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Extraction and Characterization of Biogenic Nano-Calcium Derived from *Daysciena albida* and *Otolithes ruber*: Potential Applications

Running head: Extraction and Characterization of Nano-calcium from *Daysciena albida* and *Otolithes ruber*

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SUMMARY

Research background. In India, widespread dietary deficiencies in calcium and vitamin D present a significant public health concern. Over the past five decades, evidence suggests declining dietary calcium intake across rural, tribal, and urban populations. This prolonged deficiency poses serious risks to bone health, contributing to the development of rickets, osteoporosis, and osteopenia, as well as potential disruptions in metabolic rates and physiological functions. A key factor in this decline appears to be the reduced consumption of calcium-rich dairy products. As a result, research is exploring alternative, highly bioavailable calcium sources, such as those derived from fish bone waste. Additionally, the potential of nano calcium supplements to enhance absorption and improve bone density, compared to traditional supplements, is an area of active investigation.

Experimental approach. Nano calcium powder was synthesized from the bones of two commercially available fish species *Daysciena albida* (DNC) and *Otolithes ruber* (ONC), ethically sourced from the Kerala coast, following relevant regulations. The alkali extraction method was

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employed and the resulting nano-calcium powder was characterized using various physiological and chemical analyses.

Results and conclusions. The production process yielded differently for both samples. Notably, both samples exhibited distinct characteristics in color, proximate composition, and SEM-EDX analysis. DNC additionally contains slightly more calcium and phosphorus than ONC. The DNC nanoparticles (153.8 nm) were also smaller than the ONC nanoparticles (337.1 nm). Interestingly, further analysis using techniques like FTIR, Zeta potential, TGA, and XRD revealed significant similarity between the DNC and ONC samples, despite the initial differences in yield, composition, and particle size. This finding suggests that while the choice of fish species significantly influences the yield, composition, and characteristics of the synthesized nano-calcium powder, DNC appears to be a more favorable source, both types may exhibit similar functionality and warrant further investigation.

Novelty and scientific contribution. This is the first report on extracting and characterizing biogenic nano-calcium from two commercial fishes, *Daysciena albida* and *Otolithes ruber* of the Malabar coast. The extracted nano-calcium powders from these two fishes will provide a good source of calcium and help overcome calcium-related disorders.

Keywords: fish bones; biogenic nano-calcium; particle size analysis; zeta potential; XRD analysis

INTRODUCTION

Calcium is the most abundant mineral found in the bones and teeth of the body (*1*). During the growth phase of humans and animals, bones are formed and remodeled with constant absorption and deposition of calcium. The Institute of Medicine states that the daily calcium requirement varies from 600 to 1300 mg/day based on age and gender (*2*). The Indian Council of Medical Research recommends a daily calcium intake of 600 mg/day for both genders (*3*). The typical daily calcium consumption ranges from 400 to 500 mg/day in South Asia and India, while it is between 900 and 1000 mg/day in the USA and Northern Europe (*4*,*5*). Calcium plays a crucial role in maintaining the proper functionality of both circulatory and neuromuscular systems, which acts as a cofactor for several hormones and enzymes also exerting influence on the immunological system. However, inadequate calcium in the body can cause disrupted bone growth, osteoporosis, and osteomalacia (*6,7*). Despite health hazards, Asians consume a negligibly low amount of calcium <500 mg/day (*8*).

Calcium deficiency and metabolic bone diseases are prominent co-morbidity in celiac disease, with around three-quarters of newly diagnosed patients exhibiting reduced bone mineral density. As

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a result, osteopenia and osteoporosis can be telltale signs of atypical celiac disease presentation (*9*). To rectify this, calcium should be supplemented for celiac patients. However, the exorbitant treatments and calcium supplements taken for prolonged periods like calcium sulfate (gypsum) and calcium carbonate (limestone) compounds can cause side effects like breast cancer, heart problems, *etc*. (*10*). To reduce this, the usage of natural sources of calcium is an effective way. Lactose intolerance, a condition stemming from inadequate production of the enzyme lactase, can hinder the digestion of lactose present in dairy products, leading to potential side effects such as diarrhoea, flatulence, abdominal cramps, abdominal distention, and increased bowel movements in affected individuals (*11*). As a solution, calcium is extracted from by-products of food processing such as eggshells and fish bones (*12*). Meanwhile, cow and pig bone-derived calcium was frequently linked to health issues and religious restrictions (*13*). However, calcium from seafood is considered a promising supplement for a healthy diet (*12*).

Fish bones, commonly considered waste in the seafood industry, contain high calcium and phosphorous levels (*14*). Fish bones contain valuable minerals like calcium phosphate, creatine phosphate, and hydroxyapatite ($Ca_{10}(OH)_2(PO4)_6$), which are crystalline structures attached to collagen fibres (*15*). Fishbone contains nearly 60-70 % mineral of its total mass (*16*) and 34–36 % calcium (*17*), making it a promising source of calcium supplements for humans. Transforming fish bone waste into bioactive materials offers a novel and sustainable method of production in materials science (*18*). One of the significant demersal finfish resources that are exploited along the coast of Kerala is the sciaenids which contribute 6.9 % of the demersal landings (*19*,*20*). Thus, the present study aims to produce and characterize nano-calcium from two commercial fishes: *Daysciena albida* (two-bearded croaker) and *Otolithes ruber* (tiger-toothed croaker) of the Kerala coast.

MATERIALS AND METHODS

Chemicals

NaOH was procured from HiMedia (Maharastra, India) and HCl was procured from Emplura grade (Ahmedabad, India). Petroleum benzene, boric acid, sulphuric acid, copper sulphate, potassium sulphate, methyl orange, and nitric acid were procured from HiMedia (Maharastra, India).

Extraction of nano-calcium from Daysciena albida and Otolithes ruber

Fishes were purchased from a local fish market (Chambakkara, Kochi, India). Fishes were cleaned and filleted and the bones were separated from the meat. Fish bones were stored in a freezer until use. The production process of nano-calcium powder is illustrated in Fig. S1. Frozen fish bone

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was thoroughly washed and boiled for an hour (*21*). Adhering meat pieces along with the bones are separated and dried in a hot air oven (Kemi, Model-KOS-6, Kerala, India) at 50 ºC for 24 h. The fishbone was autoclaved (Equitron, Model-7431 SLEFA, Mumbai, India) at 121 ºC for 3 h followed by drying in a hot air oven at 50 ºC for another 24 h. The fishbone was smashed in a mortar and pestle and ground in a blender for 1 min. The obtained powder was referred to as coarse calcium powder and the yield (%) was computed.

HCl (1 N) was added to the obtained powder (1:5 *m/V*) (*15*) and stirred for 1 h (Kemi, Model-KMS-350, Kerala, India) followed by incubation (Binder, Model-CBS 260, Hongkong, Japan) for 24 h at room temperature. The solution was centrifuged (ThermoFisher Scientific, Model-Sorvall™ ST 16, Deutschland, Germany) at 1,711×*g* for 15 min to remove HCl from the coarse bone meal. The collected supernatant was mixed with NaOH in the ratio of 1:5 (*m/V*) heated at 100 ºC for 60 min and centrifuged. The process was repeated thrice. After the process, the supernatant was collected and adjusted to neutral pH (OAKTON, Model-pH 550 benchtop, West Bengal, India) using HCl (1N) and then centrifuged. Subsequently, the sediment was transferred to a ceramic tray and dried in a hot air oven at 50 ºC for 15–18 h. After drying, the sediment underwent a refining process for 45 min in a ball miller (Retsch GmbH, Model-PM 100, Haan, Germany) and was sieved through a 50 µm-mesh sieve. The obtained powder from *Daysciena albida* and *Otolithes ruber* were referred to as *Daysciena* nano-calcium (DNC) and *Otholithes* nano-calcium (ONC), respectively. The resultant yield was computed.

