium from Wadaslintang Reservoir, based on particle size, did not include nanocalcium, but microcalcium. Calcium extracts from Mozambique tilapia bones of Sentani Lake and Rawa Pening Lake include nanocalcium. The formation of nanocalcium is caused by the precipitation process that produced smooth and nano-sized calcium deposits. The process of bone softening using solvents at high temperatures (boiling) can change the bone texture due to the dissolving of several organic compounds (Kim & Mendis 2006).

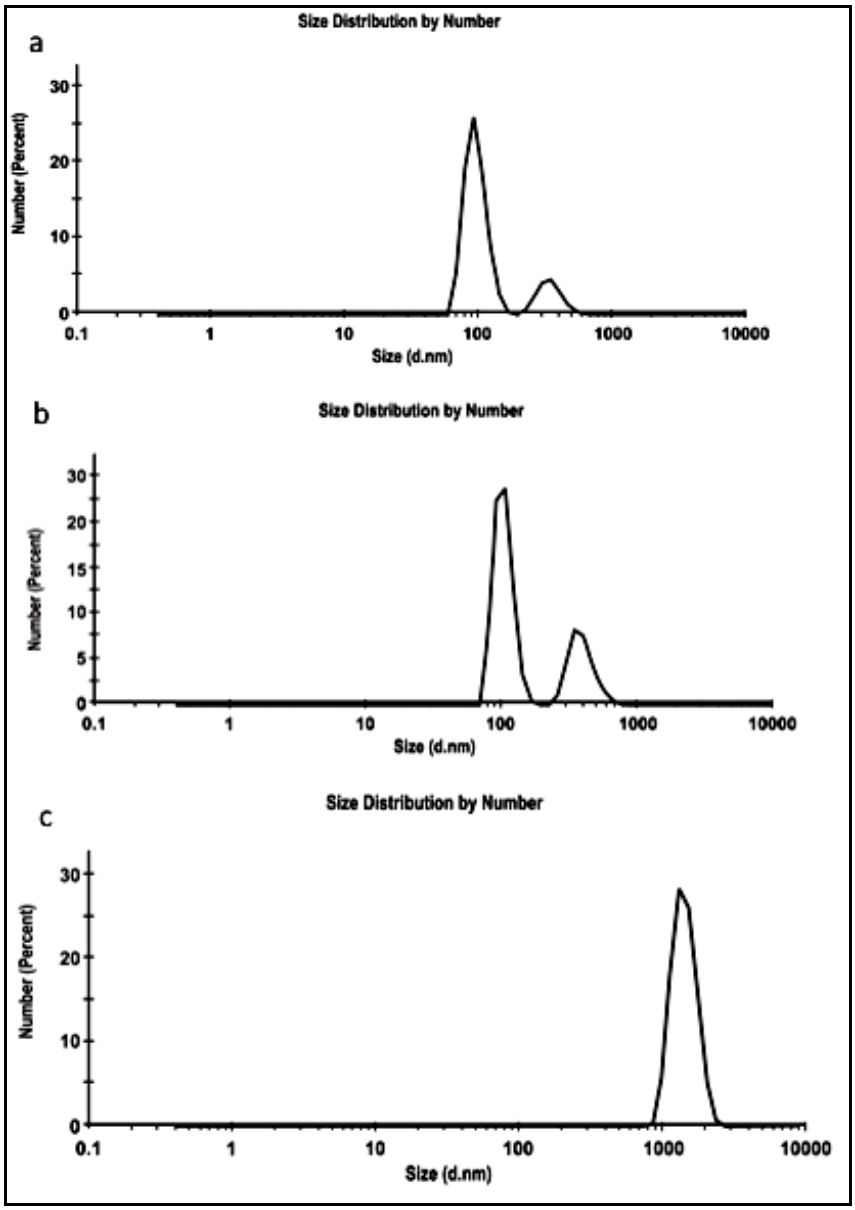

Figure 1. Particle size distribution of biocalcium from *Oreochromis mossambicus* bones from: a - Sentani Lake; b - Rawa Pening Lake; c - Wadaslintang Reservoir.

The results of this study produced biocalcium powder with a smaller size than that of Benjakul & Karnjanapratum (2018), who produced biocalcium from tuna bones with size of 17.07 to 20.29 µm. Jeong et al (2013) explained that nanocalcium is safe for consumption and has higher bioavailability than macrocalcium. Nanocalcium has a higher suspension stability in water and the grittiness can be removed. Thus, nanocalcium from fish bones could be used for calcium enrichment of beverages without reducing the sensory value of the product.

