



Valorization of Tigertooth Croaker (*Otolithes ruber*) Bones into Biological Hydroxyapatite: A Physicochemical and Organoleptic Assessment

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ABSTRACT

Background: The seafood industry generates a large quantity of fish processing waste which causes environmental risks, despite high nutritional importance. Present study investigated the extraction of biological hydroxyapatite derived from the bones of Tigertooth croaker (*Otolithes ruber*) using three different extraction methodologies like, water boiling, alkaline boiling and extracting by means of autoclaving method.

Methods: Several physicochemical like, water holding capacity, oil holding capacity, bulk density, tapped density, compressibility and organoleptic like instrumental colour and whiteness index measurement was performed.

Result: The water boiled sample showed higher yield value (60.29%), though the alkaline boiled samples exhibited significant higher physical and functional parameters. Out of all the treatments, the AB4 (boiling with 2% NaOH) samples had the overall higher parameters specifically, highest water holding capacity (144.02%), oil holding capacity (67.64%), bulk density (669.66 kg/L), tapped density (759.00 kg/L). Also, AB4 sample had the highest lightness and whiteness index among all the treated samples. Moreover, the PCA bi-plot assured the high correlation of AB4 and AB6 sample towards physicochemical parameters. The study suggest that using 2% NaOH for extraction of biological hydroxyapatite is an effective method for valorizing Tigertooth croaker bone waste and mitigate the environmental impacts.

Key words: Bone powder, Hydroxyapatite, *Otolithes ruber*, Physicochemical parameters, Valorization.

INTRODUCTION

The global aquaculture and fisheries production rose to a whopping volume of 235 million tonnes in 2024, out of which 195 million tonnes of production came from aquatic animals. These aquatic animals contribute majorly in the human diet, comprising 89% utilization for human consumption, the rest being considered as waste (FAO, 2026). Fish significantly contributes to daily human life as a potent source of nutrient (Tiwari *et al.*, 2023). Therefore, there is a rising trend in fish processing industries, which substantially increases the fishery by-products, accounting 70% of the processed fish. These by-products are mainly contributed by heads (9-12%), bones (9-15%), skin (1-3%), viscera (12-18%) and scales (5%) respectively (Karayannakidis and Zotos, 2016). Also, roughly around 60% of the fish is being regarded as by-catch and rejected from the final fish processing (Sinaga *et al.*, 2026). These by-products are indiscriminately discarded in open areas or utilized for the production of low valued product like fish meal, animal feed and often enhance the environmental impacts. These components are considered as food losses which mean the reduction of human consumable edible portions (Yarali, 2026). To reduce the environmental hazards, there is an optimum need to introduce alternative usage, such as the production of biological Hydroxyapatite. Fish bone contains the primary inorganic constituent Hydroxyapatite, comprising 60-70%

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of the total mineral phase (Murugan and Ramakrishna, 2005). Hydroxyapatite derived from biological matter shows

many advantages over synthetic hydroxyapatite like better bio-compatibility. Therefore, it can be extensively utilised to retard osteoporosis disease and promotes bone integration (Sadat-Shojai *et al.*, 2013). Currently, researchers are showing interests in extracting Hydroxyapatite from natural resources, which include bones of bovine and porcine, marine shells, egg shells, fishery by-products. Out of these, hydroxyapatite extracted from fish waste showed significant considerations as it mitigates the environmental impacts, as well as its physico-chemical properties.

Croaker, belonging to the Sciaenidae family, is one of the commercially important marine teleost food fish contributing 10-12% of the total demersal catch of India (Renuka *et al.*, 2016) and a significant 25% of the total demersal fish landings of West Bengal (Roul *et al.*, 2023). *Otolithes ruber*, commonly known as the Tigertooth croaker, is extensively distributed along the coasts of India, likely in the Arabian Sea, as well as in the Bay of Bengal. Tigertooth croaker is a highly economically valuable candidate species contributing to the artisanal as well as commercial fishery sector. The species is in high demand due to its nutritional richness, including a substantially high content of protein and essential fatty acids. The species is mainly caught by using trawler and gill net gear. This generates significant amount of processing discards which include bones, frames, heads and viscera, which is largely not utilized or discarded as waste in open areas. However, these bioresources can be valorized widely for the production of bioactive molecule which can be applicable to food fortification, nutraceuticals, healthcare and pharmacological industry and hence, increasing the economic value.

