Research Article

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Bioavailability of Nano-calcium from Parang Fishbone Extracted with Alkaline Solvent at Varying Ratio of NaOH and Extraction Time

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ABSTRACT: Calcium is an important nutrient that supports various biological functions of the body. Calcium plays a role in osmoregulation, muscle contraction, bone mineralization, blood clotting, and regulating the body's acid and base balance. Calcium absorption can be optimal in the body if it has a smaller particle size, namely nano-sized. Parang fish bones have great potential to be used as a source of calcium in the form of nano-calcium. Nano-calcium is calcium in the form of small particles, completely absorbed into the body, and can optimally meet the body's calcium needs. This research aims to evaluate the effect of variations in alkaline solvent concentration and extraction time on particle size and calcium levels of the resulting nano-calcium and determine the absorption of Parang fishbone nano-calcium in the in vitro bioavailability process of the inverted intestinal sac. The nano-calcium extraction method combines a sample ratio of NaOH at 1:3, 1:4, and 1:5 with extraction times of 30, 60, and 90 minutes. The in vitro reverse gut method tested the obtained nano-calcium for bioavailability. The concentration of the basic solvent and the optimum extraction time influence the nano-calcium produced due to the optimal contact time between the material and the solvent during the extraction process. Nano-calcium treatment with a sample ratio: NaOH of 1:4 with an extraction time of 60 minutes, resulted in a water content of 5.67%, ash content 74.20%; protein content 3.52%; fat content 0.117%; calcium content 35.46%; yield 44%, particle size 62 nm; and bioavailability at a 10 minute absorption time of 9.105%, a 20 minute absorption time of 9.222% and a 30 minute absorption time of 9.334%. The sample ratio treatment: NaOH 1:4 with an extraction time of 60 minutes is the best treatment because it can produce a smaller nano-calcium size and high bioavailability.

Keywords: nano-calcium, *Parang* fishbones, bioavailability, inverted gut sacs

INTRODUCTION

Calcium is an important nutrient that supports various biological functions within the body. It is vital for sustaining strong bones and promoting healthy teeth, and is responsible for regulating several cellular functions. The average daily calcium intake among Asian populations is approximately 500 mg, which is significantly lower than the FDA-recommended intake of 1000-1200 mg. According to Benjakul *et al*. (2018), calcium deficiency is a global issue affecting all countries worldwide. Calcium deficiency problems can arise due to lifestyle or activities. Insufficient calcium intake in intestinal absorption can lead to chronic problems, including decreased bone mass. In many Asian communities, such as Indonesia, dairy products are often avoided due to widespread lactose intolerance. Obtaining calcium from natural sources, like fish bones, is the most effective method. Fish bones can be highly beneficial as a source of nano-calcium. The creation of calcium from fish bone waste could address two problems simultaneously: providing calcium for individuals who are lactose intolerant and offering a means to recycle fishery waste that can harm the environment, produce unpleasant odors, and impact human health. Parang's fish (*Chirocentrus dorab*) has a fairly wide range and is found in almost all marine waters in Indonesia. Data on Parang-parang fish production in the Southern Sumatra region is 17,393 tons annually, producing 5,218 tons of waste annually. The potential of parang fish is usually used as raw material for making pempek and crackers. It can be used as an alternative to the proper use of waste to provide a source of calcium. To optimize calcium absorption in the body, calcium must be transformed into nano-calcium (Halimah *et al*., 2016).

Nano-calcium is calcium in the form of small particles that can be completely absorbed into the body, which is more efficient than particle size and can optimally meet the body's calcium needs. Due to its extremely small size (10^{-9} m), nano-calcium exhibits enhan ced solubility in the gastrointestinal tract. Thereby facilitating rapid absorption into the bloodstream (Ranjan *et al*., 2019). The amount of calcium that the body can absorb influenced by the solubility of calcium when absorbed in the duodenum and

proximal jejunum. According to Suptijah *et al*. (2012), nano-calcium has a particle size of around 37-127 nm, which allows for high bioavailability. This small size allows calcium to easily enter the receptors and be optimally absorbed into the body.

Extraction of fish bones has been achieved by various researchers using a concentrated alkaline solution, including Anggraeni (2019), who used a 1:3 ratio NaOH 1N: sample on tilapia bone. Kusumawati *et al.* (2022) applied a 1:5 ratio of NaOH to sample on grouper, while Amitha *et al*. (2019) utilized 2% NaOH on grouper as well. Nemati *et al*. (2017) employed 2% NaOH on yellowfin tuna bones, and Prinaldi *et al*. (2018) used 1.5 N NaOH on yellowfin tuna. Lastly, Sumarto *et al.* (2021) worked with 1.5 N NaOH on various species of *Clarias sp*. Alkaline treatment has been shown to be an effective method for eliminating protein-based materials from the bone matrix (Idowu, 2020). Benjakul *et al*. (2017) compared calcination and alkaline extraction methods for assessing the natural calcium level of tuna bones, and recommend alkaline extraction as the best approach for producing edible calcium with high bioavailability.