Functional groups. The characteristic bands of biocalcium were observed at 563.21, 1033.85, 1458.18, 1651.07, 2854.65, 2924.09 cm^{-1}, indicating the presence of calcium phosphate and calcium carbonate (Figure 2). Venkatesan et al (2015) reported that calcium phosphate and collagen in fish bones appear in bands 566, 601, 717, 1038, 1102, 1159, 1458, 1649, 1745, 2857, 2926, 3008 and 3431 cm^{-1}. Meanwhile, the bands 1000 to 1100 cm^{-1} showed the stretching of PO_4 vibration, while 567 cm^{-1} is a PO stretching vibration of a PO_4 group. The OH stretching at hydroxyapatite group was observed in the band near 3400 cm^{-1}. Furthermore, the bands 1560, 1421, and 1456 cm^{-1} indicate the carbonate group.

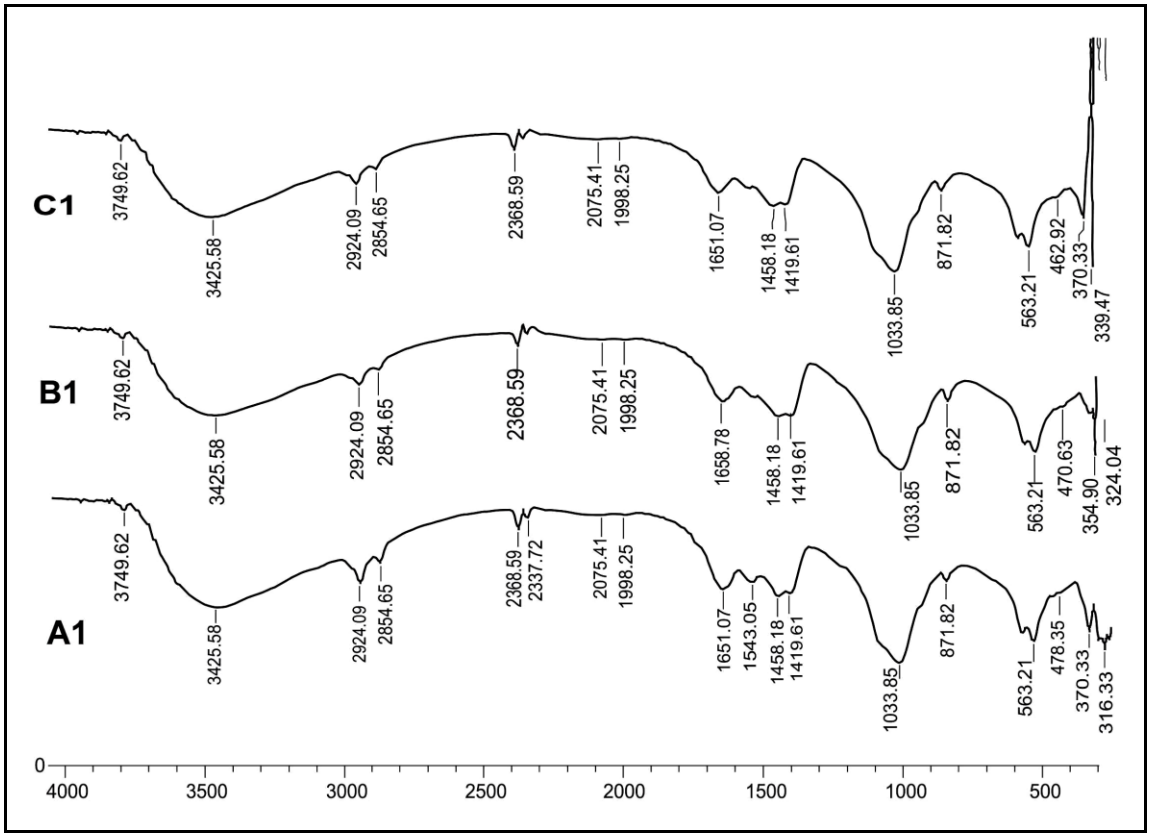

Figure 2. Functional groups of nanocalcium from Oreochromis mossambicus bones from: A1 - Lake Sentani; B1 - Lake Rawa Pening; C1 - Lake Wadaslintang.