Yield percentage analysis

The yield percentage analysis of the extracted nano-calcium powders was calculated using the following formula (*12*):

$$\text{Yield} = \left(\frac{\text{Net mass of nanocalcium}}{\text{Net mass of dried bone}}\right) \cdot 100 \qquad /1/$$

Color analysis

*L** (lightness), *a** (redness/greenness), and *b** (yellowness/blueness) color values of the extracted nano-calcium powder were assessed using a colorimeter (HunterLab, Model-MiniScan EZ 4000, Virginia, USA). To measure the total color difference (∆*E**), the following equation was computed:

$$\Delta E^* = 100 - \sqrt{(\Delta L *)2 + (\Delta a *)2 + (\Delta b *)2} \quad /2/$$

Where ∆*L**, ∆*a**, and ∆*b** are the differences between the corresponding sample as compared to the white standard (*L**=93.63, *a**=−0.94, and *b**=0.40).

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Particle size analysis

The particle size of different samples was measured as described by Yin *et al*. (*16*) with slight modifications. The sample (50 mg) was dissolved in 50 mL ultrapure water and adjusted to pH 2.0 using 1 N HCl to facilitate particle disaggregation. The mixture was homogenized (Ika, Model-Ultra-Turrax 50T, Staufeu, Germany) at 1,000 rpm for 15 min. Subsequently, the particle size distribution of the powder samples was measured using a laser diffraction technique by a Particle Size and Zeta Potential Analyzer (Horiba, Model-nanoPartica SZ-100V2, Kyoto, Japan).

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX) analysis

EDX spectrum and elemental mapping were evaluated using a Scanning Electron Microscope attached to an energy-dispersive X-ray spectroscope (EDX) detector (Joel, Model-JSM-5400, Tokyo, Japan) at 15 kV as outlined by *Benjakul et al*. (*17*) with a slight modification.

Proximate composition analysis

Proximate analysis of moisture (AOAC 950.46), protein (AOAC 920.153), fat (AOAC 960.39), and ash (AOAC 928.08) content of nano-calcium powder was carried out following the method of AOAC (*22*).

Inductively coupled plasma–mass spectrometry (ICPMS) analysis

The determination of major and trace minerals such as Na, K, Mg, P, Ca, Fe, Cu, and Zn from the obtained nano-calcium powders was carried out with the use of an Inductively Coupled Plasma-Mass Spectrometer (Thermo Fisher Scientific, Model-iCAP RQ, Massachusetts, USA) with Helium KED (Kinetic Energy Discrimination) mode following the method of Leme *et al*. (*23*) with slight modification.

Fourier transform infrared spectroscopy (FTIR) analysis

The functional groups of obtained nano-calcium powders were examined using a Fourier Transmission Infra-Red Spectrophotometer (Perkin Elmer, Model-FT9700, Massachusetts, USA) with a scan range spanning from 400 to 4000 cm^{-1}. The resolution was set at 0.2 cm^{-1} and each sample underwent 64 scans. The preparation of all samples was carried out using the KBr pellets method (*24*).

Zeta potential analysis

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Nano-calcium powders were diluted to 0.1/100 g using distilled water and the pH value was adjusted to 7.0 using 1 N HCL and NaOH (*16*). The Zeta potential of the nano-calcium powders was determined using a Nano Particle Analyzer (Horiba, Model-SZ-100, Kyoto, Japan) equipped with a DPSS Laser at 532 nm as the light source.

X-ray diffraction (XRD) analysis

Microstructural features of obtained nano-calcium samples were analyzed using an X-ray diffractometer (Bruker, Model-D8 Advance A25, Karlsruhe, Germany). The sample was spread over a low-background sample holder (amorphous silica holder) and secured on the sample stage within the goniometer. The instrument was configured with B-B geometry and the material was examined at an angle of 2θ=8–80°. The XRD pattern was recorded with a current and voltage set at 40 mA and 40 kV, respectively. The percentage of crystallinity was calculated using the following equation:

$$\text{Crystallinity} = \left(\frac{A(peak)}{A\,(total)}\right) \cdot 100 \quad /3/$$

Thermogravimetric analysis (TGA)

The thermal properties of the extracted nano-calcium powders were analyzed with the aid of a thermogravimetric analyzer (Hitachi, Model-STA 7300, Tokyo, Japan). A small portion of the sample was weighed in an aluminium pan and sealed. The scanning was spanning from 300 ºC up to 800 ºC at a heating rate of 10 ºC/min in a nitrogen atmosphere.

Statistical analysis

Every experiment was conducted in triplicate and IBM's Statistical Package for the Social Sciences (SPSS) version 26.0 software program (IBM, Armonk, New York, USA) was used to statistically analyze the data (*25*). The findings were presented in the form of mean value±standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to estimate the significant differences in means among the different treatments. A significance threshold of $p<0.05$ was applied.

RESULTS AND DISCUSSION

Yield percentage of NC powders

Yield percentage of the product is of significant economic importance. The yield percentage of the extracted nano-calcium varied between DNC (40.97±1.05 %) and ONC (28.00±1.5 %) samples. DNC exhibited significantly higher yield compared to ONC ($p<0.05$). This was plausibly due to the

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smaller and thinner bone structures of OCB, while DCB had larger, denser, and thicker bone particles. This variation in yield may be attributed to the higher fat content present in tiger-toothed croaker bones, which led to saponification between fat and alkali during the alkaline extraction process and subsequently increased nano-calcium powder loss (*12*). A longer heating period reduced the yield of nano-calcium powders by causing a huge loss of solid fraction from the bones. Thus, the amount of nano-calcium produced was mostly influenced by the heating method (*26*). However, nano-calcium powder extracted from starry trigger (*Abalistes stellaris*) and red snapper (*Lutjanus malabaricus*) fishbone (*27*,*28*) showed a yield of 7.55–12.94 % and 7.8 % which was lower than the obtained nanocalcium powders. Moreover, *Zainol et al*. (*29*) achieved a relatively higher yield of 68 % from tilapia fish scales by the alkali extraction method. Nano-calcium powders extracted from six commercial species exhibited a yield of 20–50 % (*12*) and *Kastuwonus pelamis* observed a yield of 40 % (*6*), closely related to the obtained nanocalcium powders. Thus, the extraction process and species affected the yield percentage.

