Chemical composition of bio calcium powders from pressure-cooked Indo-Pacific tarpon (*Megalops cyprinoides*) bone from Tarakan, North Kalimantan, Indonesia

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Abstract. Bone from Indo-Pacific tarpon, *Megalops cyprinoides*, is one of the unutilized by-products of frozen minced fish production, which is disposed as waste. It is potentially being used as raw material for making bio calcium powder. This study aimed to determine the chemical composition of bio calcium powder from pressure-cooked *M. cyprinoides* bone. The results showed that the variant of cooking time gave significant differences ($P<0.05$) in moisture, protein, fat, ash, calcium and phosphorus content. The highest levels of calcium (33.00±0.16%) and phosphorus (15.73±0.02%) were found in the variant of 1 hour cooking time. Based on the FTIR test, the bio calcium powder in all variants contained phosphate, carbonate, hydroxyl, and amide groups. Bio calcium powders from pressure-cooked Indo-Pacific tarpon can be used as an alternative source of calcium.
Key Words: bone flour, functional group, mineral, phosphorus content, proximate value.

Introduction. Indo-pacific tarpon (*Megalops cyprinoides*) is a type of fish with high salinity tolerance. Its existence is widespread in tropical and subtropical offshores and is often found in coastal waters, estuaries and mangrove forests. This fish has different names in various countries, such as Alanku (India), Isegoi (Jepang), Ileya (Sri Lanka), Abulong (Philippines) and Indo-pacific Tarpon (Australia) (Shen et al 2009; Khairul et al 2019). Meanwhile, in Indonesia, this fish is known as Bulan-bulan. *M. cyprinoides* has a relatively low selling price in the Tarakan area, North Kalimantan, because it is not one of the main catches. It has a very soft texture, making it less preferred to be consumed conventionally. However, this fish has a reasonably good taste and good nutritional value. Therefore, this fish in minced form is frequently used as raw material for food processing (Cahyani et al 2020). The procedure of producing minced fish leads to the production of waste materials such as bones, heads, skins, scales and offal, which tend to cause severe environmental pollution. The processing of fishery wastes into value-added products is an effort that can be adopted to overcome these problems.

Fish waste in the form of bones is an essential source of minerals such as calcium, which is beneficial to the health, explicitly preventing osteoporosis. However, it was recently discovered that calcium intake from food or supplements is associated with other health benefits, such as preventing hypertension, cholesterol and colorectal cancer (Cormick & Belizan 2019). Generally, calcium is obtained from dairy products and their derivatives. Therefore, an alternative source is needed, particularly for those suffering from lactose intolerance. These alternative sources can be derived from marine products and are being widely developed, because they are easy to obtain, abundant, safe and they promote beneficial biological activities (Xu et al 2020).

Recent studies on the use of fishbone waste as a source of calcium have been reported, including bio calcium powder from Skipjack tuna bones (Benjakul et al 2017), nano calcium powder from skipjack tuna bones (Harmain et al 2018), bio calcium powder and hydroxyapatite from salmon bones (Idowu et al 2020), bio calcium from Mozambique tilapia bones (Kurniasih et al 2020) and bio calcium powder from Asian sea bass bones (Wijayanti et al 2021). However, no scientific studies have been reported on the utilization of *M. cyprinoides* bone waste as bio calcium. Production of bio calcium powder from fishbones can use n-hexane as a solvent to remove the fat component. However, this study used the cooking with pressure method to minimize the use of chemicals in removing fat components. Cooking with pressure is intended to remove some organic components, such as fat and protein in fishbone, thus obtaining a higher percentage of calcium content in fishbone powder (Kusumaningrum et al 2016). This work is a preliminary study meant to determine the chemical composition of bio calcium powders from pressure-cooked *M. cyprinoides* bone.

Material and Method

Collection and preparation of pressure-cooked bones. *M. cyprinoides* bones were obtained from the local processor of frozen minced fish in Tarakan City, North Kalimantan. The bones were wrapped in a polyethylene bag and brought to the Fisheries Product Technology laboratory, Faculty of Fisheries and Marine Sciences, Borneo Tarakan University, within 30 minutes. The preparation of pressure-cooked bones referred to Kusumaningrum et al (2016), with modifications. *M. cyprinoides* bones were cut manually, using a knife, to remove the head and fins. Afterward, they were washed to remove adhering flesh and other debris and then drained. After draining, they were boiled for 30 minutes at an initial temperature of 800°C. They were re-boiled 4 times, each time after a replacement of the boiling water. Furthermore, they were cooked using a pressure cooker (Maxim, Indonesia) for 0, 1 and 2 hours.