Extraction using NaOH allows calcium to precipitate in the bone matrix in large quantities, and the fishbone calcium produced increases, thereby increasing the amount of nano-calcium, the yield, and the size of the nano-calcium particles. This research used parang fishbones as raw material for making nano-calcium, whose bioavailability was tested using an *in vitro* method using an inverted gut sac. The *in vitro* measurement method is a simulation of the process of food digestion in the gastrointestinal tract.

MATERIALS AND METHODS

Material

The main material used in this research was Parang fishbones (*Chirocentrus dorab*) obtained from UKM Sejahtera, Lampung, Indonesia. All fishbone waste is collected with random internal factors such as size, sex, or fish age. NaOH 1 N (Merck, 1310-73-2), NaCl, pepsin enzyme, and HCl (Mallinckrodt, 7647-01-0) were used in this study. The subjects were male Wistar rats with a weight of 200 ± 20 g, which were obtained from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

Bone Powder Production

The production of coarse brown powder was conducted following the method by Anggraeni (2016) with modifications. Each type of fish bone was washed in flowing water. The fishbone was then boiled for one hour, thoroughly cleaned, and dried in a cabinet dryer at 50 °C for a period of 24 hours. Afterward, the fishbone was autoclaved at 121 °C for 3 hours and dried again in a cabinet dryer at 50 °C for an additional 24 hours. The fishbone was pulverized using a mortar and then ground with a multifunction disintegrator for 1 min. The coarse calcium powder was subsequently analyzed for its yield percentage and prepared for additional testing.

Fishbone nano-calcium

The nano-calcium in powder form was produced according to Anggraeni's method (2016). The coarse calcium powder was immersed in 1 N HCl (using a sample-to-solvent ratio of 1:5, w/v) and stirred for 1 hour using a magnetic stirrer until a homogeneous solution was achieved. The sample was then incubated at ambient temperature for 24 hours. The crude bone meal was processed to eliminate HCl through centrifugation. The supernatant was eliminated, and the remaining concentrated crude bone meal pellet was placed into a beaker. The subsequent step involved the addition of 1 N NaOH solution in proportions of 1:3, 1:4, and 1:5. The pellets were then hydrolyzed on a hotplate stirrer at 100 °C for 30, 60, and 90 minutes, repeated three times. Each hydrolysis repetition was conducted using the centrifugation method to isolate the supernatant from the sediment. The hydrolyzed sediment was combined with distilled water and subsequently adjusted to a pH of 7.0-7.1 using 1 N HCl. The sediment was placed in a ceramic tray and subjected to drying in a controlled setting at 50 °C for 16 hours. After drying, the sediment was finely pulverized to produce fish bone nano-calcium.

Proximate analysis

The moisture level was evaluated with a moisture analyzer (MB-90, Ohaus, Parsippany, NJ, USA). The protein level was measured through Kjeldahl digestion, as outlined in AOAC 920.153. Fat content was determined using Soxhlet extraction according to AOAC 960.39, while ash content was quantified using gravimetric techniques following AOAC 928.08.

Calcium solubility

Calcium solubility analysis refers to the method of Santoso *et al*. (2006). This test was carried out by adding 0.5 grams of the sample to 40 ml of ion-free distilled water. Then, the sample solution was adjusted to pH levels of 2, 3, 4, 5, 6, 7, 8, 9, and 10 using 1N HCl and 1N NaOH. The sample was stirred for 20 minutes at room temperature at

a speed of 200 XG. The samples were then incubated in a water bath at 37 ℃ for 2 hours. The sample was then centrifuged for 10 minutes at a speed of 3000 XG and subsequently filtered using Whatman 42 filter paper. The dissolved calcium content of the filter results was measured using atomic absorption spectrophotometry (AAS) with a wavelength of 420 nm.

Scanning electron microscopy

The samples were first placed on a bronze stub and then covered with a gold layer using a sputter coater (SPI-Module, West Chester, PA). Visualization of the specimens was conducted using a scanning electron microscope (SEM) from Thermo Fisher Scientific Prisma E, MA, USA, set at an accelerating voltage of 10 kV and a magnification of × 25,000.

Particle size analysis

The dimensions of the nano-calcium particles were determined using a Zetasizer Nano (Malvern Zetasizer, Instrument Ltd, UK). The particle size analysis method follows the procedure described by Yin *et al*. (2016), albeit with some adjustments. A 50 mg sample was dissolved in 50 ml of ultrapure water, and the pH was adjusted to 2.0 by adding 1 N HCl. Subsequently, the mixture was placed in a homogenizer (Ika Ultra-Turrax 50T Basic, Selangor, Malaysia) and processed at 300 XG for 15 minutes. The particle size distribution was evaluated using dynamic light scattering with a Malvern Zetasizer Nano ZS device (Malvern, UK).