Assessing the physico-chemical and organoleptic parameters of biological hydroxyapatite derived from the bones of *Otolithes ruber* is important to measure its suitability for application in food industry, pharmaceuticals and biomedical sector. Also, very fewer studies were observed for comparing different extraction methods of hydroxyapatite from the bones of fishes. Thus, the present study therefore aims to extract the natural hydroxyapatite from the bones of Tigertooth croaker (*Otolithes ruber*) fish using three different extraction approaches and assessing the physicochemical and sensory parameters of the hydroxyapatite samples. Despite the large interests on production of natural fish derived hydroxyapatite, majority of the researches mostly covered few species, specifically, tilapia, sardine, tuna, salmon etc. (Da Cruz *et al.*, 2020; Surya *et al.*, 2021; Venkatesan and Kim, 2010; Shi *et al.*, 2018). In contrast, a very little number of researches conducted in fish from Sciaenidae family, although these fishes are highly potent commercial species extensively processed in Indian coasts and generated a large quantity of fish processing wastes. Also, there is very less evidence is present on comparative assessment of hydroxyapatite extracted using different processing techniques from waste of fishes belonging to Sciaenidae family, signifying a potent research gap that the present study is going to address.

MATERIALS AND METHODS

Collection of samples

Fresh Tigertooth croaker (*Otolithes ruber*) fish was brought from local fish market (Garia fish market) by simple random sampling and used for the present study. These freshly collected fishes were carefully transferred in insulated box under iced condition to the laboratory of fish processing technology, Faculty of fishery sciences within 1 to 2 hours of collection and stored under frozen storage condition before further processing.

Pre-processing

Before processing, the samples were thawed properly at ambient temperature. At first, the remaining flesh present on the frame of the Tigertooth croaker fish was mechanically scrapped by using knife after defrosting the frames. The cleaned frames were submerged in boiling water for about 2-3 min and the bones were completely cleaned by washing it with chilled water. At last, the Tigertooth croaker bones were stored under frozen storage condition before preparing the bone powder.

Extraction of biological hydroxyapatite

Bone powder extracted by water as boiling media

The water extraction method was opted by the method suggested by Amitha *et al.* (2019) with minor modifications. Tigertooth croaker fish bones were washed and boiled in distilled water for about 30 minutes at 80-90°C. The meat present on the frames was cleaned thoroughly and washed with water. After cleaning and washing, the bones were dried under hot air oven (mechanical dryer) at 100°C for 3 hours. The dried Tigertooth croaker fish bones further ground and packed in plastic container at room temperature.

Bone powder extracted by alkaline as boiling media

The alkaline extraction of fish bone powder was followed the process given by Nemati *et al.* (2017) with some modifications. Different proportion of alkaline media and boiling times were taken for extracting the bone powders. The bones were boiled in 0.5%, 1%, 1.5%, 2%, 2.5% and 3% sodium hydroxide solution (NaOH) for 5 min, 10 min, 20 min, 30 min, 40 min and 50 min respectively at a ratio of 1 part of Tigertooth croaker bone to 3 parts of NaOH solution (w/v). The samples in this study were denoted as AB1, AB2, AB3, AB4, AB5 and AB6 following the conditions mentioned before. The immersed bones were first filtered using a filter cloth and further washed with mineral acid (1% Hydrochloric acid) and deionized water to neutralize the bones completely. It was then dried in hot air oven at 100°C for 2 hour. At last, the dried bones were crushed into finer bone powder by using a 20 mesh sized sieve.

Bone powder extracted by autoclaving

The autoclaving method for extraction of Tigertooth croaker fish bone powder was followed by the process suggested

by Yin *et al.* (2016) with some modifications. The bones which were stored under frozen storage condition transformed to normal room temperature by thawing. The bones were washed under running water to remove the dirt, slimes and blood from it. After that, it was submerged into potable water and boiled at 121°C for 1 hour using a vertical autoclave machine. The resultant juice was removed after boiling and the It was again washed with running water. Further the bones were finely grounded using a industrial grade mincer machine. Ice water was poured into the fish bone mince at the weight ratio of 3:10. It was then again grounded for formulating a paste. The paste was further dried in a hot air oven at a temperature and time combination of 105°C for 6 h. The paste was dried and grounded again using grinder and 20 mesh sized sieve was used for screening purposes.