Color of NC powders

The highest *L**- value is preferred for the nano-calcium powder (Fig. S2), resembling hydroxyapatite crystal products (*29*). *L**, *a**, *b*,* and Δ*E** of DNC and ONC are represented in Table 1. From Table 1, the nano-calcium powder from brackish water fish (DNC) exhibits a significantly brighter color when compared to marine fish (ONC) ($p<0.05$). This was mostly due to the amount of fat content of OCB, which was higher than DCB (Table 1). The higher the fat content, contributed to a slightly darker hue in its nano-calcium powder (ONC) ($p<0.05$). The presence of organic materials, particularly protein and fat content, in fish bones plays a crucial role in influencing the color of nano-calcium powder, resulting in a deeper tint (*17*). The color values obtained for nano calcium powders from six commercial species, as reported by *Kusumavati et al*. (*12*), align with the findings of this study. In contrast, the *L** value was higher than in the previous reports (*17*,*30*). Amos *et al.* (*31*) highlight that the most critical factors influencing the quality of fishery products are time and temperature tolerance.

Fig. S2

Proximate composition of NC powders

Chemical composition comparisons between coarse fish bone and nano-calcium powder are detailed in Table 1. Both types can maintain a moisture content of 0.5–0.7 %. The increased ash content in nano-calcium powder, compared to coarse bone powder, suggests that alkali-based chemical extraction results in lower protein and fat content ($p<0.05$). Chemical composition

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comparisons across freshwater (*Daysciena albida*) and seawater (*Otolithes ruber*) fish did not reveal significant differences. DNC and ONC exhibited ash content of 75 and 63.78 %, lower than nano-calcium from *Katsuwonus pelamis*, Indonesian commercial fish bone wastes, *Lutjanus malabaricus,* and *Channa striata* bones, which reported ash content of 85.72, 76.15–86.76, 87.08, and 98.09–99.04 %, respectively (*6*,*12*,*28*,*32*). The higher ash content corresponds to a negligible amount of organic matter, such as fat and protein. DNC and ONC had protein content of 4.72 and 6.86 % and fat content of 1.66 and 8.87 %, respectively, consistent with the study by Kusumavati *et al*. (*12*). However, the previous works had shown that the protein and fat content of fishbone nano-calcium powder varied, depending on pre-treatment and fish species (*33*-*35*).

Table 1

Particle size of NC powders

Nano-calcium powders derived from the two fish species exhibited a particle size of less than 600 nm, with an average particle size of 153.8 nm for DNC and 337.1 nm for ONC. This aligns with the outcomes reported by Zhang *et al*. (*36*) and *Kusumavathi et al*. (*12*), in which the particle size falls within the range of 100-120 nm and 87.37-281.4 nm respectively. In contrast, Anggraeni *et al*. (*28*), Gnanasekaran *et al*. (*37*), Rashad *et al*. (*38*), Ho *et al*. (*39*), *Anggraeni* (*21*), *Prinaldi et al*. (*30*), and Benjakul *et al*. (*33*) reported varying particle size distributions of 440.41nm, 50–80nm, 250–2500 nm, 39.42 nm, 500 nm, 259–397 nm, and 590–38,780 nm, respectively. The nano-size, being smaller than the microscopic size, facilitates quicker entry into mineral receptors and uptake by cells, as noted by Sumarto *et al*. (*40*). Smaller particle sizes are associated with greater degradation of the collagen matrix and an increased surface area, leading to higher particle solubility when introduced into a solution.

SEM-EDX morphology and elemental composition of NC powders

The SEM-EDX images of both DNC and ONC are depicted in Fig. 1. SEM-EDX scans reveal that nano-calcium powders from all fish species tended to agglomerate in the dry state, displaying an asymmetrical shape with a size ranging from 100 to 600 nm. The SEM-EDX analysis of elemental composition showed significant amounts of O and C elements in addition to Ca and P elements, along with other trace elements such as Na, Cl, Mg, Fe, and Zn. The mass percentage of Ca varied between 12.25 and 2.62 % for DNC and ONC, respectively. DNC and ONC had phosphorus content of 8.31 and 1.59 % respectively. Interestingly, the Ca/P mole ratio of the DNC and ONC were 1.47 and 1.64 respectively, which differed from the stoichiometric value of hydroxyapatite (HAP) of 1.67 and referred to as calcium-deficient hydroxyapatite (CDHA) by *Yusuf et al*. (*41*). The Ca/P mole ratio of tiger-

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toothed nano-calcium (1.64) was closest to the stoichiometric value of hydroxyapatite. Similar findings were reported by *Benjakul et al*. (*17*) and *Kusumavati et al*. (*12*), with Ca/P ratios ranging from 1.62-1.65 and 1.41–1.57, respectively. In contrast, Wijayanti *et al*. (*35*) reported that the Ca/P mole ratio of Asian seabass bio-calcium powder ranged from 1.29–1.31 which was lower than the current results. However, Rashad *et al.* (*38*) and Ho *et al.* (*39*) reported that the Ca/P ratio of Egyptian Nile tilapia and *Lates calcarifer* bone were 2.25 and 1.845 which was higher than the stoichiometric value of HAP indicating that they belong to B-type biological hydroxyapatites. FTIR analysis supported the SEM-EDX test, revealing a substantial proportion of carbon (C) and oxygen (O) components in these fishbone nano-calcium powders. These powders contain phosphate groups and various organic components, including lipids and proteins, as corroborated by the proximate results.

Fig. 1

Mineral composition of NC powders

Trace mineral composition analysis (Table 2) revealed that DNC and ONC had increased calcium and phosphorus content due to alkali extraction's heightened ash content. The bones of brackish and saltwater fish exhibited no significant chemical makeup differences, except for the tiger-toothed croaker bone, which displayed notably different fat values. Petroleum ether defatting of tiger-toothed croaker bone could enhance mineral extraction. The results showed that phosphorus and calcium were the most abundant minerals in DNC and ONC, followed by sodium and magnesium, along with trace elements like Fe, Zn, and Cu. Similar results were obtained in the study conducted by Ho *et al.* (*39*) where nano-hydroxyapatite from *Lates calcarifer* bone had a presence of major and trace elements like Fe, K, Mg, Na, Zn, and Se. Compared to other studies, lower mineral values were observed, such as sodium content of 0.19 % in nano-calcium from *Litopenaeus vannamei* shells (*42*) and calcium and phosphorus content of 2.9 and 6.84 %, respectively, in nano-calcium from *Katsuwonus pelamis* (*6*). Nilsuwan *et al*. (*26*) reported that the scales prepared using the heating/thermal method still had high quantities of retained organic compounds, especially collagen, which resulted in a lower proportion of minerals and contain higher content of protein.

Table 2

FTIR spectra of NC powders

FTIR spectra of DNC and ONC (Fig. 2) exhibited no discernible differences between the two fish species. The spectra indicated strong phosphate absorption in the 1,023.5 cm^{-1} region, affirming the existence of hydroxyapatite crystals (HAP). The split-shaped phosphorus absorption bands in the 560.67 and 601.61 cm^{-1} areas indicated the presence of hydroxyapatite crystals. The FTIR spectra also revealed the presence of carbonate, amine, hydrocarbon, and hydroxyl groups, suggesting the

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presence of organic compounds like protein, fat, and water in minute amounts. Prior research has identified various spectral peaks indicating the presence of specific functional groups in nano-calcium products: Phosphate group: Peaks at 564 cm^{-1} (*16*), 603 cm^{-1} (*43*), and 1,033 cm^{-1} (*44*); Carbonate ions: Peak at 1,414.93 cm^{-1} (*16*); Amide group: Peak at 1,533 cm^{-1} (*29*); Organic material (C-H): Peaks at 2,852 and 2,922 cm^{-1} (*45*); Water content: Bands above 3,300 cm^{-1} with low intensity (*46*). These findings suggest the presence of minute amounts of organic compounds like protein, fat, and water in the nano-calcium products. Notably, the consistent extraction method resulted in identical FTIR spectra across all samples, indicating a highly uniform chemical composition.