Bioavailability nano-calcium

Bioavailability nano-calcium analysis followed the Natalijah (2017) method and was slightly modified. These mice were given food and drink *ad libitum* and then allowed to acclimate for 1 week before the start of the experiment. The intestine was recorded as measuring 7 cm and contained 1.4 ml of serosal fluid (0.9% w/v NaCl solution). The upper end of the intestine is connected to tube B (the cannula part of the Crane and Wilson tube) and secured with thread to prevent it from coming loose easily. The intestine that had been given serosal fluid was placed in a Crane and Wilson tube filled with 25 ml of mucosal fluid (a pepsin HCl solution prepared by dissolving 0.5 g of pepsin in 100 ml of 0.1 N HCl). This mucosal fluid contains 2 g of sample mixed with 25 ml of pepsin HCl solution and is incubated at 37 ℃ in a waterbath shaker for 90 minutes. Tubes containing mucosal fluid with samples and controls were placed in a water bath shaker at 37 ˚C for incubation. Throughout the experiment, each part of the intestine was continuously submerged in mucosal fluid and provided with oxygen gas (through pipe C) at a rate of approximately 100 bubbles per minute. The test solution (containing nano-calcium samples) at 10, 20, and, 30 minutes, serosal fluid was taken via pipe cannula B and put into a vial, then the intestine was filled again with 1.4 ml of 0.9% w/v NaCl. The control portion (without a sample), after 10 minutes, the serosal fluid was collected via cannula and placed into a vial. Then, each liquid in the vial was tested for absorbed calcium levels with atomic absorption spectrophotometry (AAS).

Statistical analysis

All experiments in this research were conducted in triplicate. Statistical evaluation was carried out using SPSS (IBM SPSS version 25, IL, USA), and means were compared utilizing Duncan's multiple range test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

The characteristics of fish bone nano-calcium were carried out physically and chemically, including proximate analysis, particle size, calcium solubility, nano-calcium morphology, and nano-calcium bioavailability *in vitro* using an inverted gut sac. The results of the proximate composition analysis for nano-calcium, considering different concentrations of alkaline solvent and varying extraction times, are presented in Table 1.

Moisture content

Table 1 shows that the nano-calcium moisture content varies between treatments, ranging from 5.62% to 6.30%. The highest water content of 6.30% was found in nano-calcium with a sample:NaOH ratio of 1:5 and an extraction time of 90 minutes. The lowest water content, 5.62%, was found in nano-calcium with a sample:NaOH ratio of 1:3 and an extraction time of 30 minutes. This water content value is lower than that of research by Benjakul *et al*. (2017), which indicates that the water content of fish bone calcium is 7.35%. The amount of water content can be influenced by the drying time and temperature of the nano-calcium deposits as well as the hygroscopic properties of the material. The water content can be reduced due to the heat transfer and mass transfer processes that occur during the drying process. The concentration of nano-calcium in the water content of Parang fish exceeds that documented by Kusumawati *et al*. (2022) for tilapia (1.66%), catfish (2.16%), and tuna (2.47%). Differences in water content can be attributed to variations in raw materials, methods of producing nano-calcium, and the drying methods employed.

Table 1. Nano-calcium powder chemical constituents

Ratio	Extraction time	Proximate composition				
		Moisture	Ash	Protein	Fat	Calcium
1:3	30 min	5.62±0.08^{f}	72.30±0.08^{a}	3.62±0.01^{a}	0.21±0.01^{a}	35.28±0.02^{e}
1:4		5.64±0.04^{ef}	72.36±0.01^{e}	3.52±0.01^{c}	0.18±0.09^{b}	35.28±0.04^{e}
1:5		5.68±0.01^{d}	72.39±0.01^{e}	3.50±0.01^{cd}	0.18±0.09^{b}	35.84±0.09^{c}
1:3	60 min	5.63±0.02^{f}	74.16±0.02^{d}	3.59±0.01^{b}	0.13±0.02^{c}	35.35±0.20^{de}
1:4		5.67±0.02^{ef}	74.20±0.01^{d}	3.49±0.01^{e}	0.13±0.01^{d}	35.46±0.05^{d}
1:5		6.14±0.01^{c}	74.25±0.09^{c}	3.47±0.09^{e}	0.12±0.02^{e}	35.73±0.01^{c}
1:3	90 min	6.12±0.02^{c}	74.33±0.01^{b}	3.52±0.02^{c}	0.12±0.01^{de}	38.11±0.01^{b}
1:4		6.23±0.01^{b}	74.66±0.01^{a}	3.47±0.02^{e}	0.11±0.09^{f}	38.41±0.05^{a}
1:5		6.30±0.08^{a}	74.70±0.02^{a}	3.44±0.01^{f}	0.11±0.01^{f}	38.43±0.01^{a}