Huang et al (2011) also reported that phosphate groups (PO_4^{3-}) were found in the regions of 563, 957, and 1030 cm^{-1}, while the apatite carbonate groups (CO_3^{2-}) were found in the bands 876 and 1412 to 1547 cm^{-1}. Phosphate groups were present near bands 1030 (v3), 600 (v2), and 560 (v4) cm^{-1}. Meanwhile, carbonate ion substitution was observed in bands 875 cm^{-1} and from 1410 to 1450 cm^{-1} (D'Elia et al 2013; Bonadio et al 2013). The absorption of organic material (CH) was indicated by wavenumber values of 2855 and 2925 cm^{-1} (Boutinguiza et al 2012).

Biocalcium morphology. Nano-calcium extract from the bones of Mozambique tilapia from Sentani Lake and Rawa Pening Lake has a square shape with several large particles

visible. Biocalcium extract from Wadaslintang Reservoir in micrometer size also presents squared-shape particles, but they are larger (Figure 3). Different results were obtained by Yin et al (2015), where micrometer-sized fish bones particles have a spherical shape, with a placement of several small particles between large particles. In nanometer sizes, particles have a polyhedron shape. Calcium citrate has various sheet shapes, being relatively smooth (Li et al 2016). The shape of these particles is influenced by the low crystallinity of calcium citrate.

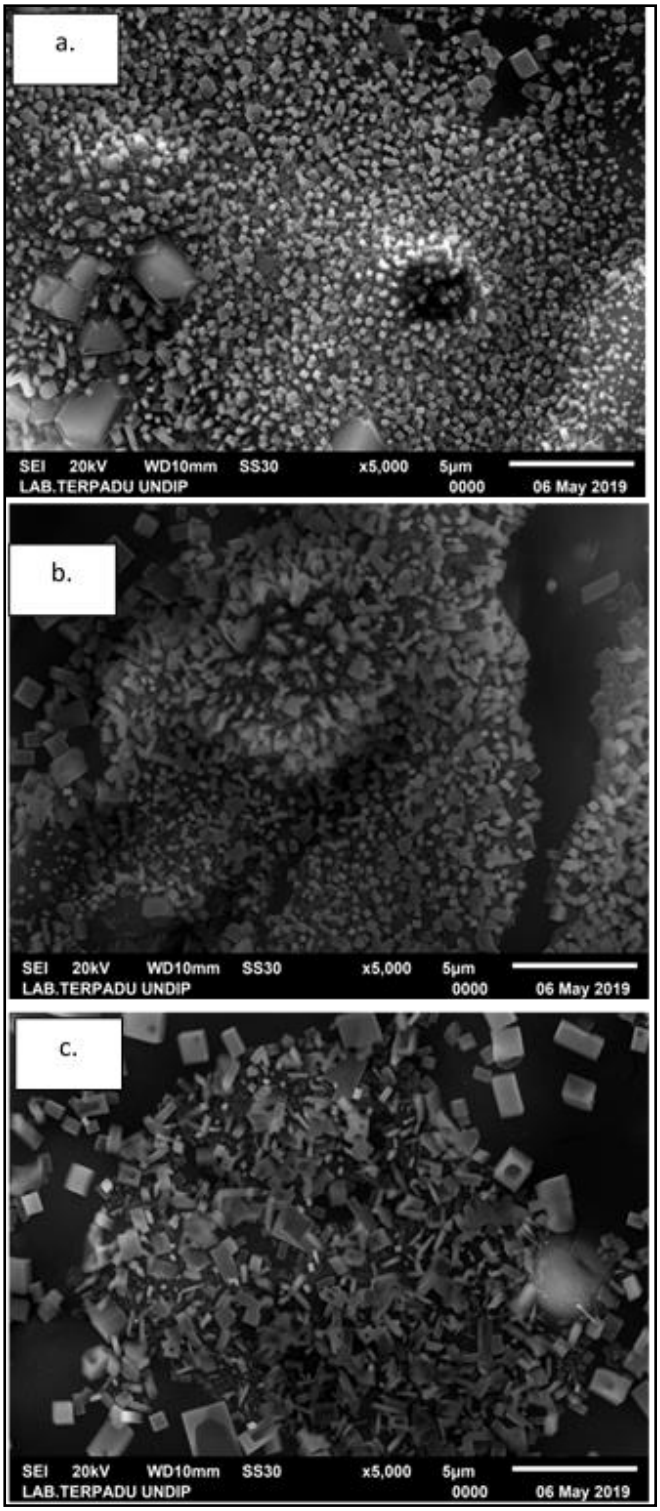

Figure 3. Morphology of biocalcium from *Oreochromis mossambicus* bones from: a - Sentani Lake; b - Rawa Pening Lake; c - Wadaslintang Reservoir.