Fig. 2

Zeta potential of NC powders

Zeta potential, a measure of electric potential difference across a particle's surface, plays a crucial role in the stability of colloidal suspensions like nano-calcium. ZETA potential of DNC and ONC were −34.4 and −39.2 mV respectively, indicating good stability in dispersions and suspensions. The negatively charged surfaces demonstrated excellent biocompatibility, aligning with studies showing that values above −30 mV generally provide good stability (*47*,*48*). Furthermore, negatively charged surfaces, like those of DNC and ONC, have been linked to promoting the differentiation of osteogenic cells, according to Xu (*49*). This suggests potential biocompatibility advantages for these nano-calcium products.

XRD patterns of NC powders

X-ray diffraction (XRD) analysis reveals the crystallinity of DNC and ONC nano-calcium (Fig. 3). Both samples exhibit similar peak patterns around 2θ=10–80° (31–64°), indicating shared crystal phases and a hexagonal system. This result was in line with Prayitno *et al*. (*50*), representing successful nanoparticle formation. The crystallinity percentage of DNC (73.7 %) and ONC (78.1 %) highlight sharp and thin peaks, indicating its high crystallinity (>70 %). The degree of crystallinity of both the samples in this study was higher than that of bio-calcium from skipjack tuna, yellowfin tuna, and Asian seabass (*17*,*33*,*51*) prepared by alkaline pretreatment. However, 60–90 % crystallinity indicated the hydroxyapatite in biomedical applications (*52*). Therefore, these fish bone nano-calcium products hold potential for biomedical use. Interestingly, while the alkaline extraction method maintained the crystal phase, it significantly increased the degree of crystallization. This finding underscores the effectiveness of this approach for producing highly crystalline nano-calcium for potential biomedical applications.

Fig. 3

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TGA patterns of NC powders

Analyzing the thermal stability of DNC and ONC with TGA revealed four distinct weight loss stages across 30–800 °C (Fig. 4 and Table 3). Initial water release (1–6 %) occurred around 80 °C, followed by decomposition of organic compounds like proteins and fats at 250–244 °C. The similar reports were documented for various fish bones (*53*,*54*). Higher temperatures triggered a major weight loss (10–15 %) due to complex molecular degradation, with ONC exhibiting slightly higher resistance. Finally, decarbonation above 750 °C caused a final mass loss (5–6 %). Interestingly, despite minor variations in weight loss at specific stages, both DNC and ONC displayed remarkable thermal stability, retaining over 75 % of their mass even at 800 °C. Nevertheless, the thermal resistance of pure hydroxyapatite (HAp) sourced from *Sardinella longiceps* falls within the range of 600-1000 °C, consistent with this research (*55*). This outstanding resistance highlights their potential for applications requiring high thermal performance. In summary, the comprehensive analysis of the nano-calcium powders extracted from fish bones reveals their potential for various applications, including biomedical uses, owing to their stability, elemental composition, and suitable crystallinity.

Table 3 and Fig. 4

CONCLUSIONS

The extracted nano-calcium powders from two Malabar Coast croakers, *Daysciena albida* (DNC) and *Otolithes ruber* (ONC), while sharing significant similarities in their FTIR, ZPA, TGA, and XRD patterns, revealed distinct differences in chemical composition, physiological characteristics, and Ca/P ratios. While both are suitable raw materials for nano-calcium production, DNC emerged as the preferred choice. These findings suggest that the bones of the two commercial croaker species from the Malabar coast can serve as suitable raw materials for the production of nano calcium, exhibiting an average particle size ranging from 153.8 nm to 337.1 nm. Notably, the bone of the two-bearded croaker (DNC) emerged as the preferred choice for further study. Its superior calcium content, higher yield percentage, and average particle size make it a promising candidate for further research and development in this area. This paves the way for exciting advancements in utilizing DNC as a valuable source for nano-calcium production.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY

Data were not shared.

ETHICS AND CONSENT TO PARTICIPATE

Not applicable.

AUTHORS' CONTRIBUTION

Vaisshali Prakash Arul Prakasam and Radhika Rajasree SR designed the work and conducted the experiments. Vaisshali Prakash Arul Prakasam drafted the article, and Radhika Rajasree SR interpreted and evaluated the data and carried out the review and editing. Radhika Rajasree SR conceptualized the study, provided resources, supervised, offered funding, and critically revised the manuscript. Both authors revised and approved the final version of the article.

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REFERENCES

1. Pravina P, Sayaji D, Avinash M. Calcium and its role in the human body. Int J Pharm Sci. 2013;4(2):659-68.

2. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011; 96(1):53-58.
https://doi.org/10.1210/jc.2010-2704

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3. Sesikeran B. Nutritional Requirements and Recommended Daily Allowances for Indians: A Report of the Expert Group of the Indian Council of Medical Research. Jamai-Osmania PO, Hyderabad: National Institute of Nutrition, Indian Council of Medical Research; 2009.

4. Wang Y, Li S. Worldwide trends in dairy production and consumption and calcium intake: Is promoting consumption of dairy products a sustainable solution for inadequate calcium intake? Food Nutr Bull. 2008;29(3):172-85.
https://doi.org/ 10.1177/156482650802900303

5. Balk EM, Adam GP, Langberg VN, Earley A, Clark P, Ebeling PR, *et al.* International Osteoporosis Foundation Calcium Steering Committee. Global dietary calcium intake among adults: a systematic review. Osteoporos Int. 2017;28(12):3315-24.
https://doi.org/10.1007/s00198-017-4230-x

6. Harmain RM. Nanocalsium Characterization of Cakalang Fish Bone Flour (*Katsuwonus pelamis L*). Int J Innov Res Techn. 2020;3(10):306-08.

7. Bas M, Daglilar S, Kuskonmaz N, Kalkandelen C, Erdemir G, Kuruca SE, *et al.* Mechanical and biocompatibility properties of calcium phosphate bioceramics derived from salmon fish bone wastes. Int J Mol Sci. 2020;21(21):8082(1-14). https://doi.org/10.3390/ijms21218082

8. Cormick G, Belizán JM. Calcium intake and health. Nutrients. 2019;11(7):1606(1-16). https://doi.org/10.3390/nu11071606

9. Krupa-Kozak U, Drabińska N. Calcium in gluten-free life: health-related and nutritional implications. Foods. 2016;5(3):51(1-11).
https://doi.org/10.3390/foods5030051

10. Nemati M, Huda N, Ariffin F. Development of calcium supplement from fish bone wastes of yellowfin tuna (*Thunnus albacares*) and characterization of nutritional quality. Int Food Res J. 2017;24(6):2419-26.

11. Kettawan A, Sungpuag P, Sirichakwal P, Chavasit V. Chicken bone calcium extraction and its application as a food fortificant. Journal of the National Research Council of Thailand. 2002;34(2):163-80.