Note: *Dry weight basis. Values are seen as mean ± SD (n = 3). Different superscript alphabets show significant differences determined by Duncan's test ($p < 0.05$)

Ash content

Extraction of fish bones with NaOH solvent effectively reduces organic matter in coarse bone powder. Additionally, immersing coarse fish bone powder in HC prior to alkaline extraction can speed up the process of protein hydrolysis. The nano-calcium ash content of parang fishbones obtained results between 72.16 -74.70%. The results of this study are lower than those of Putranto *et al*. (2015), who achieved an ash content of 88.52% by using NaOH for 2 hours during the extraction of belida fish bones. Research by Suntornsaratoon *et al*. (2018) indicated that calcium extracted from tuna fish bones has an ash content of 69.50% lower than that obtained from parang fish bones. The increased ash content in nano-calcium powder suggests that the alkali treatment successfully lowers the levels of protein and fat in nano-calcium. The ash content increases with extraction time. The longer the extraction duration, the higher the ash content of the material, because the longer the extraction duration, the longer the product will be in contact with the solvent. As a result, more non-mineral components will be dissolved.

Protein content

Protein must be removed as much as possible through the protein hydrolysis process to make bone meal. The nano-calcium protein content of parang fish bones was found to be 3.44% to 3.62%. Parang fishbones, without undergoing the extraction process, have a protein content of 27.83%, which is higher when compared to the nano-calcium protein content of parang fishbones that go through the extraction process. The protein concentrations observed in this study exceeded those documented in the research conducted by Kusumawati *et al*. (2022). The protein levels in tilapia fish are 0.67%, while snapper fish contain 1%, and grouper fish have a protein content of 1.08% when extracted with 1 N NaOH. Differences in protein levels can be caused by variations in the samples used and the time required for soluble extraction. The process of alkaline hydrolysis and the duration of extraction greatly influence the levels of protein and fat, as reactions involving denaturation and the saponification of free fatty acids with alkali considerably reduce their quantities. The alkali hydrolysis treatment has a dual effect, reducing organic matter through saponification and denaturing proteins and enzymes, including proteases and lipases, that can compromise quality and color stability during enzymatic processing (Kusumawati *et al*., 2022)

Fat content

The fat component in the process of making nano-calcium must be reduced to produce a good fish bone meal product. The decrease in fat content that occurs with increasing extraction time indicates that high temperatures and the use of alkaline solvents can oxidize fat. The nano-calcium extraction process using NaOH can reduce the fat content of the material because the fat dissolves during the extraction process. Testing the nano-calcium fat content of parang fishbones yielded results ranging from 0.11% to 0.21%. Parang fishbones, without undergoing the extraction process, have a higher fat content, specifically 5.66%. A study conducted by Idowu *et al*. (2020), the fat content of salmon bones extracted with an alkaline solution for 1 hour resulted in a fat content of 0.33%, and salmon bones extracted for 6 hours had no detectable fat or 0 %. The nano-calcium fat content of parang fishbones is lower when compared to research by Lekahena *et al*. (2014); tilapia fish bones extracted with NaOH solution produced a fat content of 0.38%. The type and size of the species used influence differences in fat content. The fat content present in fish bones is closely linked to the body