Production of bio calcium powder. Bio calcium production referred to Kurniasih et al (2020), with modifications. The pressure-cooked bones were extracted using 1.5 N NaOH, at a ratio of 1:2 (w/v) and a temperature of 600°C for 2 hours, in a Waterbath (Memmert, Germany). Furthermore, the residue was separated from the filtrate and washed under running water, until neutral. The bones were dried at 650°C for 48 hours, using an electric oven (Memmert, Germany). Afterward, they were ground using a grinder and sieved with a 100 mesh sieve.

Proximate analysis. Proximate analysis of bio calcium powder, including moisture, ash, protein and fat contents, referred to Hasan et al (2020). The proximate value is expressed based on the wet weight (wet basis).

Determination of calcium and phosphorus content. Determination of calcium and phosphorus content referred to AOAC (2011). A total of 0.5-1.0 g of the sample was weighed in a vessel, and 10 mL of concentrated HNO_3 was added. The vessel was closed and put into microwave digestion. Samples were destroyed at 150^0C for 10 minutes (Ramp) and at 150^0C for 15 minutes (Hold). The digestion results were transferred to a 50 mL volumetric flask and 0.5 mL of a conentration of 100 mg L^{-1} internal standard yttrium was added. The solution was diluted within an aqua bidest to the mark and homogenized. The solution was filtered using filter paper and then measured in the ICP-OES system (Agilent 720, US).

Determination of functional group. The Fourier Transform Infrared Spectroscopy (FTIR) instrument (Agilent Cary 630, US) was used to determine the functional groups or specific components contained in the sample based on the appearance of peaks at certain wavenumbers. Spectrum profiling was carried out in the region of 4,000 to 400 cm^{-1}.

Statistical analysis. This study was conducted using a completely randomized design and presented as mean ± standard deviation (SD). Proximate, calcium, and phosphorus values were analyzed using the ANOVA test at 95% (P<0.05) confidence level, followed by a Duncan's post hoc test to examine differences between all treatment pairs. Statistical analysis was performed using SPSS version 25. Meanwhile, the functional groups were analyzed descriptively and presented in a figure.

Results and Discussion

Proximate value of bio calcium powder. The proximate values of bio calcium powders from pressure-cooked *M. cyprinoides* bone are shown in Table 1. According to the ANOVA test, it was showed that cooking time (0, 1, 2 hours) has an effect on water, protein, fat and ash content of bio calcium powder from pressure-cooked *M. cyprinoides* bone (P<0.05). A further Duncan's post hoc test showed significant differences in moisture content at all variants of cooking time. These results indicate that the variant in cooking time of 2 hours is the best because it produced the lowest water, protein and fat content and the highest ash content in the resulting bio calcium powder.

Table 1

Proximate value of bio calcium powders

Variant	*Moisture (%)*	*Protein (%)*	*Fat (%)*	*Ash (%)*
CT0	3.35 ± 0.02^{a}	14.57 ± 0.11^{a}	0.62 ± 0.03^{a}	78.42 ± 0.44^{a}
CT1	3.13 ± 0.03^{b}	12.29 ± 0.27^{b}	0.51 ± 0.04^{b}	80.72 ± 0.47^{b}

Variant of cooking time: CT0 - cooking time 0 hour; CT1 - cooking time 1 hour; CT2 - cooking time 2 hours.
Data are presented as mean±SD; different superscripts indicate significant differences (p<0.05).

The moisture content of bio calcium powders from pressure-cooked *M. cyprinoides* bone was 3.34±0.02%, 3.13±0.03%, and 2.98±0.12%, respectively. It implied that the longer the cooking time, the lower the moisture content, which was associated with some water loss during the thermal treatment. The use of high temperatures to reach the boiling point of water during the cooking process causes a decrease in the moisture content of the material. The heating process of food can remove free water, which has a vital role in the microbial growth. Microbes require a slightly lower moisture content inside the cell than outside. When the free water outside is reduced, the free water inside flows out of the cell until reaching the equilibrium. These conditions cause osmotic shock and plasmolysis in cells. Thus, microbial cells do not grow and food spoilage can be slowed (Erkmen & Bozoglu 2016). Moisture content plays an important role in maintaining the stability of powder products and shelf life. Reduction of moisture content aims to preserve food from microbiological and chemical damage. The moisture content, required to get a longer shelf life of the powder, is maximum 10% (Ojo et al 2017).