12. Kusumawati P, Triwitono P, Anggrahini S, Pranoto Y. Nano-calcium Powder Properties from Six Commercial Fish Bone Waste in Indonesia. Squalen Bull Mar Fish Postharvest Biotechnol. 2022;17(1):1-12.
https://doi.org/ 10.15578/squalen.601.

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13. Jung WK, Shahidi F, Kim SK. Calcium from Fish Bone and Other Marine Resources. In: Barrow C, Shahidi F, editors. Marine nutraceuticals and functional foods. New York, US: CRC Press; 2007. pp .419-30.
https://doi.org/10.1201/9781420015812.ch17

14. Sriuttha M, Chanshotikul N, Hemung B. Calcium extraction from catfish bone powder optimized by response surface methodology for inducing alginate bead. *Heliyon*. 2024;*10*(9):e30266(1-12).
https://doi.org/10.1016/j.heliyon.2024.e30266

15. Huang YC, Hsiao PC, Chai HJ. Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells. Ceram Int. 2011;37(6):1825-31.
https://doi.org/10.1016/j.ceramint.2011.01.018

16. Yin T, Park JW, Xiong S. Physicochemical properties of nano fish bone prepared by wet media milling. LWT-Food Sci Technol. 2015;64(1):367-73. https://doi.org/10.1016/j.lwt.2015.06.007

17. Benjakul S, Mad-Ali S, Sookchoo P. Characteristics of biocalcium powders from pre-cooked tongol (*Thunnus tonggol*) and yellowfin (*Thunnus albacores*) tuna bones. Food Biophys. 2017a;12:412-21.
https://doi.org/10.1007/s11483-017-9497-0

18. Alshemary AZ, Cheikh L, Çardaklı İS. Extraction and degradation rate analysis of calcium phosphate from diverse fish Bones: A comparative study. J Saudi Chem Soc. 2024;28(3), 101859(1-7).
https://doi.org/10.1016/j.jscs.2024.101859

19. Manojkumar PP. Fishery of sciaenids with some observations on the biology and stock assessment of *Johnieops sina* (Cuvier, 1830) exploited along the Malabar coast. J Mar Biol Assoc India. 2011;53(1):68-74.

20. Asokan PK, Venkatesan V, Said Koya KP, Abdussamad EM, Lakshmi Pillai S, Najmudeen TM, *et al.* Status of marine fisheries of Kerala. Mar Fish Infor Serv T & E Ser. 2015;(226):22-26.
http://eprints.cmfri.org.in/id/eprint/10979

21. Anggraeni N. Bioavailability of Nano Calcium Extracted from Nile Tilapia Bones (*Oreochromis niloticus*) with Variations in Base Solvent Concentrations and Extraction Time [PhD Thesis]. Special Region of Yogyakarta, Indonesia: Gadjah Mada University; 2019 (in Indonesian).

22. AOAC, Official methods of analysis. Association of Official Analytical Chemists, Washington; 2000.

23. Leme AB, Bianchi SR, Carneiro RL, Nogueira AR. Optimization of sample preparation in the determination of minerals and trace elements in honey by ICP-MS. Food Anal Method. 2014;7:1009-15.

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https://doi.org/10.1007/s12161-013-9706-5

24. Corrêa THA, Holanda JNF. Fish bone as a source of raw material for synthesis of calcium phosphate. Mater Res. 2019;22:e20190486(1-5).
https://doi.org/10.1590/1980-5373-MR-2019-0486

25. Steel RG, Torrie JH, editors. Principles and procedures of statistics, a biometrical approach. Toronto, NY, UK: McGraw-Hill Book Company, Inc; 1981.

26. Nilsuwan K, Pomtong S, Chedosama A, Sookchoo P, Benjakul S. Chemical Compositions and Characteristics of Biocalcium from Asian Sea Bass (*Lates calcarifer*) Scales as Influenced by Pretreatment and Heating Processes. Foods. 2023;12(14):2695(1-15).
https://doi.org/10.3390/foods12142695

27. Husna A, Handayani L, Syahputra F. Pemanfaatan tulang ikan kambing-kambing (*Abalistes stellaris*) sebagai sumber kalsium pada produk tepung tulang ikan. Acta Aquatica: Aquat Sci. 2020;7(1):13-20.
https://doi.org/10.29103/aa.v7i1.1912

28. Anggraeni N, Dewi EN, Susanto AB, Riyadi PH. In Situ Bioavailability of Nano-calcium from Red Snapper (*Lutjanus malabaricus*) Bone Extract with Varying Extraction Duration. Int J Agric Biol. 2024;*13*(4):632-38.
https://doi.org/10.47278/journal.ijab/2024.167

29. Zainol I, Adenan NH, Rahim NA, Jaafar CA. Extraction of natural hydroxyapatite from tilapia fish scales using alkaline treatment. Mater Today Proc. 2019;16:1942-48.
https://doi.org/10.1016/j.matpr.2019.06.072

30. Prinaldi WV, Suptijah P, Uju U. Physicochemical Characteristics of Nanocalcium Extract from Bones of Yellowfin Tuna (*Thunnus Albacares*). J Pengolah Has. 2018; 21(3):385-95.
https://doi.org/10.17844/jphpi.v21i3.24708

31. Amos B, Sector F, Einarsson H, Eythorsdottir A. Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement. United Nations University, Uganda, 2007;45:1-35. http://www.unuftp.is/static/fellows/document/amos06prf.pdf

32. Widiastuti I, Sudirman S. Hydroxyapatite characteristics from snakehead fish (*Channa striata*) bone via alkali treatment followed by calcination method. Trop J Nat Prod Res. 2024;*8*(2):6147-51.

33. Benjakul S, Mad-Ali S, Senphan T, Sookchoo P. Biocalcium powder from precooked skipjack tuna bone: Production and its characteristics. J Food Biochem. 2017b;41(6):e12412(1-8).
https://doi.org/10.1111/jfbc.12412

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34. Idowu AT, Benjakul S, Sinthusamran S, Sae-leaw T, Suzuki N, Kitani Y, Sookchoo P. Effect of alkaline treatment on characteristics of bio-calcium and hydroxyapatite powders derived from salmon bone. Appl Sci. 2020;10:4141(1-12).
https://doi.org/1–12.10.3390app10124141

35. Wijayanti I, Benjakul S, Sookchoo P. Preheat-Treatment and Bleaching Agents Affect Characteristics of Bio-calcium from Asian Sea Bass (*Lates calcarifer*) Backbone. Waste Biomass Valor. 2021;12:3371–82.
https://doi.org/10.1007/s12649-020-01224-w

36. Zhang J, He S, Kong F, Huang S, Xiong S, Yin T, *et al*. Size reduction and calcium release of fish bone particles during nanomilling as affected by bone structure. Food Bioprocess Technol. 2017;*10*:2176-87.
https://doi.org/10.1007/s11947-017-1987-z

37. Gnanasekaran R, Yuvaraj D, Muthu CM, Ashwin R, Kaarthikeyan K, Kumar VV, *et al*. Extraction and characterization of biocompatible hydroxyapatite (Hap) from red big eye fish bone: Potential for biomedical applications and reducing biowastes. Sustainable Chemistry for the Environment 2024;*7*:100142(1-9).