Yield study

Yield percentages were calculated during different steps of fish bone powder preparation. Yield of raw fish, separated backbones weight, clear frames weight after removal of residual meat on it and finally fish bone powders weight was measured to calculate the yield of fish bone powder from raw Tigertooth croaker fish.

Estimation of pH

The pH value was recorded using a pH meter, according to the method of EIC. Homogenise 10 g of fish bone powder samples with 20 ml distilled water. As a general rule the ratio of water to fish should be 2:1. Measure pH of homogenous solution and correct to zero dilution according to the following formula:

$$pH(0)=pH(D)-0.068 D^{0.5}$$

Where,

pH (0) = pH at zero dilution.

pH (D) = pH determined on the homogenate.

D = Dilution ration = Vol. of water added to the sample in ml/wt. of sample in gm.

Estimation of bulk density

The bulk density of the Tigertooth croaker fish bone powder was determined by measuring the weight of the powder and the corresponding volume without tapping, following the method suggested by Nijdam and Langrish, (2005). One gram of bone powder was added to a 10-ml graded measuring cylinder. For calculating the bulk density of the bone powder sample the below mentioned formula was used.

$$\text{Bulk density} = \frac{\text{Weight of the dry bone powder}}{\text{Bulk volume of the sample}}$$

Estimation of tapped density

Tapped density for Tigertooth croaker bone powder was achieved by mechanically tap the measuring container, containing the sample. It allows the particle to settle and create the compactness. To determine tapped density, the

preliminary volume or mass of the sample was noted down, followed by manual tapping of the measuring cylinder or container. This process is repeated until a stable volume or mass reading is obtained, indicating minimal further change. The tap density can then be calculated using the following equation given by Sharma *et al.* (2021):

$$\text{Tapped density} = \frac{\text{Weight of dry powder}}{\text{Tapped volume}}$$

Estimation of compressibility index (CI)

Percent compressibility of the powdered excipients was determined directly from the following formula proposed by Rahman *et al.*, (2017).

Determination of Hausner's ratio

Hausner's ratio serves as a reliable indicator of a powder's flowability, calculated by dividing the tapped density by the bulk density (Sharma *et al.*, 2021). This ratio provides valuable insight into the powder's flow characteristics, with lower values signifying better flowability. In essence, a smaller Hausner's ratio denotes a more free flowing powder, while a larger ratio indicates poorer flow characteristics.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Water holding capacity (WHC) and oil holding capacity (OHC)

The water holding capacity of Tigertooth croaker fish bone powders was determined according to the previously described method by Antu *et al.* (2024). Around 1 g of bone powder sample was added in 10 mL of distilled water. The mixture was then opted with continuous stirring at a rate of 300 rpm for 24 hour for homogenization purposes. The homogenized solution was further centrifuged at 3000 rpm for 10 min. The supernatant was scrapped and the weight of the wet sample was recorded. For calculating the water holding capacity the following formula was followed.

$$\text{Water holding capacity} = \frac{(W_2 - W_1)}{W_1}$$

Where,

W_1 = Weight of the dry bone powder sample.

W_2 = Weight of wet sample.

Oil holding capacity of Tigertooth croaker fish bone powders were determined according to the previously described method given by Antu *et al.*, (2024). One gram of Tigertooth croaker bone powder was mixed with 10 mL of soybean oil. The mixture was then vigorously stirred at room temperature for a period of 30 mins. The sample was then homogenized completely and placed it in a centrifuge machine for 10 min with 3000 rpm rotation. Removal of the supernatant was done and the weight of the residual sample was recorded. Calculation of oil holding capacity of bone powders was done by using the equation:

$$\text{Oil holding capacity} = \frac{(W_2 - W_1)}{W_1}$$

Where,

W_1 = Weight of dry bone powder sample.

W_2 = Weight of oil mixed sample.

