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Characteristics of Nanocalcium Derived from Snakehead Fish (*Channa striata*) Bone Meal using Ultrasound-assisted Extraction

Herpandi Herpandi*, Agustina SP Manurung, Sabri Sudirman and Indah Widiastuti

Fisheries Product Technology, Faculty of Agriculture, Universitas Sriwijaya, Indonesia

***Correspondence to:**
Herpandi Herpandi
Fisheries Product Technology,
Faculty of Agriculture,
Universitas Sriwijaya,
Indonesia.
E-mail: herpandi@fp.unsri.ac.id

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Abstract

Snakehead fish (*Channa striata*) is commonly used as a raw material in traditional foods in South Sumatra. However, some parts of this fish, such as the bones, are not used during food processing. This study aimed to determine the physicochemical characteristics of nanocalcium derived from snakehead fish bones using different solvents. The solvents used were distilled water (dH_2O), hydrochloric acid (HCl), and natrium hydroxide (NaOH). The results showed that the smallest particle size distribution was produced by the HCl solvent (264.5 ± 29.5 nm). Different solvents significantly affected the whiteness, with the highest value of 86.79 ± 0.45% obtained from the NaOH solvent. In terms of chemical properties, the different solvents also significantly affected the moisture content (0.65 - 1.31%) and ash content (91.15 - 98.26%). The calcium (Ca) content ranged from 20.26 ± 0.95% - 21.38 ± 1.17% and the phosphorus (P) content ranged from 0.02 ± 0.01% - 0.13 ± 0.01%. The functional groups present in the snakehead fish bones included phosphate group (PO_4^{3-}), hydrogen phosphate group (HPO_4^{2-}), hydroxyl group (OH^-), and carbonate group (CO_3^{2-}). In this study, hydroxyapatite (HAp) was formed with A-type and B-type carbonate apatite. The different solvents used in the nanocalcium process significantly affect the yield, moisture content, ash content, and P content.

Keywords

Channa striata, Hydroxyapatite, Nanocalcium, Phosphate, Solvent extraction

Introduction

Over 70% of the total fish caught undergoes further processing before reaching the market. This processing-such as gutting, scaling, and filleting-varies by species due to differences in composition, size, shape, and intrinsic chemistry, and can result in substantial amounts (approximately 20 - 80%) of fish waste [1]. The waste primarily consists of muscle trimmings (15 - 20%), bones (9 - 15%), viscera (12 - 18%), skin and fins (1 - 3%), heads (9 - 12%), and scales (5%) [2]. This waste has not been properly utilized. As a result, this waste continues to increase alongside the development of the fishing industry and the rising levels of fish consumption in households [3]. These wastes can certainly be optimized as an alternative source of nutraceutical ingredients, including functional foods such as fish bone waste [4].

The consumption of freshwater fish in the community has increased, leading to growth in the fish farming industry and among them snakehead fish (*C. striata*) consumed commonly. Snakehead fish is the main ingredient in a traditional fishcake dish from South Sumatra, Indonesia, known as "pempek". Since pempek is typically made using the meat of the snakehead fish, other parts such as the bones are often discarded as waste. However, research on the potential use of fishbone waste generated during pempek production has not yet been conducted.

Fish bones are primarily composed of minerals and collagen [5, 6]. The key minerals are Ca and P, which are essential for the body, including bone and tooth formation, blood production, and the prevention of osteoporosis [7, 8]. Thus, fish bones can be utilized as a source of nutraceutical raw materials. The Ca (12.8%) and P (6.4%) contents in snakehead fish bones are relatively high, making them a potential source of minerals [9]. A previous study also reported that snakehead fish bone contains more Ca (12.78%) compared to mackerel bone (6.6%) [10]. However, Ca is generally available in micro form, which is not optimally absorbed by the body's metabolism. Therefore, to ensure that Ca products are easily absorbed and utilized by the human body, Ca needs to be converted into nanocalcium [11].

Fish bones are also known to contain HAp, which can be utilized as a biomaterial in various biomedical applications [12-14]. HA is primarily composed of Ca and P, with a Ca/P molar ratio of 1.67. Therefore, HA can be synthesized by extracting Ca and phosphate precursors from fish bones using precipitation and sol-gel methods [15]. This study focuses on the extraction of Ca and P minerals from snakehead fish bones as HA precursors, which are then used as raw materials for nanocalcium production. Previous studies reported that nanocalcium exhibits several benefits and can be applied in agriculture and human use, such as in health supplements and wound healing [16, 17].