38. Rashad RT. Simple extraction of the hydroxy-apatite from the Egyptian Nile tilapia (*Oreochromis niloticus*) fish scales. Acta Sci Malays. 2023;*7*(2):54-59.
http://doi.org/10.26480/asm.02.2023.60.65

39. Le Ho KH, Dao VH, Pham XK, Nguyen PA, Phan BV, Doan TT, Lam TH. Physicochemical properties, acute and subchronic toxicity of nano-hydroxyapatite obtained from *Lates calcarifer* fish bone. Reg Stud Mar Sci. 2022;*55*:102560(1-10).

40. Sumarto, Desmelati, Sari NI, Angraini RM, Arieska L. Characteristic of Nano-Calcium Bone from a Different Species of Catfish (*Pangasius hypophthalmus*, *Clarias batrachus*, *Hemibagrus nemurus* and *Paraplotosus albilabris*). IOP Conf Ser: Earth Environ Sci 2021;695(1):012055(1-9).
https://doi.org/10.1088/1755-1315/695/1/012055

41. Yusuf Y, Khasanah DU, Syafaat FY, Pawarangan I, Sari M, Manuntu VJ, *et al.* Biogenic based hydroxyapatite. In Ifan, editor. Sleman, Indonesia; Mada University Press; 2019

42. Suptijah P, Jacoeb AM, Deviyanti N. Characterization and Bioavailability of Nanocalcium from Vannamei Shrimp (*Litopenaeus vannamei*). Jurnal Akuatika. 2012;3(1):63-73.

43. Cahyanto A, Kosasih E, Aripin D, Hasratiningsih Z. Fabrication of hydroxyapatite from fish bones waste using reflux method. IOP Conf. Ser.: Mater. Sci. Eng. 2017; 172(1):012006(1-6).
https://doi.org/10.1088/1757-899X/172/012006

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44. Bonadio TGM, Sato F, Medina AN, Weinand WR, Baesso ML, Lima WM. Bioactivity and structural properties of nanostructured bulk composites containing Nb_2O_5 and natural hydroxyapatite. J Appl Phys 2013;113(22): 223505(1-8).
https://doi.org/10.1063/1.4809653

45. Boutinguiza M, Pou J, Comesaña R, Lusquiños F, De Carlos A, León B. Biological hydroxyapatite obtained from fish bones. Mater Sci Eng C. 2012;32(3):478–86.
https://doi.org/10.1016/j.msec.2011.11.021.

46. Nam PV, Van Hoa N, Trung TS. Properties of hydroxyapatites prepared from different fish bones: A comparative study. Ceram Int. 2019;45(16):20141-47.
https://doi.org/10.1016/j.ceramint.2019.06.280

47. Guillen-Romero LD, Oropeza-Guzmán MT, López-Maldonado EA, Iglesias AL, Paz-González JA, Ng T, Serena-Gomez E *et al.* Synthetic hydroxyapatite and its use in bioactive coatings. J Appl Biomater. 2019;17(1):2280800018817463(1-12).
https://doi.org/10.1177/2280800018817463

48. Lu GW, Gao P. Emulsions and microemulsions for topical and transdermal drug delivery. In Kulkarni VS editor. Handbook of non-invasive drug delivery systems. Norwich, UK; William Andrew Publishing; 2010; 59-94.
https://doi.org/10.1016/B978-0-8155-2025-2.10003-4

49. Xu R. Progress in nanoparticles characterization: Sizing and zeta potential measurement. Particuology. 2008;6(2):112-15.
http://dx.doi.org/10.1016/j.partic.2007.12.002

50. Prayitno AH, Prasetyo B, Sutirtoadi A. Synthesis and characteristics of nano calcium oxide from duck eggshells by precipitation method. IOP Conf Ser: Earth Environ Sci 2020; 411(1):012033(1-6).
http://dx.doi.org/10.1088/1755-1315/411/1/012033

51. Wijayanti I, Benjakul S, Saetang J, Prodpran T, Sookchoo P. Soluble bio-calcium from Asian sea bass bone prepared with organic acids: solubility and physiochemical characteristics. Biomass Conv Bioref. 2024;1-11.
https://doi.org/10.1007/s13399-024-05402-x

52. Aminatun R. Synthesis and characterization of hydroxyapatite layer on cobalt alloys through dip coating method as a prosthetic bone implant candidate. J Optoelectron Biomed M. 2015;7(1):11-8.

53. Goto T, Sasaki K. Effects of trace elements in fish bones on crystal characteristics of hydroxyapatite obtained by calcination. Ceram. Int. 2014; 40(7):10777-85.
https://doi.org/10.1016/j.ceramint.2014.03.067

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54. Sobczak-Kupiec A, Wzorek Z. The influence of calcination parameters on free calcium oxide content in natural hydroxyapatite. Ceram. Int. 2012;38(1):641-47. https://doi.org/10.1016/j.ceramint.2011.06.065

55. Ashwitha A, Thamizharasan K, Bhatt P. Optimization of hydroxyapatite (HAp) extraction from scales of *Sardinella longiceps* and its conjugative effect with immunostimulants. SN Appl Sci. 2020;*2*:1-8. https://doi.org/10.1007/s42452-020-3057-9

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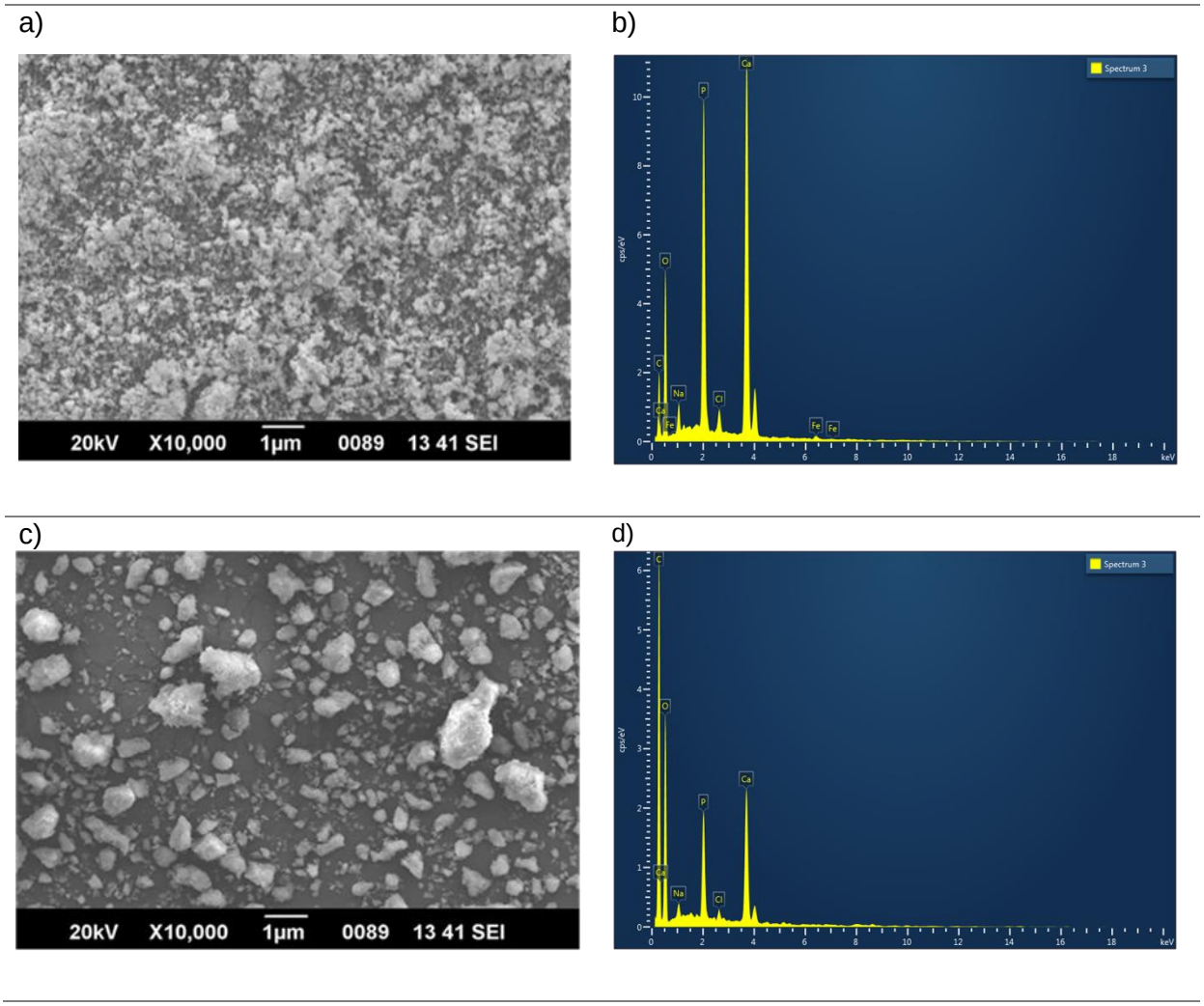