Assessment of colour and whiteness index (W.I)

The colour of the Tigertooth croaker fish bone samples was measured by using a hunter colorimeter (Model no: Colour Quest XE, Hunter Lab) at RKVY central instrumentation facility, Uttar Banga Krishi Viswavidyalaya (UBKV), Cooch Behar, West Bengal. The color parameters (L^* , a^* and b^*) were interpreted as L^* for lightness, a^* for redness or greenness and b^* for yellowness or blueness. The whiteness index (W.I) was determined using the equation given by Baycar *et al.* (2021).

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

Statistical analysis

All the data were checked for normal distribution with normality plots prior to analysis of variance (ANOVA) to determine significant differences among means at $\alpha = 0.05$ level, using statistical tools of Microsoft Office Excel (2019) and R software (Version 2.14.1). Tukey HSD was used to determine significant differences between treatments. PCA analysis was

Table 1: Yield percentage of tigertooth croaker bone powder.

Treatment	Yield (%)
WB	60.29±0.50 ^a
AC	40.36±0.14 ^f
AB ₁	51.04±0.06 ^b
AB ₂	45.70±0.02 ^d
AB ₃	41.34±0.14 ^{ef}
AB ₄	45.28±0.07 ^d
AB ₅	41.86±0.18 ^e
AB ₆	47.71±0.10 ^c

*Results are mean of five determinations (n=5) with SEM.

#Values of mean within the same column having different superscripts are statistically significant based on Tukey HSD ($p < 0.05$).

performed using R studio software version 4.6.1. The data replication taken for conducting all the experiments is n=5.

RESULTS AND DISCUSSION

Yield study

The yield percentage of differently treated fish bone powder is represented in the Table 1. The results were depicted as 60.29±0.50% yield percentage for water boiled fish bone powder samples (WB), 40.36±0.14% for autoclaved fish bone powder (AC), 51.04±0.06% for 0.5% NaOH boiled fish bone powder (AB₁), 45.7±0.02% for 1% NaOH boiled fish bone powder (AB₂), 41.34±0.14% for 1.5% NaOH boiled fish bone powder (AB₃), 45.28±0.07% for 2% NaOH boiled fish bone powder (AB₄), 41.86±0.18% for 2.5% NaOH boiled fish bone powder (AB₅) and 47.71±0.10% for 3% NaOH boiled fish bone powder (AB₆) respectively. The yield percentage of differently extracted Tigertooth croaker bone powder samples were presented in Table 1. The water-boiled bone powder samples yielded the highest percentage, whereas the chemically treated and autoclave-processed samples exhibited significantly lower yield percentages ($p < 0.05$). The water boiled samples exhibited higher yield percentage than alkaline boiled and autoclaved Tigertooth croaker fish bone powder samples as, chemical processing and high heating in autoclave exclude significant amount of organic moiety like protein and fat, which lowers the yield percentage.

pH study of tigertooth croaker bone powder

The pH values of Tigertooth croaker bone powder in this present study significantly varied ($p < 0.05$) within different treatments. The highest pH value was observed in AB-6 samples (pH 9.52±0.03), followed by AB5 (pH 9.32±0.05), AB-4 (pH 8.33±0.05), AB-3 (pH 6.79±0.03), AB-2 (pH 6.39±0.04), AC (pH 6.22±0.03), WB (pH 6.12±0.05) and AB-1 (pH 6.09±0.03) respectively. The pH results showed in the Fig 1. The boxplot diagram (Fig 2) and Tukey HSD

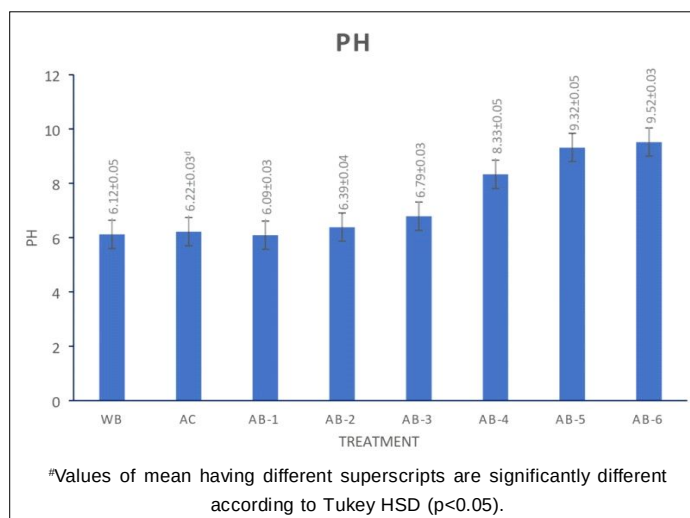


Fig 1: pH of Tigertooth croaker bone powders.