In the extraction process, several factors influence the outcome, such as the type of solvent, pH, temperature, and extraction time [18, 19]. In this study, ultrasound-assisted extraction was applied during the process. A previous study reported that ultrasound-assisted extraction typically increases the yield of Ca from fish bones compared to traditional extraction methods [20]. Besides increasing the extraction efficiency, the ultrasound technique also has some drawbacks, such as localized heat generated by cavitation, optimization of extraction parameters, and equipment cost issues [21, 22]. Previous studies have reported that Ca and P from fish bones can be extracted using acidic, saline, deep eutectic, and alkaline solvents [23-25]. A previous study reported that nanocalcium powder from fish bone was prepared using HCl and NaOH [26]. Additionally, different solvents (HCl and NaOH) resulted in varying mineral compositions of Ca hydroxide ($Ca(OH)_2$) [27]. Based on these findings, the type of solvent affects the mineral content obtained during the extraction process, including Ca and P. Therefore, we hypothesized that the type of solvent also influences the preparation of nanocalcium from snakehead fish bone meal. Thus, this study aimed to determine the physical characteristics (whiteness and particle size) and chemical characteristics (Ca, P, functional groups, and proximate composition) of nanocalcium extracted from snakehead fish (*C. striata*) bone meal using ultrasound-assisted extraction with different solvents, such as NaOH, HCl, and water.

Materials and Methods

Production of snakehead fish bone meal

The production of snakehead fish bone meal was carried out based on previous methods [28, 29]. Fish bones were thoroughly washed and boiled at 80 °C for 30 min. The boiled fish bones were then cleaned and boiled again for 3 h. Afterward, the fish bones were drained at room temperature for 15 min and dried in an oven (Memmert Universal Oven UN55, Germany) at 80 °C for 12 h. Finally, the dried fish bones were ground into a powder (80 mesh).

Production of nanocalcium

The production process of snakehead fish bone meal nanocalcium was carried out based on previous studies [28, 30]. Briefly, 10 g of fish bone meal was extracted using three types of solvents: dH_2O, HCl, and NaOH. The bone meal was extracted using an ultrasonic bath at 50 °C and 45 kHz for 45 min. The extraction results were then cooled, filtered, and neutralized (for HCl or NaOH solvents). The samples were dried in an oven at 105 °C for 3 h and then calcined in a muffle furnace (Thermo Scientific FB1410M-33, USA) at 600 °C for 5 h. The extracts were then weighed, and the yield was calculated [31].

Whiteness, particle size, and proximate analysis

The whiteness of the snakehead fish bone meal nanocalcium was measured using a chromameter (Chromameter CR-400/410) [32]. Particle size was determined using a particle size analyzer (PSA) (Beckman Coulter Delsa™ Nano) [33]. Proximate analysis, which includes moisture and ash contents, was performed according to the AOAC method [34].

Ca, P, and function group analysis

The Ca and P contents in snakehead fish bone meal nanocalcium were determined using Atomic Absorption Spectrophotometry (AAS, AA-7000 Shimadzu) [35] and the functional group analysis of nanocalcium was conducted using Fourier-transform infrared (FTIR, Bruker Tensor 27) [36].

Data analysis

The data on yield, moisture, ash, Ca, and P contents were expressed as the mean ± standard error of the mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test ($p < 0.05$) with SPSS software (ver. 22.0; IBM Corporation, USA). Functional groups and particle size were analyzed using descriptive analysis.

Results and Discussion

Yield of nanocalcium from snakehead fish bone meal

The yield of snakehead fish bone meal nanocalcium is shown in figure 1. The extract yield represents the percentage of the main raw material (fish bone meal) that is processed into the final product (nanocalcium). The yield from the dH_2O solvent (20.77 ± 1.31%) was significantly higher ($p < 0.05$) compared to the NaOH (5.54 ± 2.33%) and HCl (4.75 ± 2.90%) solvents. A previous study reported that the yield of nanocalcium from yellowfin tuna fish bone ranged from 22.25% - 30.10% [37]. The yield of nanocalcium from fish bones can vary depending on the fish species [26, 38]. A previous study reported that the use of dH_2O can reduce organic compounds in small amounts compared to acidic and