Fig. 1. The appearance of nano-calcium powder particles from two different fish species and their elemental compositions were determined by SEM-EDX: a) DNC, b) EDX spectra of DNC, c) ONC and d) EDX spectra of ONC

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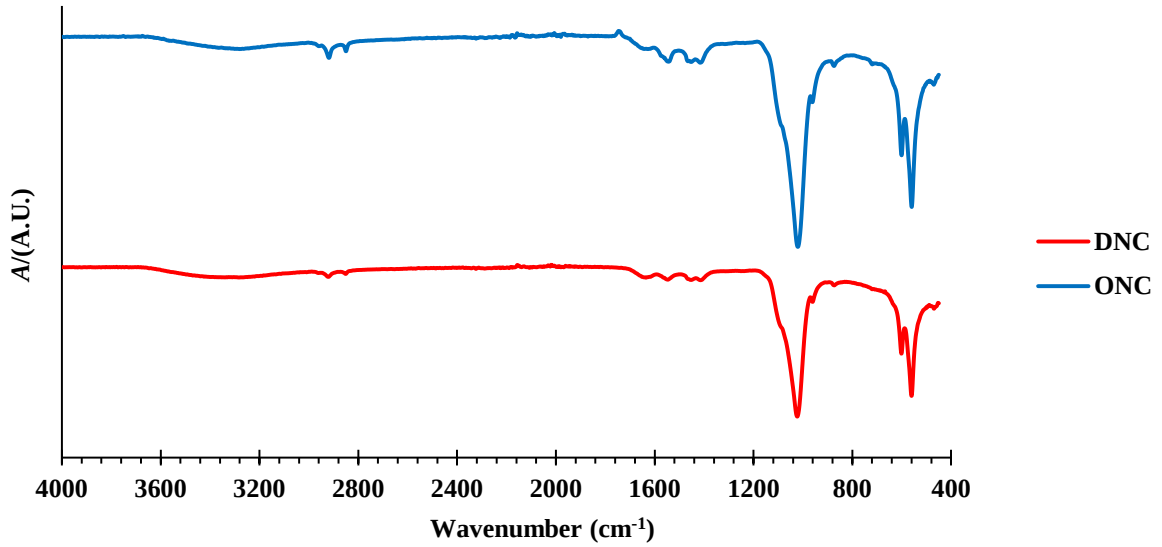

Fig. 2. FTIR spectra of nano-calcium powders from *Daysciena albida* (DNC) and *Otolithes ruber* (ONC)

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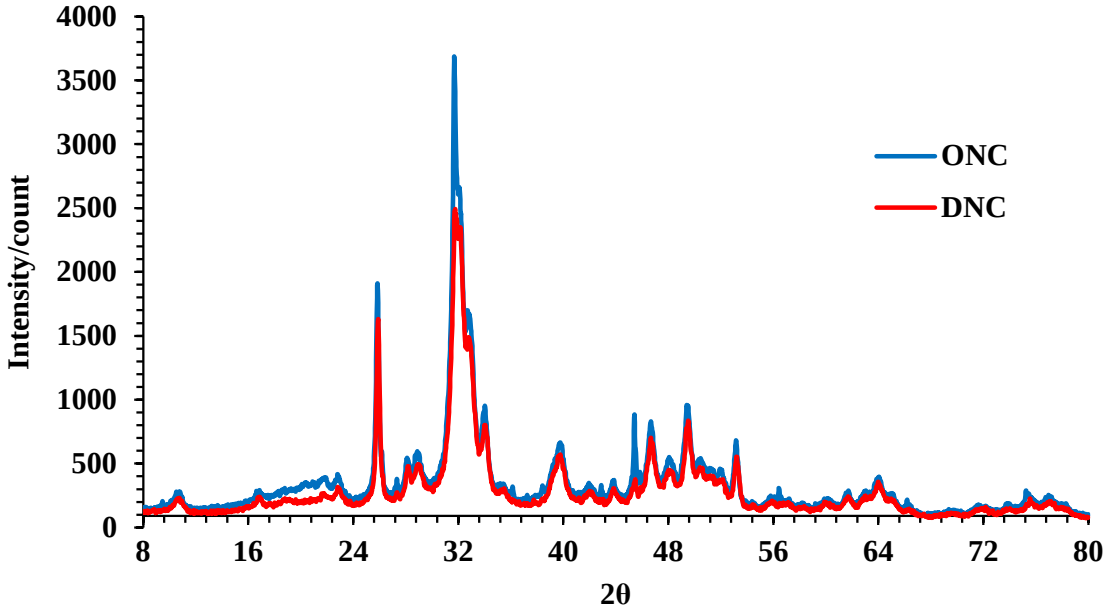

Fig. 3. X-ray diffraction patterns of nano-calcium powders from *Daysciena albida* (DNC) and *Otolithes ruber* (ONC)