result (Fig 1) reveal that the pH content of the eight differently treated bone powders exhibits significant variation ($p<0.05$) across treatments, with the notable exception of WB and AB-1 samples, as well as AB-5 and AB-6 samples, which demonstrate no statistically significant differences between them. The alkaline boiled samples showed higher values than the water boiled sample. The probable reason behind this was the application of higher concentration of NaOH during processing of fish bone powders, notably 0.5% in AB1; 1% in AB2, 1.5% in AB3; 2% in AB4; 2.5% in AB5 and 3% in AB6 samples. The AB5 and AB6 samples exhibited higher pH values, which might be attributed to higher alkalization effect in processing. The final pH value of the bone powder samples mechanistically influenced by the residual alkali present in the matrix of fish bone powder after washing.

Bulk density of bone powders

The bulk density of Tigertooth croaker fish bone powder is divided into four major parameters like untapped bulk density, tapped density, compressibility index and Hausner ratio (Table 2). The bulk density of Tigertooth croaker bone powders were as 483.33±3.66 kg/L in WB, 582.33±5.66 kg/L in AC, 495.66±4.33 kg/L in AB-1, 545.33±9.66 kg/L in AB-2, 499.66±7.21 kg/L in AB-3, 669.66±2.66 kg/L in AB-4, 579.00±4.93 kg/L in AB-5 and 614.66±5.54 kg/L in AB6. On the other hand, the tapped density of bone powder samples was 560.33±5.33 kg/L in WB, 700.66±7.26 kg/L

in AC, 590.66±8.19 kg/L in AB-1, 645.66±12.10 kg/L in AB-2, 622.33±2.66 kg/L in AB-3, 759.00±5.29 kg/L in AB-4, 700.66±7.26 kg/L in AB-5 and 705.66±8.33 kg/L in AB-6 respectively. The compressibility index (CI) of bone powder samples were within the range of 11.77±0.76 to 19.71±0.89, exhibiting the maximum value (19.71±0.89) ($p<0.05$) obtained in AB-3 Tigertooth croaker bone powder samples. Similarly, the Hausner ratio values of Tigertooth croaker bone powder samples showed highest value ($p<0.05$) in AB-3 samples as 1.24±0.015 and the lowest value (1.13±0.01) ($p<0.05$) in AB-4 samples respectively. Table 2 showed differences in bulk density and tapped density which signifies that the treatments significantly influenced the physical attributes of the bone powder samples. Higher values of bulk density might inform lower particle sizes of the bone powder samples. This is occurred due to the reduction of vacuum spaces within the particles (Wu *et al.*, 2012). As, more particles can occupy the void space within the particle, the bulk density increases. The highest bulk density showed in AB4 samples, which is due to higher compactness of the particles. The tapped density was increased due to mechanical tapping, which made the samples more compact structure. AB4 samples showed higher tapped density due to better particle size. Two major parameters which influence highly the flow characteristics of Tigertooth croaker bone powder was Compressibility index and Hausner ratio. The better flow characteristics were observed in case of AB4 samples;

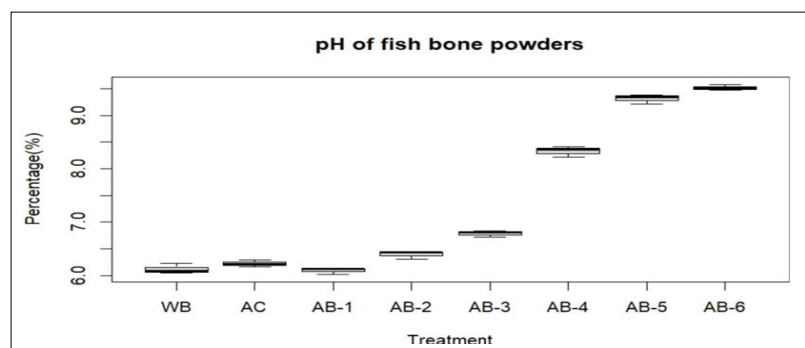


Fig 2: Boxplot diagram of pH of tigertooth croaker bone powder.