Conclusions. Different Mozambique tilapia fish farming locations had a significant effect ($p<0.05$) on yield, content, and whiteness of calcium from Mozambique tilapia bone. Mozambique tilapia bones from Sentani Lake produced biocalcium with the highest yield and whiteness value; they also had smaller particles (nanometer size), compared to biocalcium from fish from the other locations. Based on the FTIR test, biocalcium in all samples is available in the form of calcium phosphate and calcium carbonate. The difference in farming location did not affect the morphology of biocalcium produced; all samples had square-shaped particles.

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References

Benjakul S., Karnjanapratum S., 2018 Characteristics and nutritional value of whole wheat cracker fortified with tuna bone bio-calcium powder. Food Chemistry 259:181-187.

Benjakul S., Mad-Ali S., Senphan T., Sookchoo P., 2017 Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics. Journal of Food Biochemistry 41(6):e12412, 8 p.

Bonadio T. G. M., Sato F., Medina A. N., Weinand W. R., Baesso M. L., Lima W. M., 2013 Bioactivity and structural properties of nanostructured bulk composites containing Nb_2O_5 and natural hydroxyapatite. Journal of Applied Physics 113:223505, 8 p.

Boutinguiza M., Pou J., Comesana R., Lusquinos F., de Carlos A., Leon B., 2012 Biological hydroxyapatite obtained from fish bones. Materials Science and Engineering: C 32(3):478-486.

Darmanto Y. S., Riyadi P. H., Susanti S., 2017 Characteristic of taro (*Colocasia esculenta*) and eeaweed (*Eucheuma cottoni*) based analogue rice fortified with fishes bone collagen (a promising anti-diabetic functional food). Journal of Engineering and Applied Sciences 12(12):3055-3060.

D'Elia N. L., Gravina A. N., Ruso J. M., Laiuppa J. A., Santillan G. E., and Messina P. V., 2013 Manipulating the bioactivity of hydroxyapatite nano-rods structured networks: Effects on mineral coating morphology and growth kinetic. Biochemica et Biophysica Acta (BBA) - General Subjects 1830(11):5014-5026.

Halimah S. N., Suryani R. A., Wijayanti S. W., Pangestu R. A., Deni G. D., Romadhon, 2016 Fortification seaweed noodles [*Eucheuma cottonii* (Weber-van Bosse, 1913)] with nano-calcium from bone catfish [*Clarias batrachus* (*Linnaeu*s, 1758)]. Aquatic Procedia 7:221-225.

Harmain R. M., Dali F. A., Husain R., 2018 Nanocalcium characterization of skipjack fish bone flour (*Katsuwonus pelamis L*). International Journal of Innovative Science and Research Technology 3(10):306-308.

Hemung B. O., 2013 Properties of tilapia bone powder and its calcium bioavailability based on transglutaminase assay. International Journal of Bioscience, Biochemistry, and Bioinformatics 3(4):306-309.

Herawati V. E., Ayu R., Darmanto Y., Rismaningsih N., Windarto S., Karnaradjasa O., 2019 Amino acid and fatty acid profiles of Mozambique tilapia (*Oreochromis mossambicus*) in different aquaculture systems from Indonesian waters. AACL Bioflux 12(5):1771-1778.

Huang S., Chen J. C., Hsu C. W., Chang W. H., 2009 Effects of nano calcium carbonate and nano calcium citrate on toxicity in ICR mice and on bone mineral density in an ovariectomized mice model. Nanotechnology 20:375102, 7 p.

Huang Y. C., Hsiao P. C., Chai H. J., 2011 Hydroxyapatite extracted from fish scale: effects on MG63 osteoblast-like cells. Ceramics International 37(6):1825-1831.

Huda N., Li Leng A., Yee C. X., Herpandi, 2010 Chemical composition, colour, and linear expansion properties of Malaysian commercial fish cracker (keropok). Asian Journal of Food and Agro-Industry 3(5):473-482.