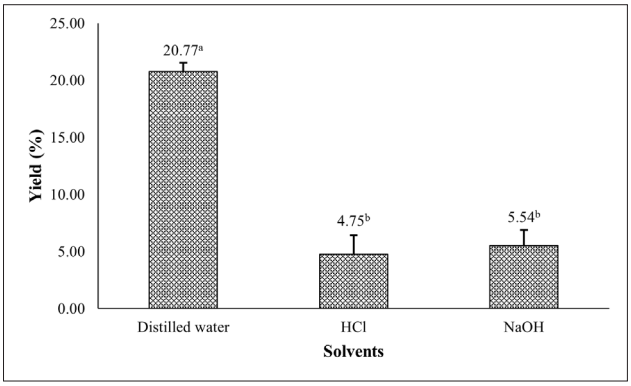

Figure 1: Yield of nanocalcium from snakehead fish (*C. striata*) bone nanocalcium. Data are expressed as mean ± SEM (n = 3). Different superscript letters on the bars indicate significant differences ($p < 0.05$).

basic solvents [37]. Nanocalcium yields decrease when soaked in acidic or alkaline solutions because bone constituent components, such as minerals and organic compounds, are more easily hydrolyzed. Additionally, a previous study has reported lower yields in the manufacture of fishmeal with pH > 7 or pH < 7, compared to pH = 7. This is due to the rapid denaturation and demineralization processes that occur, resulting in a lower yield compared to water [39].

In this study, ultrasound-assisted extraction was also applied during the process. A previous study reported that ultrasound-assisted extraction typically increases the yield of Ca from fish bones compared to traditional extraction methods [20]. Ultrasound induces the formation of cavitation bubbles on the material's surface, which generate microjets, shear stress, and turbulence. These phenomena result in surface delamination, erosion, and fragmentation of the material [40]. As the surface area increases, mass transfer is enhanced and promotes the release of cellular content. This improved accessibility allows solvents to better interact with the substrate, accelerating biochemical reactions and, in turn, increasing extraction efficiency and yield [41, 42].

Whiteness

The average whiteness of snakehead fish bone meal nanocalcium ranged from 82.55 ± 0.16% - 86.79 ± 0.45% is shown in figure 2. The three types of solvents used had no significant ($p > 0.05$) effects on the degree of whiteness of nanocalcium. The whiteness value was found to be highest in the NaOH solvent (86.79 ± 0.45%), although this value is smaller than the whiteness value of commercial Ca carbonate ($CaCO_3$) powder, which is ≥ 92% [37]. The presence of organic compounds in fish bones gives the product a less white color. The use of alkaline solvents can hydrolyze these organic compounds, producing Ca that settles on the bone matrix and results in a whiter color. In contrast, when an acidic solution is used in the soaking process, the pH of the solution affects the color stability [43]. Acid can dissolve color pigments in the material, causing the brightness of the color pigments to decrease with increasing concentration and extraction time. Additionally, the concentration of NaOH used also affects the degree of whiteness [44].

Particle size

The PSA results for snakehead fish bone meal nanocalcium are shown in figure 3. Different extraction solvents resulted in differences in the particle size of snakehead fish bone meal nanocalcium. The HCl solvent produced the smallest particle size (264.5 ± 29.5 nm), compared to NaOH (502.5 ± 50.9 nm) and dH_2O (715.8 ± 163.8 nm). This result can be attributed to the strong acidic nature of HCl, which more effectively breaks down the bone matrix and removes non-Ca components, such as proteins and fats. This process facilitates greater Ca release and results in finer particle dispersion. Furthermore, the production of nanocalcium powder typically involves demineralization with HCl, which aids in the removal of compounds bound to Ca, thereby improving its solubility [45]. Minerals can be extracted using various acidic solutions, including HCl, sulfuric acid, and lactic acid [46]. A previous study reported that the smallest nanocalcium particle size from acid solvents was 259 nm [37]. Another study also reported that the particle size of nanocalcium from catfish ranged from 339.30 nm - 464.73 nm [9]. Additionally, the particle size of nanocalcium prepared from tuna species ranged from 650 nm - 38,780 nm [47]. A product can be categorized as nano-sized when the particle size is between 10 nm and 1,000 nm [37, 48]. Smaller particle sizes typically exhibit better absorption when the product enters the body. In PSA measurements, particles are measured in a liquid medium dispersed in a dispersant, and these conditions greatly affect the measurement process. Ad-