Table 2: Bulk density of tigertooth croaker bone powder.

Treatment	Bulk density (Kg/L)	Tapped density (Kg/L)	Compressibility index	Hausner ratio
WB	483.33±3.66 ^e	560.33±5.33 ^e	13.70±0.75 ^{cde}	1.15±0.008 ^{bcd}
AC	582.33±5.66 ^c	700.66±7.26 ^b	16.89±0.52 ^{abc}	1.20±0.003 ^{ab}
AB ₁	495.66±4.33 ^e	590.66±8.19 ^{de}	16.08±0.75 ^{bcd}	1.19±0.008 ^{ab}
AB ₂	545.33±9.66 ^d	645.66±12.10 ^c	15.54±0.82 ^{bcd}	1.18±0.012 ^{bc}
AB ₃	499.66±7.21 ^e	622.33±2.66 ^{cd}	19.71±0.89 ^a	1.24±0.015 ^a
AB ₄	669.66±2.66 ^a	759.00±5.29 ^a	11.77±0.76 ^e	1.13±0.010 ^d
AB ₅	579.00±4.93 ^c	700.66±7.26 ^b	17.36±0.14 ^{ab}	1.21±0.011 ^{ab}
AB ₆	614.66±5.54 ^b	705.66±8.33 ^b	12.89±0.60 ^{de}	1.14±0.008 ^{cd}

*Results are mean of five determinations (n=5) with SEM.

#Values of mean having different superscripts are significantly different according to Tukey HSD ($p<0.05$).

whereas, poor characteristics observed for AB3 samples.

Water holding capacity (WHC) and oil holding capacity (OHC) of Bone powders

Water holding capacity (WHC) and Oil holding capacity (OHC) of fish bone powder is of paramount importance, as these properties significantly impact its functionality and applicability in various industries. WHC and OHC are critical indicators of the powder's ability to retain moisture and lipids, respectively, which in turn affects its texture, stability and overall performance in food products, cosmetics and pharmaceuticals. The WHC of bone powders were $80.56 \pm 4.43\%$ in WB, $112.45 \pm 6.99\%$ in AC, $92.60 \pm 5.77\%$ in AB-1, $107.41 \pm 1.86\%$ in AB-2, $99.34 \pm 8.39\%$ in AB-3, $144.02 \pm 4.08\%$ in AB-4, $109.81 \pm 6.47\%$ in AB-5 and $128.08 \pm 2.35\%$ in AB-6 respectively. On the other hand, the oil holding capacity of bone powder samples were $56.00 \pm 1.52\%$ in WB, $63.00 \pm 0.14\%$ in AC, $56.86 \pm 0.10\%$ in AB-1, $61.39 \pm 0.23\%$ in AB-2, $58.82 \pm 0.14\%$ in AB-3, $67.64 \pm 0.18\%$ in AB-4, $61.76 \pm 0.13\%$ in AB-5 and $66.34 \pm 0.11\%$ in AB-6 respectively. Water holding capacity (WHC) and oil holding capacity (OHC) results showed in Table 3. These parameters can enhance the powder's emulsifying and stabilizing properties, making it an ideal ingredient in the fortification of food products. The Boxplot diagram showed the highest water holding capacity and oil holding capacity was obtained for AB4 samples (Fig 3). Thus, powders with smaller particle size have a larger surface area compared to larger particles. Also, the water holding capacity and oil holding capacity of fish bone powders depends on size of the particle, processing methods and surface area (Huey *et al.*, 2021).

Instrumental colour assessment

The colorimetric properties of Tigertooth croaker bone powders were evaluated using four distinct parameters: lightness (L^*), redness (a^*), yellowness (b^*) and whiteness index (WI). The L^* values of the bone powder samples were found to be 85.62 ± 0.52 for WB, 82.42 ± 0.20 for AC, 87.6 ± 0.17 for AB-1, 84.55 ± 0.06 for AB-2, 85.87 ± 0.13 for AB-3, 89.32 ± 0.17 for AB-4, 88.7 ± 0.17 for AB-5 and 86.7 ± 0.40 for AB-6. Tukey HSD analysis (Table 4) revealed significant differences ($p < 0.05$) in L^* values among all treated Tigertooth croaker bone powders. The redness