The moisture content of all treatments complied with the Indonesian National Standard for the bone powder, which required a maximum moisture content of quality I bone powder of 8% (NSAI 1992). Following the present results, a previous study also demonstrated that the Starry triggerfish bone powders had a low moisture content, ranging from 3.79 to 4.15% (Husna et al 2020). Meanwhile, another study found the moisture content of milkfish bone powder ranging from 5.19 to 5.49% (Wulandari & Kusumasari 2019). The different results could be caused by the raw materials being derived from different species with varying chemical compositions. Furthermore, the different moisture contents could be caused by differences in the drying methods (Sumarto et al 2021).

The protein content of bio calcium powders from pressure-cooked *M. cyprinoides* bone was 14.57±0.11%, 12.29±0.27% and 11.39±0.21%, respectively. The longer the cooking time, the lower the protein content. It was assumed that the protein was being denatured and disappeared with the water that came out during the cooking process, thus reducing the protein content in the bio calcium powder. These results reflected these of Kusumaningrum et al (2016), who also found that the protein content of Belida fish bone powder decreased along with the increasing frequency of boiling. The protein content of Belida fish bone powder ranged from 9.87 to 15.18%.

Another study reported higher protein levels in sardine and catfish bone powder, of 19.47% and 20.40% (Rosidi et al 2021), due to the manufacturing process, during which the deproteination method with alkaline was not applied. The alkaline method is considered more effective in hydrolyzing proteins. Nevertheless, the alkaline treatment can not eliminate 100% of the protein content from the bone powder. Thus, protein will remain in lower amounts (Qisti et al 2021). Bone contains stromal protein, which is difficult to remove by acid or alkaline treatment (Kusumaningrum et al 2016). The organic compounds in fish bones are dominated by collagen and non-collagen proteins such as osteocalcin, osteopontin, osteonectin, fibronectin, thrombospondin and proteoglycans. Different chemical compositions were exhibited by the fishbone powder manufactured from various processes. Moreover, pre-cooking treatment affected the protein content produced (Benjakul et al 2017).

The fat content of bio calcium powders from pressure-cooked *M. cyprinoides* bone was 0.62±0.03%, 0.51±0.04% and 0.42±0.02%, respectively. The longer the cooking time, the lower the fat content. It was presumably due to the thermal treatment for a longer time, which could cause many fats to melt and disappear with the water during the cooking process. In general, the fat melts at 30 to 400°C, whereas the cooking process with pressure occurs at approximately 1,000°C. High cooking temperatures also cause fat to be oxidated, which forms volatile compounds, thus they can decrease fat content. The loss of fat content in fishbone occurs during boiling, autoclaving and heating in chemical substances. The loss of fat content can increase the shelf life of the fishbone powder (Talib & Zailani 2017).

The fat content in all treatments complied with the Indonesian National Standard for bone powder, which required maximum fat content for quality I bone powder of 3% (NSAI 1992). Benjakul et al (2017) reported the lower fat content was found on Skipjack tuna bio calcium powder of 0.21%. They confirmed that the differences of method could affect the fat content of bio calcium powder. They used the method of immersion in n-hexane solvent to remove fat from fishbone, whereas this study only relied on cooking treatment with pressure. Another study reported a fat content of 0.33% in bio calcium powder from Salmon bones, with the alkaline treatment, lower than în bio calcium powder without alkaline treatment of 1.70%. Hydrolysis by alkaline treatment also contributes to reducing fat in fishbone because the trapped one might escape from the bone matrix with the treatment (Idowu et al 2020). Nevertheless, the fat content in fishbone powder can not be entirely removed by simply soaking in an alkaline solution, because the fat has a complex bond. Crude fat fills fishbone tissue, reaching 6% (Hemung 2013). The fish powder must have a low enough fat content to prevent rancidity, while producing durable fish bone powder (Sumarto et al 2021).