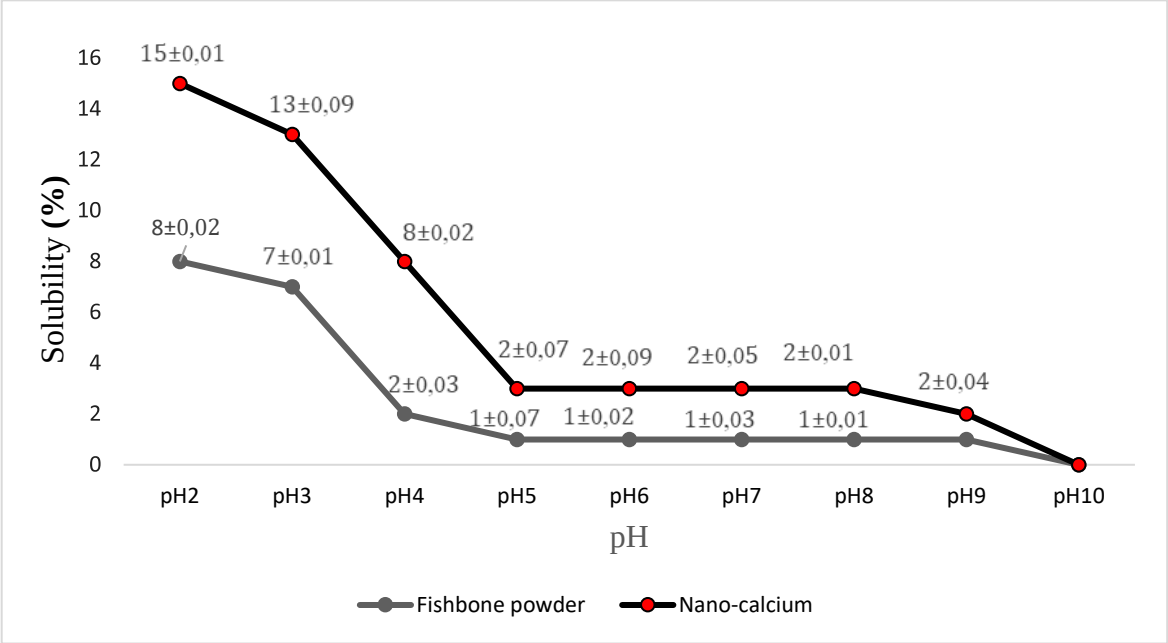

Figure 1. Calcium Solubility

fat of different species, and generally, larger and older fish are likely to have elevated fat levels. (Toppe *et al*., 2007).

Calcium content

The calcium content in parang fishbones ranges from 35.28 to 38.43% (Table 1), less than the calcium level of Mozambique tilapia bone powder, which is 74.75 to 83.25%. However, this result was higher than the ribbon fish (*Trichiurus savala*) bone meal concentration, 19.33-27.81% (Logesh *et al*., 2012). The calcium levels found in fish bones differ based on the species (Huda *et al*., 2010), the technique employed to create calcium powder, and the kind of solvent utilized (Logesh *et al*., 2012), in addition to the concentration of the solvent and the duration of boiling (Nemati *et al*., 2017). The increase in calcium levels, along with the length of extraction time, is caused by the increasing amount of protein dissolved during the extraction process using NaOH. The extraction process that involves NaOH or another alkaline solution may lead to the precipitation of calcium within the bone matrix, thus raising the calcium content percentage in the material.

Calcium solubility

Calcium solubility and pH value are the main factors that can influence calcium absorption. Calcium with a pH of 6 can dissolve in the body, so calcium can only be absorbed if it is in a water-soluble form. The solubility test utilized two materials: fish bone meal and fish bone nanocalcium. The nanocalcium used for the solubility test was treated with a sample ratio: NaOH 1:4, with an extraction time of 60 minutes. Allen and Wood (1994) stated that calcium solubility can increase in an acidic environment (pH 2), but dissolved calcium ions will precipitate in the jejunum and ileum at a pH close to neutral, namely pH 6-7, and water-soluble calcium can be absorbed optimally by the body. Calcium solubility in this study was used pH 2 to pH 10 as the parameter. Parang fishbone nano-calcium has different calcium solubility at pH levels, as seen in Figure 1. The percentage of calcium solubility increases as the pH value decreases. Calcium solubility has the highest solubility value at pH 2. This is due to the use of acid, which can facilitate the release of calcium, which is used into Ca^{2+} ions, which are more easily absorbed in the intestine (Talib *et al*., 2014). Fish bone meal has calcium solubility at a pH of 2-8 %. Meanwhile, nano-calcium fish bones have a pH 2 calcium solubility percentage of 15%.

Scanning Electron Microscopy

The morphological results of parang fishbone flour particles or powder showed that the fishbone flour was irregular, dense, and lump-shaped due to the combination of several calcium granules. The surface morphology of nano-calcium has an irregular shape, solid, smaller crystal size, and is in the form of chunks. The morphology of the fish bone meal sample has a larger crystal size and is the form of chunks. The findings from the morphological analysis of nano-calcium particles in parang fishbones indicate that the nano-calcium exists as aggregates. The nano-calcium particles are shaped like elongated grains, positioned irregularly and varying in size. The morphology of the resulting nano-calcium is spindle-