values (a^*) of the bone powder samples were determined to be 3.45 ± 0.25 for WB, 8.17 ± 0.26 for AC, 2.15 ± 0.16 for AB-1, 4.62 ± 0.19 for AB-2, 3.12 ± 0.27 for AB-3, 1.57 ± 0.22 for AB-4, 1.37 ± 0.18 for AB-5 and 2.32 ± 0.08 for AB-6. Tukey HSD results indicated significant variations ($p < 0.05$) in redness values among treatments. Conversely, the yellowness values exhibited significant differences ($p < 0.05$) among treatments, with the highest value observed for AC (44.12 ± 0.38) and the lowest for AB-5 (15.57 ± 0.34). The whiteness index results showed similar significant variations among treatments, with values ranging from 51.81 ± 0.13 for AC to 80.7 ± 0.06 for AB-5. Notably, higher L^* values corresponded to lighter, brighter, or whiter products, resulting in higher whiteness index (WI) values, whereas higher a^* and b^* values signified darker products.

PCA analysis

The principal component analysis curve showed the cumulative variance occurred with PC1 and PC2 is 86.92% with PC1 contributing 60.97% and PC2 with 25.95% respectively (Fig 4). The graph showed that the parameters like, bulk density, tapped density, water holding capacity (WHC), oil holding capacity (OHC) and Whiteness Index (WI) were positively correlated and provide a significant contribution to PC1. On the other hand, some parameters like, yield, compressibility index and Hausner ratio were inversely related with these operational traits. Also, the

Table 3: Water holding capacity and Oil holding capacity of tigertooth croaker bone powders.

Treatment	WHC (%)	OHC (%)
WB	80.56 ± 4.43^d	56.00 ± 1.52^e
AC	112.45 ± 6.99^{bc}	63.00 ± 0.14^b
AB ₁	92.60 ± 5.77^{cd}	56.86 ± 0.10^{de}
AB ₂	107.41 ± 1.86^{bc}	61.39 ± 0.23^{bc}
AB ₃	99.34 ± 8.39^{cd}	58.82 ± 0.14^{cd}
AB ₄	144.02 ± 4.08^a	67.64 ± 0.18^a
AB ₅	109.81 ± 6.47^{bc}	61.76 ± 0.13^b
AB ₆	128.08 ± 2.35^{ab}	66.34 ± 0.11^a

*Results are mean of five determinations (n=5) with SEM.

#Values of mean having different superscripts are significantly different according to Tukey HSD ($p < 0.05$).

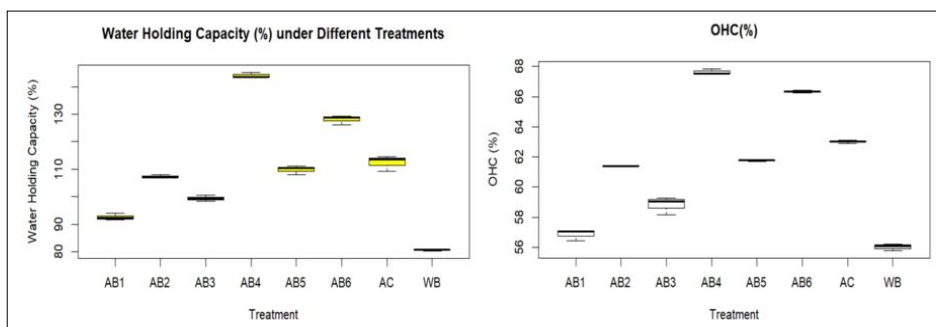


Fig 3: Boxplot diagram of water holding capacity (WHC) and oil holding capacity (OHC) of tigertooth croaker bone powders.

Table 4: Instrumental colour assessment of tigertooth croaker bone powders.