The ash content of bio calcium powders from pressure-cooked *M. cyprinoides* bone was 78.42±0.44%, 80.72±0.47% and 82.40±0.41%, respectively. The longer the cooking time, the higher the ash content. It was assumed that the longer the cooking process, the fewer the organic constituents, thereby increasing the percentage of inorganic compounds in the material. It also showed that the protein content in this study decreases along with the increase of the ash content. The ash content indicates the total minerals in the fishbone powder produced.

The previous study stated that calcium supplements from tuna fish bones boiled in 2% NaOH solution in a ratio of 1:3 (w/v) for 30 minutes produced an ash content of 77.97% (Nemati et al 2017). Alkaline solvents such as sodium hydroxide have a fairly good ability to increase the ash content of the material. Fishbone powder extracted with alkaline produced a much higher ash content than fishbone powder extracted with water (Amitha et al 2019). It was confirmed by the research of Talib et al (2014) that boiling treatment followed by an extraction process using water and acetic acid on Yellowfin tuna bone resulted in a lower ash content than in this study: 56.65% and 8.21%, respectively.

Talib & Zailani (2017) also performed the extraction using sodium hydroxide solvent, resulting in the highest ash content of Yellowfin tuna bone powder (56.70%) compared to using citric acid (55.70%), acetic acid (55.10%) and hydrochloric acid (52.30%) as solvents. The findings of a great deal of previous work corroborated this study: the alkaline hydrolysis method is an effective method for removing organic components, such as protein and fat, thereby producing a relatively high purity fishbone powder.

Calcium and phosphorus content of bio calcium powder. The calcium and phosphorus content of bio calcium powder from pressure-cooked *M. cyprinoides* bone are presented in Figure 1. According to the ANOVA test, the cooking time (0, 1, 2 hours) has an effect on the calcium and phosphorus content of the bio calcium powder from pressure-cooked *M. cyprinoides* bone ($P<0.05$). A further Duncan's post hoc test showed significant differences in calcium and phosphorus content for all variants of cooking time. The results pointed out that the cooking process with pressure affected the calcium content produced, but extending the cooking time to 2 hours had an insignificant effect and contradicted the previous results of the ash analysis. A similar behavior was observed for the phosphorus, where the levels decreased for 2 hours of cooking. It presumably occurred due to some calcium and phosphorus in the form of ions that dissolve in water, during the cooking process. Boiling can cause loss of minerals due to the diffusion of certain minerals into the water, causing mineral levels to decrease. Loss of minerals during cooking is not caused by mineral destruction, yet it is caused by cooking water which can leach minerals (Lee et al 2019).

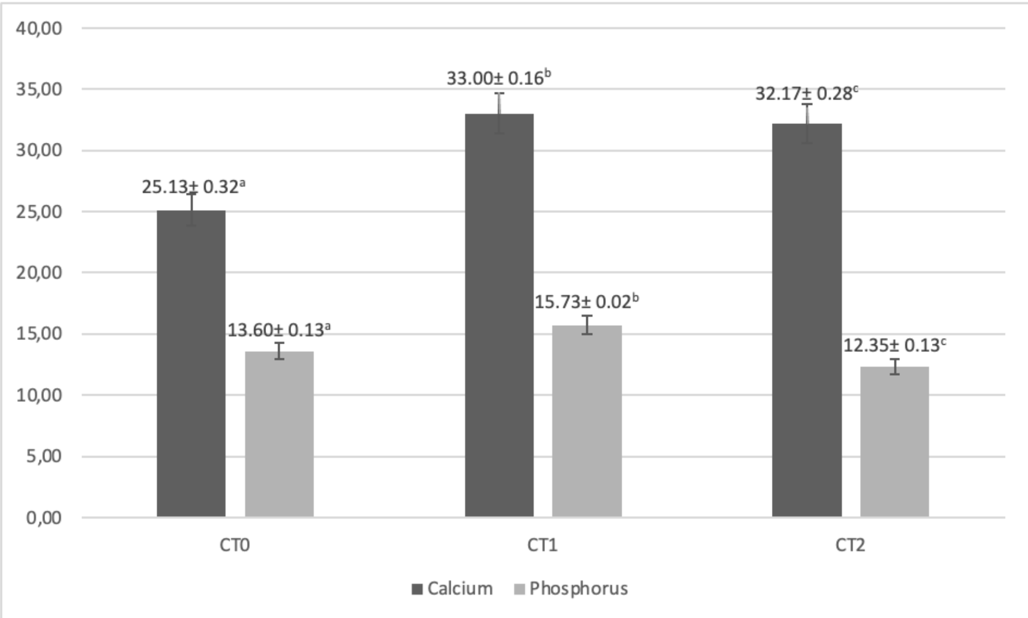

Figure 1. Calcium and phosphorus content of bio calcium powder: CT0 - cooking time 0 hour; CT1 - cooking time 1 hour; CT2 - cooking time 2 hours. Data are presented as mean±SD; different superscripts indicate significant differences ($p<0.05$).