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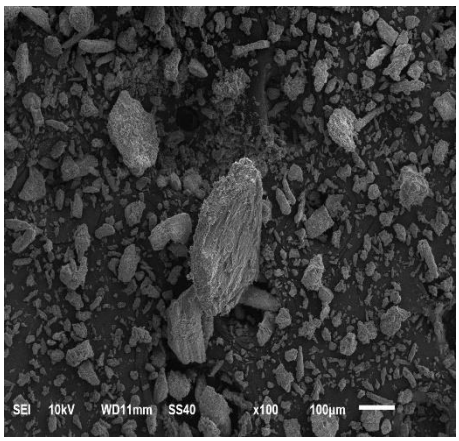
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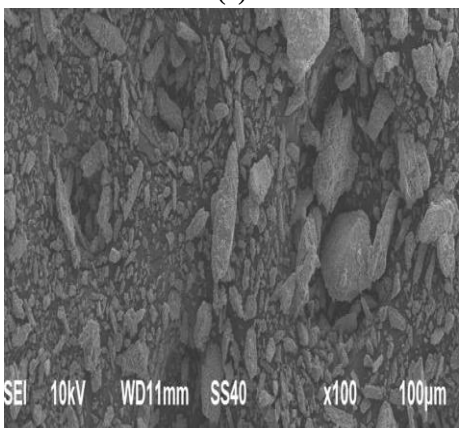
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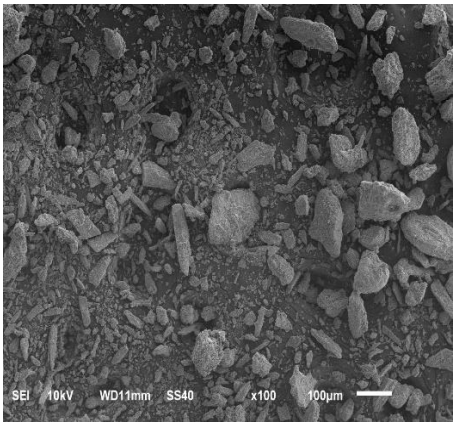
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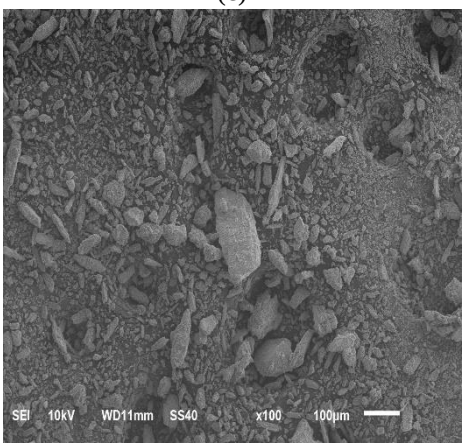
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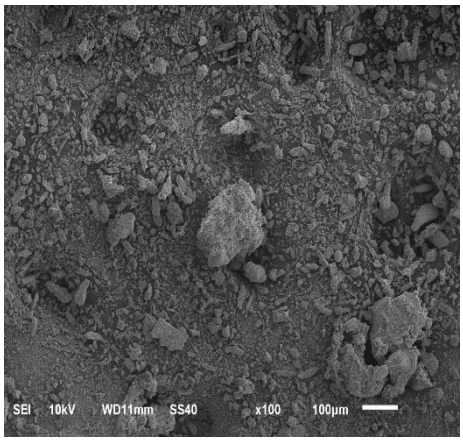
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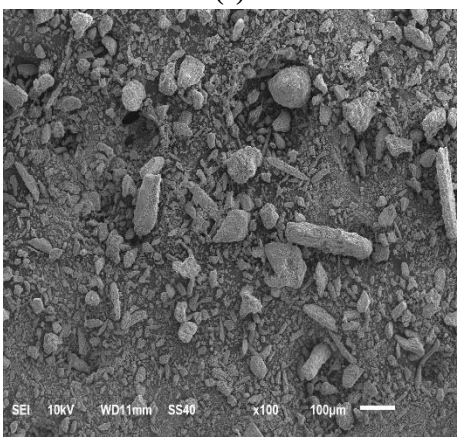
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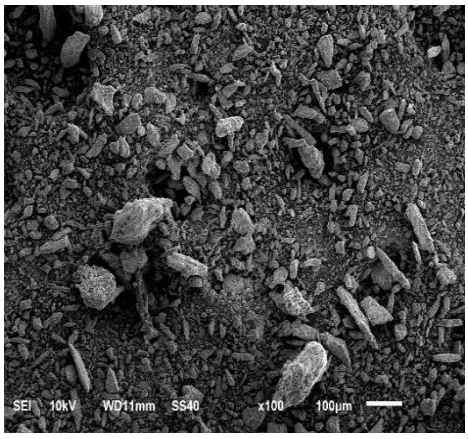
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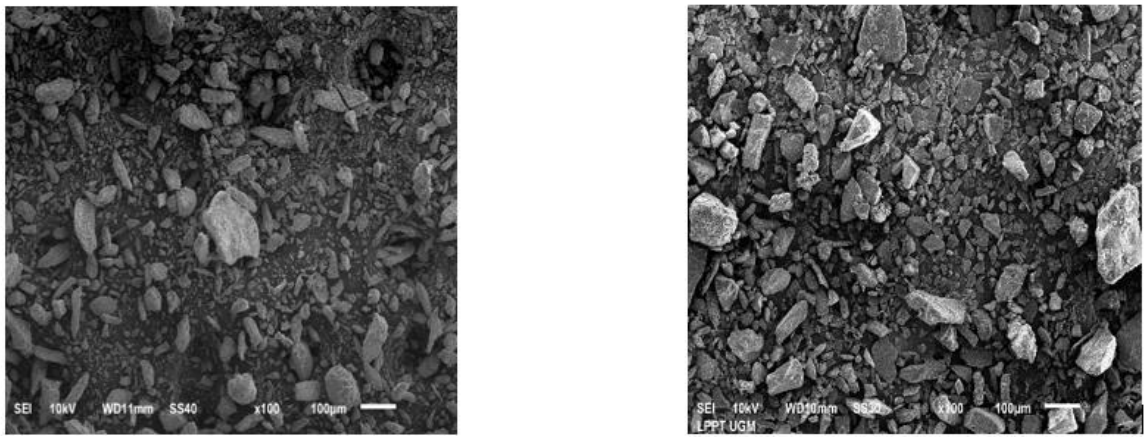