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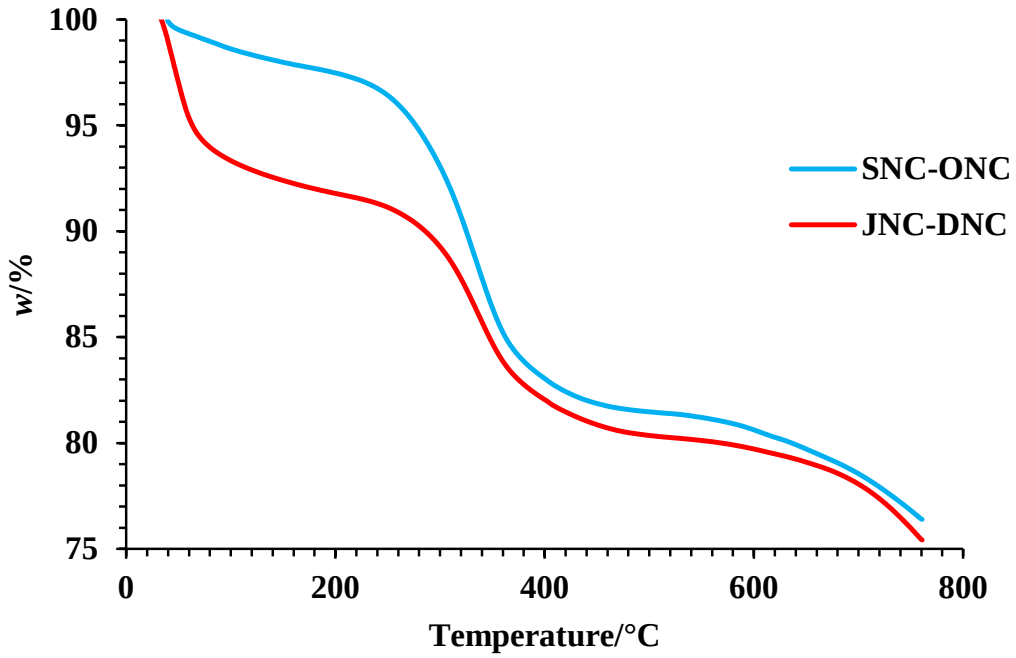

Fig. 4. Thermogravimetric analysis of nano-calcium powders from *Daysciena albida* (DNC) and *Otolithes ruber* (ONC)

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Table 1. Color and proximate composition of coarse fishbone and nano-calcium powders

Samples	*L**	*a**	*b**	Δ*E**	Moisture	Ash	Fat	Protein
DCB	(54.390±0.755)^{c}	(10.260±0.205)^{a}	(22.963±0.773)^{a}	(46.717±1.023)^{a}	(0.049±0.004)^{b}	(54.530±2.610)^{c}	(7.020±0.040)^{c}	(19.763±0.683)^{b}
DNC	(78.056±0.551)^{a}	(3.306±0.115)^{d}	(13.813±0.568)^{b}	(21.088±0.475)^{c}	(0.081±0.032)^{b}	(75.000±3.605)^{a}	(1.666±0.473)^{d}	(4.720±0.490)^{d}
OCB	(53.296±0.909)^{c}	(7.726±0.120)^{b}	(23.926±0.597)^{a}	(47.569±1.035)^{a}	(0.732±0.418)^{a}	(54.565±2.715)^{c}	(22.560±0.970)^{a}	(25.672±1.838)^{a}
ONC	(70.740±0.427)^{b}	(3.760±0.390)^{c}	(12.306±0.375)^{c}	(26.303±0.402)^{b}	(0.078±0.037)^{b}	(63.785±2.548)^{b}	(8.872±1.148)^{b}	(6.860±0.870)^{c}

Results are expressed as mean value±S.D. (*N*=3). Different letters in the same column indicate significant differences ($p<0.05$). DCB= *Daysciena* croaker bone, DNC=*Daysciena* nanocalcium, OCB=*Otolithes* croaker bone, ONC=*Otolithes* nanocalcium

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Table 2. Elemental composition of nano-calcium from *Daysciena albida* & *Otolithes ruber*

Sample	*w*/%							
	Na*	K*	Mg**	P	Ca	Fe	Cu	Zn
DNC	0.28	0.0018	0.14	19.76	9.93	0.61	BDL	0.002816
ONC	0.27	0.0026	0.16	18.89	9.80	0.61	0.000074	0.006679

*indicates isotope used for analysis, BDL=below detection limit (0.000001 %)

Table 3. Thermal degradation temperatures (T_d/°C), mass loss (Δm/%), and residue (%) of nano-calcium extracted from DNC and ONC

Samples	Δ_1		Δ_2		Δ_3		Δ_4		Residue
	T_{d1}, onset	Δm_1	T_{d2}, onset	Δm_2	T_{d3}, onset	Δm_3	T_{d4}, onset	Δm_4	
DNC	80.05	6.05	250.52	2.84	471.00	10.54	758.36	5.06	75.41
ONC	88.08	1.19	244.33	2.20	482.56	15.05	755.47	5.89	75.58

Δ_1, Δ_2, Δ_3, and Δ_4 denote the first, second, third, and fourth stage mass loss, respectively of films during the TGA heating scan (30–800 °C). DNC=*Daysciena* nano calcium, ONC=*Otolithes* nano calcium

Fig. S1. Production process of nano-calcium powder

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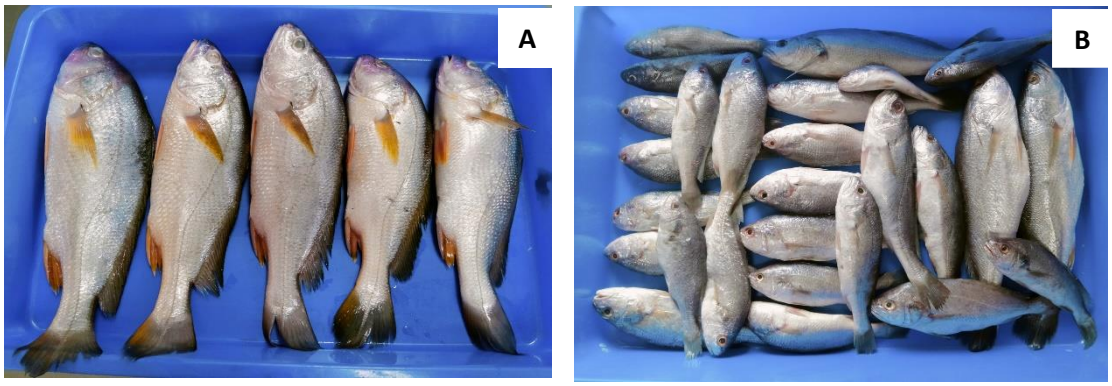

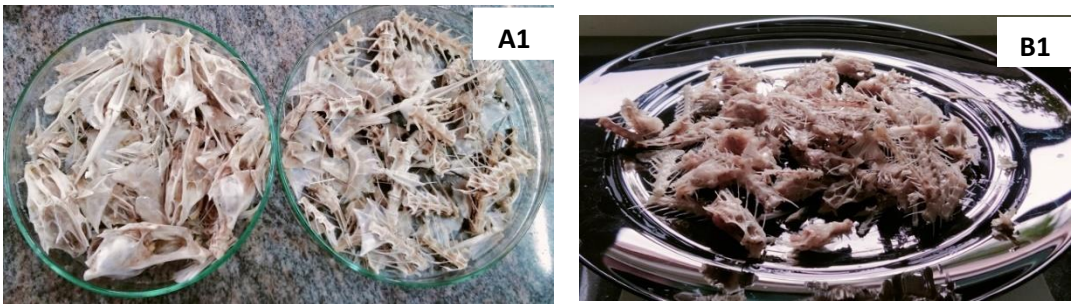

Fig. S2. Fresh fish (A) and (B), Dried fish bones (A1) and (B1) and Nano-calcium (A2) and (B2) of different species. A. *Daysciena albida* and B. *Otolithes ruber*