The percentages of calcium content were 25.13±0.32%, 33.00±0.16% and 32.17±0.28%, respectively. These results were lower than the calcium content of Starry triggerfish bone powder, of 35.75% (Husna et al 2020). The calcium content of bio calcium powder from Asian sea bass ranged from 33.43 to 33.72% (Wijayanti et al 2021). The difference in calcium levels is probably due to the different types of raw materials used. Calcium levels are strongly influenced by species, nutrient content in a feed, tendon fat on the bone surface, the amount of bone marrow and cartilage (Nemati et al 2017). In addition, fishbone calcium levels are also influenced by the preparation method of fishbone powder (Rosidi et al 2021). Talib et al (2014) reported that yellowfin bone powder with acetic acid treatment resulted in lower calcium levels, of 16.64%, compared to the water treatment, where it was of 23.61%. The calcium content of all treatments complied with the Indonesian National Standard for bone powder, which required a minimum calcium content of quality I bone powder of 20% (NSAI 1992).

The percentages of phosphorus content were 13.60±0.13%, 15.73±0.02% and 12.35±0.13%, respectively. These results were lower than the phosphorus content of Sardine bone powder, ranging from 16.57 to 17.20% (Logesh et al 2012). It was presumably due to the different processing methods of bio calcium powder, which used NaOH and ethanol solvents, without any cooking process. Other factors that can affect the mineral content in the fish body include fish metabolism, the availability of minerals in body water, and the fish ability to absorb them (Pateiro et al 2020). The phosphorus content in bio calcium powder can be influenced by the non-collagenous phospho-protein content in the fish bones used. In addition, foods containing phosphate salts such as monosodium phosphate, monocalcium phosphate and monoammonium phosphate can also trigger an increase in phosphorus levels in bones (Wijayanti et al 2021). The ratio between calcium and phosphorus (Ca/P) is important, increasing the calcium absorption in the body. The ratio for calcium absorption in the intestinal cavity is 1:1 to 3:1 (Kusumaningrum et al 2016). The ratio of Ca/P of this study ranged from 1.85 to 2.61. A lower ratio was reported by Benjakul et al (2017), which states that the Ca/P ratio of bio calcium powder from Skipjack tuna bone is 1.65. Vertebrate bone contains 60-65% hydroxyapatite crystals found in cross-linked collagen fibrils matrix. Pure hydroxyapatite has a Ca/P ratio of 1.67 (Idowu et al 2020). It showed that the bio calcium powders in this study had a low purity level but still complied with the requirements for the ratio of calcium absorption in the intestinal cavity.

Functional group of bio calcium powder. Functional groups can be predicted using FTIR, through absorption transmission, due to changes in the vibrational mode of molecules that produce specific energies, affected by the type of atoms and by the bond strength between them (Lolo et al 2022). The FTIR spectra of bio calcium powder from pressure-cooked *M. cyprinoides* bone are shown in Figure 1. The absorption peaks indicated the presence of a hydroxyapatite (HA) structure. It was observed at wavenumbers 1015, 1017, 1020 cm^{-1} as the energy for the bending vibration of the phosphate group. Benjakul et al (2017) reported that at wavenumbers 1040 and 1046 cm^{-1}, showed the bending modes of P-O bonds. It was confirmed by Berzina-Cimdina & Borodajenko (2012) that there was a bending vibration of $PO4^{-3}$ at 1000 to 1120 cm^{-1}. Carbonate groups were also detected in this research, showed by the presence of vibrations at wavenumbers of 1409, 1414, 1415 cm^{-1} as well as 870, 876 and 882 cm^{-1}. Fernandez-arias et al (2021) reported that carbonate groups were observed at wavenumbers 1400 and 1460 cm^{-1} as well as at approximately 714 and 875 cm^{-1}. The absorption peaks at 1400 and 1460 cm^{-1} are the energies for bending vibrations in type B hydroxyapatite. It is due to the replacement of the PO_4^{-3} by the CO_3^{-2} group. Stretching vibrations of the O-H group were also observed in the FTIR spectra with low intensity at wavenumbers 3411 and 3484 cm^{-1} and correlated with the hydroxyl groups in the hydrated water. This finding was in line with Correa & Holanda (2019): the absorption peaks at wave numbers 3410 and 1645 cm^{-1} had a weak energy and indicated the presence of O-H group vibrations originating from free and bound water molecules through hydrogen bonds in the sample.