Treatment	Lightness (L*)	Redness (a*)	Yellowness (b*)	Whiteness index
WB	85.62±0.52 ^{de}	3.45±0.25 ^c	34.72±0.45 ^d	62.26±0.09 ^e
AC	82.42±0.20 ^f	8.17±0.26 ^a	44.12±0.38 ^a	51.81±0.13 ^h
AB ₁	87.6±0.17 ^{bc}	2.15±0.16 ^{de}	31.6±0.15 ^e	65.98±0.06 ^d
AB ₂	84.55±0.06 ^e	4.62±0.19 ^b	41.75±0.50 ^b	55.24±0.03 ^g
AB ₃	85.87±0.13 ^d	3.12±0.27 ^{cd}	38.01±0.42 ^c	59.33±0.07 ^f
AB ₄	89.32±0.17 ^a	1.57±0.22 ^e	17.25±0.19 ^g	79.65±0.06 ^b
AB ₅	88.7±0.17 ^{ab}	1.37±0.18 ^e	15.57±0.34 ^g	80.7±0.06 ^a
AB ₆	86.7±0.40 ^{cd}	2.32±0.08 ^{de}	25.55±0.22 ^f	71.1±0.06 ^c

*Results are mean of five determinations (n=5) with SEM.

#Values of mean having different superscripts are significantly different according to Tukey HSD (p<0.05).

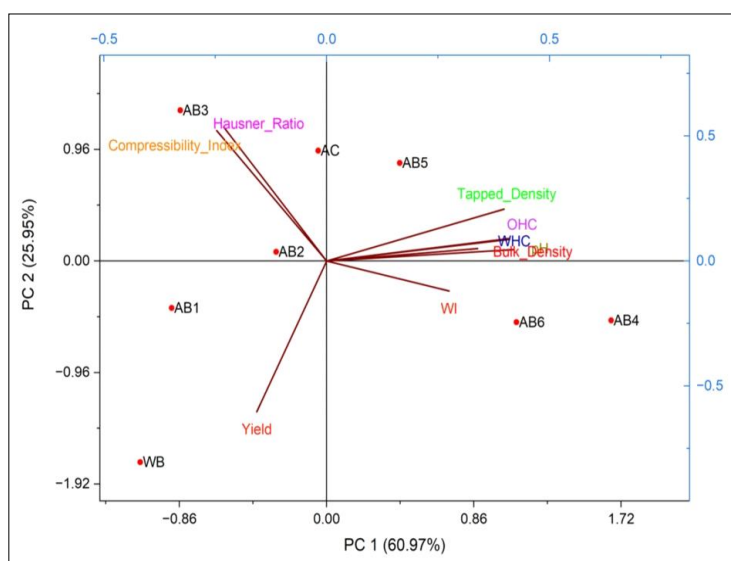


Fig 4: Bi-plot of PCA analysis of differently treated Tigertooth croaker bone powders.

score plot informed differentiation between the treated samples with AB4 and AB6 lied on the positive section of the principal component PC1 and strongly correlated with the hydration and density related parameters. Although, water boiled sample (WB) signified a strong affinity with yield (%) and relied on the negative side. The PCA curve revealed the definite functional clusters among the physicochemical parameters and distinct segregation between the treated samples. Strong correlation was observed for bulk density, tapped density, water holding capacity and oil holding capacity. Therefore, these parameters are interconnected and strongly contribute to the functional properties of the Tigertooth croaker bone powder samples. The grouping between AB4 and AB6 signified that these treatments have better attributes like, water and oil retention. On the other hand, WB showed strong affinity towards yield, indicating better yield but lesser other functional parameters. From the PCA, it can be assumed that AB4 and AB6 samples showed better functionality among the treated samples.

CONCLUSION

The current study informed that biological calcium hydroxyapatite can be easily extracted from Tigertooth croaker bone waste. This results the hydroxyapatite with good quality functional parameters and physico-chemical attributes. Out of all the treatments, the WB samples exhibited higher yield, but the 2% NaOH treated samples showed higher functional attributes among all the treated samples. The results informed that AB4 treatment obtained the maximum value of water and oil holding capacities, high number of bulk density and tapped density, better flow parameters and good whiteness index, which indicates its acceptability in food industry and biomedical applications. The PCA analysis also supported that among all the tested samples, AB4 and AB6 exhibited better physico-chemical parameters and most suitable for further applications. From this study, we can predict the conversion of Tigertooth croaker bone waste into bioactive compounds and mitigate the environmental impacts. The present study supports the production of biological

hydroxyapatite from the Tigertooth croaker bone waste and its suitability for application in food, nutraceuticals and pharmaceutical industry.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

Both the author declares that there are no conflicts of interest for the publication of this article. There is no funding or sponsorship for conducting the research of the present study.

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