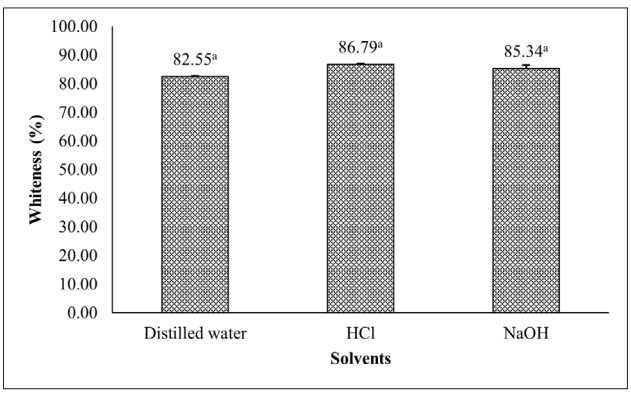

Figure 2: Whiteness value of snakehead fish (*C. striata*) bone nanocalcium. Data are expressed as mean ± SEM (n = 3). Different superscript letters on the bars indicate significant differences ($p < 0.05$).

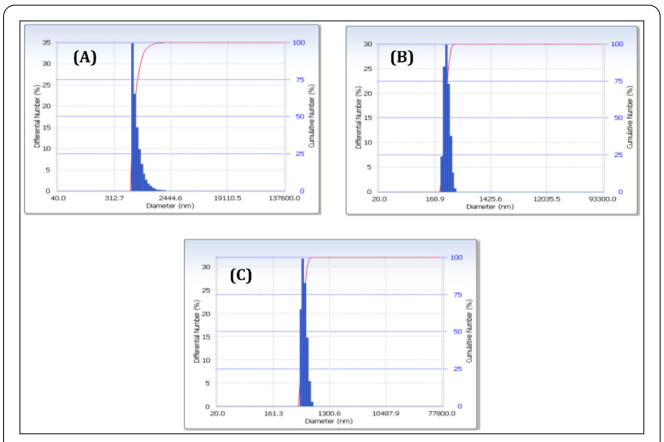

Figure 3: The particle size of snakehead fish (*C. striata*) bone nanocalcium based on the solvents: **(A)** dH_2O, **(B)** HCl, and **(C)** NaOH.

ditionally, an imperfect drying process can affect the nanocalcium, resulting in incomplete dispersion in the dispersant and leading to inconsistent particle size results, with larger sizes observed in PSA analysis [11].

Moisture and ash contents

The moisture and ash content of the snakehead fish bone meal nanocalcium are shown in figure 4. The dH_2O solvent had a significantly ($p < 0.05$) higher water content value (1.31%) compared to NaOH (0.65%) and HCl (0.67%). During the extraction process, water evaporation occurs in the filtrate, which results in free water in the sample. The decrease in water content is also due to the hydrolysis of protein in fish bone meal when mixed with acidic and basic solvents, and the heating process leads to a reduction in water bonds. A previous study reported that the low water content in tuna bone and clamshell powder was caused by protein hydrolysis during heating in the manufacture of nanocalcium powder [49].

The ash content in the NaOH solvent (98.26%) was significantly ($p < 0.05$) higher compared to the dH_2O solvent (91.15%), while no significant difference was observed in the HCl solvent (97.78%). This indicates that acid and alkaline treatments significantly affect the increase in ash content of nanocalcium. Ash content is directly proportional to the mineral content in the product [50]. A previous study reported that microcalcium from catfish bone, which was prepared using HCl and NaOH ranged from 98.25% - 98.40% [51].

Ca and P contents

The Ca and P levels of snakehead fish bone meal nanocalcium are shown in figure 5. The type of solvent used did not significantly affect the Ca levels, which ranged from 20.79% - 21.38%. A previous study reported that the Ca content of catfish bone ranged from 21% - 25% [52]. Additionally, the Ca content of nanocalcium from various fish bones ranged from 20.49% - 23.24% [26].