(i) (j)

Figure 2. The appearance of nano-calcium powder particles determined by SEM
Note: a fish powder, b (NaOH 1:3 30 min), c (NaOH 1:3 60 min), d (NaOH 1:3 90 min), e (NaOH 1:4 30 min), f (NaOH 1:4 60 min), g (NaOH 1:4 90 min), h (NaOH 1:5 30 min), i (NaOH 1:5 60 min). j (NaOH 1:5 90 min)

shaped (calcite). Research conducted by Husna *et al*. (2020) produced oyster shell nano-calcium crystals in the form of vaterite and a small amount of aragonite. Wei *et al*. (2023) produced spindle-shaped calcium crystals in shell material consisting of countless small calcite. Calcium crystals have different types of phases, namely calcite, aragonite, and vaterite. Calcite crystals stick firmly to the surface (the most stable), aragonite is easily separated from the wall, while vaterite is unstable. The difference in the shape of nano-calcium crystals can be caused by differences in the raw materials used in making nano-calcium and also the habitat of the raw materials. The findings from the morphological examination of nano-calcium particles derived from fish bone are illustrated in Figure 2.

Particle size

Nano-calcium is a solid particle that has an average size of <1 µm or <1000 µm, consisting of either crystalline or amorphous particles (Uhlemann *et al*., 2021). The smallest particle size of nano-calcium identified through testing was 62 nm, achieved with a NaOH ratio of 1:4 and an extraction duration of 60 minutes, whereas the largest particle size recorded was 183 nm. The size of nano-calcium is smaller than that of fish bone meal. It falls within the category of nano-particles. According to Mohanraj and Chen (2006), nano-particles have a size of 10–1000 nm. Suptijah *et al*. (2012) indicate that nano-calcium consists of very tiny particles, roughly measuring 500×10^{-9}, which the body can absorb completely at a rate of 100% when ingested. The outcomes regarding the particle size of fish bone nano-calcium particles are illustrated in Figure 3.

Analysis of nanocalcium particle size shows that all nanocalcium fish bone produced in this study is smaller than 1000 nm. The size of a particle in a food component is closely associated to its bioavailability. Nano-calcium is smaller in size, allowing calcium to enter receptors more quickly and absorb cells. The dimension of the nano-calcium particles observed in the study, with a NaOH ratio of 1:4 and an extraction duration of 60 minutes, measured 62 nm, which is smaller than the size of the Nyla fish nano-calcium reported by Kusumawati *et al*. (2022), which was 87.37 nm. Meanwhile, the NaOH ratio of 1:5 with an extraction time of 90 minutes was 183 nm. Tuna fish particle size distribution in the research of Benjakul *et al*. (2017), produced particle sizes of 590–780 nm.

Bioavailability

The bioavailability of nano-calcium parang fishbone are shown in Table 2. Calcium solubility shows the rate and proportion of calcium available for use in metabolic processes compared to the calcium consumed. A product's high total calcium content does not necessarily guarantee a high amount of calcium absorbed by the body, so knowledge about bioavailability is crucial. The findings from the examination of different bioavailability levels of nano-calcium derived from fish bones indicated that prolonged absorption treatment significantly impacted (p<0.05) the enhancement of bioavailability of nano-calcium produced from fish bones. The research results showed that the bioavailability of the nano-calcium produced ranged from 3.68 to 9.33%. The bioavailability value of nano-calcium is higher compared to the bioavailability of tuna bone meal in Prinaldi (2018), such as 0.86 %, but lower than research by Boutinguiza *et al*.