Huo J., Deng S., Xie C., Tong G., 2010 Preparation of biological efficacy of haddock bone calcium tablets. Chinese Journal of Oceanology and Limnology 28(2):371-378.
Jeong M. S., Cho H. S., Park S. J., Song K. S., Ahn K. S., Cho M. H., Kim J. S., 2013 Physico-chemical characterization-based safety evaluation of nanocalcium. Food and Chemical Toxicology 62:308-317.
Kim S. K., Mendis E., 2006 Bioactive compounds from marine processing by products - A Review. Food Research International 39(4):383-393.
Kurniasih R. A., Darmanto Y. S., Herawati V. E., 2019 Characteristics of surimi gel from *Oreochromis mossambicus* in different aquaculture areas. IOP Conference Series: Earth and Environmental Science 260:012111 8 p.
Li J., Liu Y., Gao Y., Zhong L., Zou Q., Lai X., 2016 Preparation and properties of calcium citrate nanosheets for bone graft substitute. Bioengineered 7(5):376-381.
Logesh A. R., Pravinkumar M., Raffi S. M., Kalaiselvam M., 2012 Calcium and phosphorus determination in bones of low value *Sardinella longiceps* (Valenciennes) and *Trichiurus savala* (Cuvier), from Parangipettai, Southeast Coast of India. Asian Pacific Journal of Tropical Disease 2:S254-S256.
Malde M. K., Bugel S., Kristensen M., Malde K., Graff I. E., Pedersen J. I., 2010 Calcium from salmon and cod bone is well absorbed in young healthy men: a double-blinded randomized crossover design. Nutrition & Metabolism 7(1):61-69.
Moses S., Agbaji E. B., Ajibola V. O., Gimba C. E., 2018 Amino acid composition and proximate analysis in tilapia (*Oreochromis mossambicus*) fish from dams and rivers in Zamfara State, Nigeria. Journal of Applied Sciences and Environmental Management 22(6):8899-8905.
Nemati M., Huda N., Ariffin F., 2017 Development of calcium supplements from fish bone wastes of yellowfin tuna (*Thunnus albacares*) and characterization of nutritional quality. International Food Research Journal 24(6):2419-2426.
Putkham A. I., Ladhan S., Putkham A., 2018 Factors affecting the particle size of bio-calcium carbonate synthesized from industrial eggshell waste. Materials Transactions 59(8):1220-1224.
Riyadi P. H., Suprayitno E., Aulanni'am, Sulistiati T. D., 2019 Optimization of protein hydrolysate from visceral waste of Nile tilapia (*Oreochromis niloticus*) by response surface methodology. AACL Bioflux 12(6):2347-2358.
Talib A., Suprayitno E., Aulani'am, Hardoko, 2014 Physico-chemical properties of Madihihang (*Thunnus albacares Bonnaterre*) fish bone flour in Ternate, North Moluccas. International Journal of Biosciences 4(10):22-30.
Talib A., Zailani K., 2017 Extraction and purification of yellowfin tuna fishbone flour as an ingredient of future traditional medicine. IOSR Journal of Pharmacy 7(11):08-14.
Venkatesan J., Lowe B., Manivasagan P., Kang K. H., Chalisserry E. P., Anil S., Kim D. G., Kim S. K., 2015 Isolation and characterization of nano-hydroxyapatite from salmon fish bone. Materials 8(8):5426-5439.
Widiany F. L., 2019 Nutritional content of food containing various mixtures of eel (*Monopterus albus*) flour and tempeh flour as supporting nutrients for hemodialysis patients. Pakistan Journal of Nutrition 18(9):900-905.
Yin T., Park J. W., 2014 Effects of nano-scaled fish bone on the gelation properties of Alaska pollock surimi. Food Chemistry 150:463-468.
Yin T., Park J. W., Xiong S., 2015 Physicochemical properties of nano fish bone prepared by wet media milling. LWT - Food Science and Technology 64(1):367-373.
***AOAC (Association of Official Analytical Chemists), 2005 Official methods of analysis of AOAC International. 18th Edition. Horwits W., Latimer G. W. (eds), Gaithersburg, USA.
***Marine and Fisheries Ministry, 2019 [Fisheries production 2017]. Jakarta, Indonesia. [In Indonesian].

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