The absorption peak at wavenumber 1567, 1573 and 1592 cm^{-1} indicated the presence of amide groups in the collagen structure. Benjakul et al (2017) reported that N-H vibrations in fishbone collagen were observed in the 1636 and 1550 cm^{-1}. Furthermore, the stretching vibrations of the C-H group in the protein were also detected at the wavenumbers of 2918 and 2928 cm^{-1}. Aside from that, the C≡C group was evident at 2100 and 2105 cm^{-1}. Absorption peaks are also observed at wavenumbers 2847 and 2857 cm^{-1}, which were related to the presence of collagen structures in fishbones.

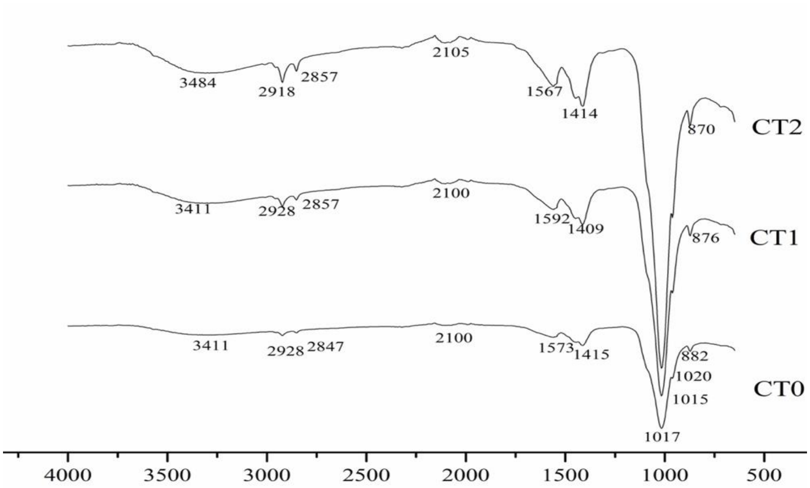

Figure 2. FTIR spectra of bio calcium powder: CT0 - cooking time 0 hour; CT1 - cooking time 1 hour; CT2 - cooking time 2 hours.

According to Cahyanto et al (2017), the compounds found in fishbone include calcium phosphate, carbonate, collagen fibers and HA. It was also confirmed by Kurniasih et al (2020) that the peak of bio calcium absorption was observed at wavenumbers 563, 1034, 1458, 1651, 2855 and 2924 cm^{-1}, which indicated the presence of calcium phosphate and collagen. Husna et al (2020) added that the FTIR spectra of Starry triggerfish bone powder indicated the presence of HA structure, marked by the absorption peaks of 874, 1410, 1458 and 3633 cm^{-1}. The results of the FTIR spectra proved that there were similar combinations with functional groups obtained from the bio calcium powders in all cooking time variants, namely phosphate, carbonate, hydroxyl and amide groups, that might correlate with the presence of collagen and HA compounds.

Conclusions. This study has successfully produced bio calcium from pressure-cooked *M. cyprinoides* bone and determined its chemical composition. The experiments confirmed that the variant of 1 hour cooking time was the best for an optimal production of calcium and phosphorus in the bio calcium powder. It is unfortunate that, according to the ratio of Ca/P, the bio calcium powders still had a low purity level. Notwithstanding the shortcoming, the study suggests the bio calcium powder can be used as an alternative source of calcium, as it qualifies for its calcium content in the bone powder and calcium absorption in the intestinal cavity, based on the ratio of Ca/P. Considerably more work will need to be done to find a more effective method to produce bio calcium powder of a high purity.

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Conflict of interest. The authors declare no conflict of interest.

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