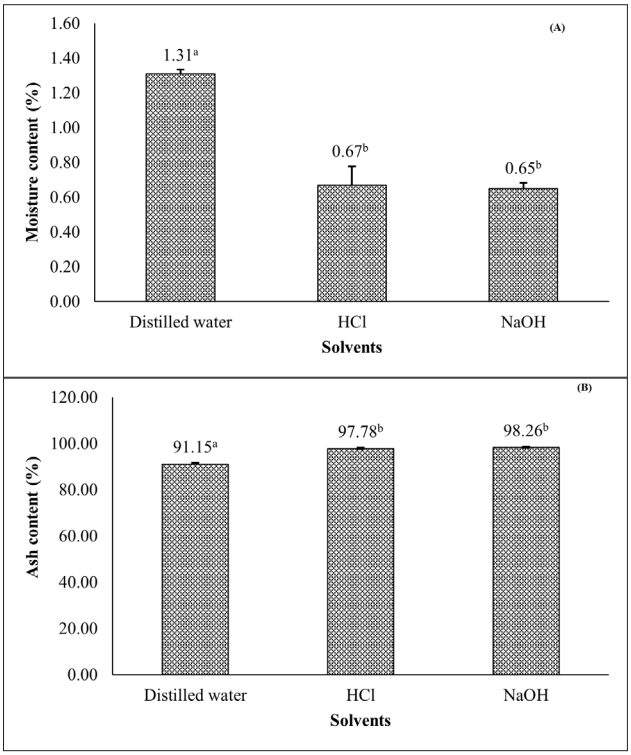

Figure 4: The **(A)** moisture and **(B)** ash contents of nanocalcium of snakehead fish (*C. striata*) bone nanocalcium. Data are shown as mean ± SEM (n = 3). Different superscript letters on the bars indicate significant differences ($p < 0.05$).

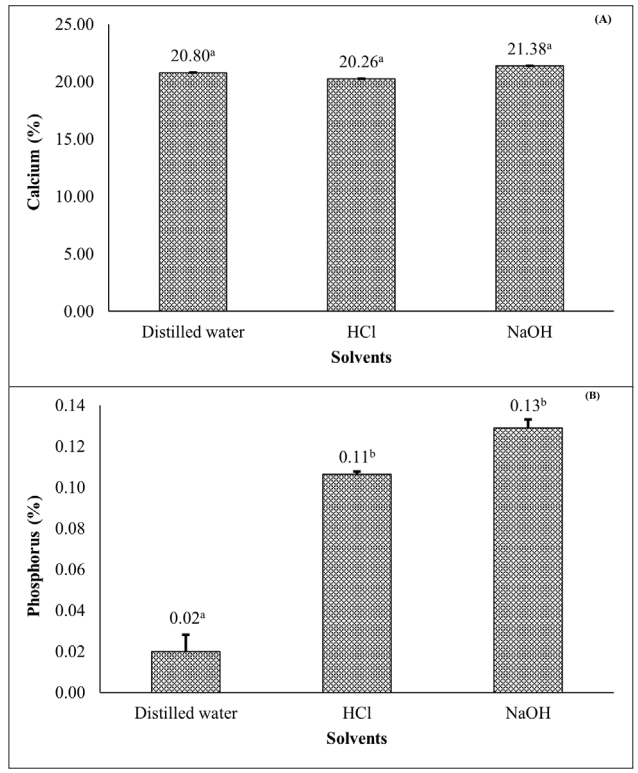

Figure 5: The **(A)** calcium and **(B)** phosphor contents of nanocalcium of snakehead fish (*C. striata*) bone. Data are shown as mean ± SEM (n = 3). Different superscript letters on the bars indicate significant differences ($p < 0.05$).

The type of solvent used had a significant ($p < 0.05$) effect on the resulting P levels, with the highest level in the NaOH solvent (0.13%) and the lowest in dH_2O (0.02%), while the HCl solvent had a P level of 0.11%. A previous study reported that the P content of catfish bone was about 10% - 11% [52]. The level of P obtained in snakehead fish bone meal nanocalcium is inversely proportional to the level of Ca. The P content of nanocalcium is lower than that of Ca [53].

Ca and P are closely related to metabolism and function. The regulation of Ca and phosphate balance is crucial for proper cellular activity, metabolic processes, and overall organ function. Low P levels are better digested by the body than high P levels, as high P can inhibit the Ca absorption process [54, 55]. A good Ca-to-P ratio of about 1:1 - 3:1 ensures that the Ca-to-P absorption process is more effective [56, 57]. The presence of Ca and P in fish bone meal nanocalcium depends on the fish species and processing methods, including the solvent used [9, 25, 58].