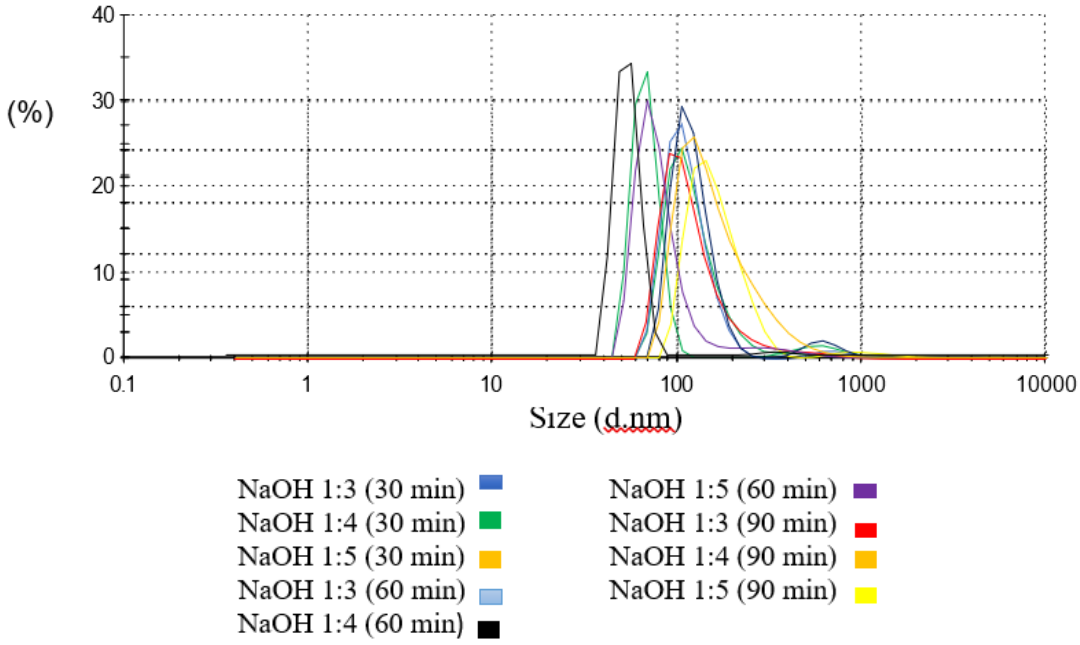

Figure 3. The particle size distribution of the nano-calcium powder

Tabel 2. Bioavailability nano-calcium (%)

Bioavailability	Time	absorption	(min)
	10	20	30
Control	0	0	0
Sample: NaOH 1:3 30 min	6.85±0.05^{b}	6.90±0.05^{b}	6.95±0.02^{b}
Sample: NaOH 1:3 60 min	6.51±0.02^{b}	6.61±0.06^{b}	6.86±0.09^{b}
Sample: NaOH 1:3 90 min	6.47±0.09^{b}	6.52±0.07^{b}	6.57±0.07^{b}
Sample: NaOH 1:4 30 min	8.07±0.02^{c}	8.28±0.01^{c}	8.31±0.01^{cd}
Sample: NaOH 1:4 60 min	9.10±0.01^{d}	9.22±0,02^{d}	9.34±0.01^{d}
Sample: NaOH 1:4 90 min	7.87±0.05^{bc}	7.89±0.05^{bc}	7.97±0.02^{bc}
Sample: NaOH 1:5 30 min	5.79±0.06^{a}	5.84±0.02^{a}	5.89±0.03^{a}
Sample: NaOH 1:5 60 min	5.68±0.06^{a}	5.78±0.05^{a}	5.81±0.08^{a}
Sample: NaOH 1:5 90 min	5.68±0.09^{a}	5.69±0.02^{a}	5.71±0.05^{a}

Note: Values are seen as mean ± SD (n = 3). Different superscript alphabets show significant differences determined by Duncan's test ($p < 0.05$)

(2012) 9.53% for fish bone nano-calcium. This is because the nano-sized particles in nano-calcium powder can increase calcium absorption. As noted by Erfanian *et al*. (2014), it is advisable to decrease particle size to enhance calcium absorption and its bioavailability. A reduction in particle size results in an increased surface area relative to its volume. As the surface area expands, the particles dissolve faster since they are only located on the outer layer of each particle, which boosts the rate of absorption.

In the digestive system, one of the things that determines calcium absorption is the size of the calcium particles. The smaller the calcium particle dimensions, the higher the absorption efficiency. The dimensions of the particles in the solution influenced calcium's solubility; smaller particles resulted in a more significant breakdown of the collagen matrix, increased the surface area of the particles interacting with the solution, and improved particle solubility. Based on the findings of Sumarto *et al*. (2021), the size of food particles is closely associated with their bioavailability. Therefore, it is essential to assess the particle size of the nano-calcium powder. The tiny size of nano-calcium enables rapid entry into mineral receptors, facilitating efficient cellular absorption.

CONCLUSION

The base solvent ratio and extraction time have an influence on the nano-calcium produced. As the NaOH solvent ratio increases with the extraction time, the water

content and calcium content increase. Protein content and fat content decrease due to hydrolysis. Meanwhile, the particle size will reach its smallest size with an optimal concentration ratio of sample to NaOH and an optimal extraction time. The optimum extraction time is achieved with NaOH at a concentration of 1:4, with a duration of 60 minutes, resulting in a calcium content of 35.46% and a particle size of 62 nm. The best bioavailability of fish bone nano-calcium is the treatment with an extraction time of 60 minutes with a sample:NaOH ratio of 1:4, namely, absorption times of 10 minutes, 20 minutes and 30 minutes have values of 9.10%, 9.22%, and 9.34 %. With its extremely small size, nano-calcium can quickly enter mineral receptors and be readily absorbed by cells.

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