Functional groups of nanocalcium

The FTIR spectra of snakehead fish bone meal nanocalcium is shown in figure 6 and table 1. The FTIR spectra was analyzed to determine the functional groups present in the nanocalcium based on previous study by Prinaldi et al. [37].

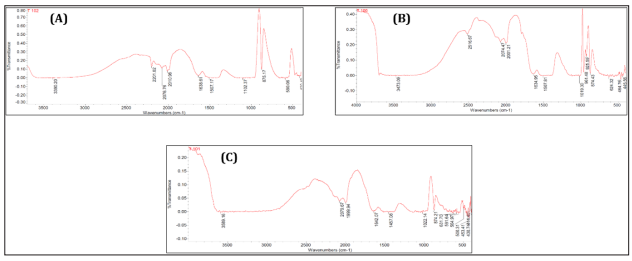

Figure 6: The FTIR spectra of nanocalcium of snakehead fish (*C. striata*) bone nanocalcium with different solvents: **(A)** dH_2O, **(B)** HCl, and **(C)** NaOH.

Table 1: The FTIR peaks of nanocalcium of snakehead fish (*C. striata*) bone.

Functional groups	Solvents		
	dH_2O (cm^{-1})	HCl (cm^{-1})	NaOH (cm^{-1})
v_1 PO_4^{3-}	-	961.48	-
v_2 PO_4^{3-}	420.40	464.76	414.40
v_3 PO_4^{3-}	1102.37	1019.30	1022.14
v_4 $PO4^{3-}$	590.06	-	564.97
A-type	1507.17	1507.81	-
B-type; HPO_4^{2-}	875.17	874.43	874.21; 1457.08
OH^-	3390.20	3473.09	3589.16

The FTIR analysis detected the presence of OH^-, CO_3^{2-}, PO_4^{3-}, and HPO_4^{2-} in the nanocalcium. The functional groups in nanocalcium are part of the HAp compound, with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ [59, 60]. In HAp compounds, there is an inorganic component found in bones called carbonate apatite [61]. In the present study, the symmetric stretching of PO_4^{3-} (v_1 PO_4^{3-}) was detected only in HCl, and the asymmetric bending of PO_4^{3-} (v_4 PO_4^{3-}) was detected in NaOH and dH_2O. Meanwhile, the symmetric bending (v_2 PO_4^{3-}) and asymmetric stretching (v_3 PO_4^{3-}) of PO_4^{3-} were detected in all solvents. Carbonate apatite is divided into two types: type A carbonate apatite (A-type carbonate) and type B carbonate apatite (B-type carbonate). The A-type carbonate apatite is formed when CO_3^{2-} substitutes for OH^-, while the B-type carbonate apatite is formed when carbonate replaces PO_4^{3-} [62, 63]. In the present study, A-type carbonate was detected in HCl and dH_2O, whereas B-type carbonate was detected in all solvents.

Overall, nanocalcium is successfully extracted from snakehead fishbone using different solvents such as NaOH, HCl, and water. The snakehead fish nanocalcium contains Ca and P. Previous studies reported that Ca and P from fish bones can be extracted using acidic (HCl), saline, deep eutectic, and alkaline (NaOH) solvents [23, 26]. Nanocalcium is a material with nanoscale dimensions [64]. Due to its small particle size and high surface area, nanocalcium offers several potential applications in human health, including bone health, dental care, and drug delivery [65]. It can also be used as a raw material for nutraceutical products. Additionally, nanocalcium is being explored for its potential in promoting tissue regeneration and as a hemostatic agent [66, 67].

Conclusion

Nanocalcium extracted from the fishbone of snakehead fish (*C. striata*) using an acidic solvent (HCl) exhibits better properties than that extracted using an alkaline solvent (NaOH) or dH_2O. Extraction of nanocalcium with HCl produces the smallest particle size, measuring 264.5 ± 29.5 nm. The type of solvent used in the nanocalcium extraction process significantly affects the yield, moisture content, ash content, and P content. FTIR analysis shows that A-type carbonate apatite is present in samples treated with neutral and acidic solvents. Snakehead fish nanocalcium shows significant promise in various biomedical applications due to its nanoscale properties. Future research should focus on optimizing its synthesis methods, enhancing its biocompatibility, and evaluating its safety through both in vitro and in vivo studies.

Acknowledgements

None.

Conflict of Interest

None.

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