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Development, characterization, and principal component analysis of fish bone-based fortified refined wheat flour tortilla and its organoleptic attributes

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Abstract

This study aimed to develop nano-sized-biogenic hydroxyapatite (HAp) from fishbone; followed by calcination at 850°C. The core part of the research “ultra-sonication” was done following different levels of operating variables. Characterization of resulted HAp was done using different techniques. FTIR spectra confirmed PO_4^{3-} peak at 1031.77 cm^{-1}. As per the findings of atomic absorption spectroscopy (AAS) and particle size distribution (PSD), 128.04 ± 0.19 ppm calcium and 50–400 nm particle size were determined. Scanning transmission electron microscopy (STEM) confirmed agglomerated crystal lattice, whereas in XRD 2θ crystalline behavior was noted at 300 a.u. Laser-induced breakdown spectroscopy (LIBS) derived Ca/P-Ratio was 1.69. HAp was further used to develop nano-sized-biogenic HAp fortified refined wheat flour tortillas with four formulations at 0%, 0.2%, 0.4%, and 0.6% levels. Tortilla was characterized for moisture (%) and spread ratio lied within 6.20 ± 0.05 to 8.15 ± 0.83 and 5.95 ± 0.08 to 5.80 ± 0.03, respectively. Best average acceptance was seen in a formulation named NBio-FB1 (0.2) which could thus prove a better choice for end-users regarding the potential to eradicate calcium deficiency. Moreover, PCA underlined 74.82% total variance.

Novelty impact statement: The ultrasonic processing method was practiced to reduce the particle size of HAp obtained from the discarded fish bone. With Ca/P ratio of nano-sized-biogenic HAp was determined by LIBS. Moreover, fortification of tortillas with nano-sized-biogenic HAp powders developed from fishbone may play a role to improve bone health.

1 | INTRODUCTION

The growing trend in the consumption of fast food in most cases does meet the consumer demand regarding taste but lacks proper nutrition and the trend is also rendering most of the parts of food commodities useless. So, in this regard, the need of the hour for food scientists is the development of fortified foods that follow the zero waste theory without compromising the consumer acceptability and nutritional profile (Huey et al., 2021). Nanotechnology, in this regard, serves both purposes. In the last few years, zero waste theory is a challenging strategy for researchers to recycle waste resources into alternative useful forms (Pietzsch et al., 2017; Song et al., 2015). Production of the huge amounts of low-valued food by-products and food waste from fish and meat items is becoming a matter of concern for food and environmental scientists around the globe (Lucarini et al., 2020). Similarly, we too would have to take care of our own types of food waste and strategies to tackle them.

Fishing is important to many people in developing countries. The estimated demand for consuming fish might be approximately 9.8 billion tons by 2050 (Searchinger et al., 2019). A tremendous increase in fish consumption endorses high biological protein value and a superior source of omega-3 fatty acids. Rohu (*Labeo rohita*) is a common freshwater fish in Pakistan with high consumer preference thus ranking 10th in terms of aquaculture species (Baset, 2020; Mehar et al., 2022).

An estimated figure of around 18 to 30 million tons of waste with no financial value was recorded annually from the fish processing industry (Mukkanti & Tembhurkar, 2021). During fish processing, more than half of the fish mass has been produced as leftovers, including frames, and trimmings (containing bone, and skin) (Idowu et al., 2020). Fishbone is one of the underutilized parts and considered a waste that could be given its due value, especially regarding calcium and phosphorous components. In this regard, nanotechnology is the state-of-the-art technology that could serve the purpose of making such components worth adding to different kinds of food products without compromising their acceptability (Widyastuti & Evawati, 2019). The aforementioned minerals are important because of their important roles in the human body. Calcium deficiency could be the indicator of poor bone health that could result in the development of osteopenia and thus osteoporosis (Harmain et al., 2019). Bio-mimic substitute of calcium like Hydroxyapatites (HAp) from large animals is being extensively used in most parts of the world in the food industry (Chaiarwut et al., 2020). But there is a need to opt for sources according to each community suiting their pattern of consumption.

HAp may be synthesized from calcium-phosphorus-based inorganic precursors or natural biogenic resources. However, HAp synthesized from both resources is bioactive and is given equal consideration for in-vitro and in-vivo biomedical applications. Based on various preliminary research studies, extraction of HAp from fish bone biogenic sources were reported. Kongsri et al. (2013); Panda et al. (2014) extracted HAp from fish scales. Furthermore, Biazar et al. (2020); Hasan et al. (2020); Latif et al. (2020); Surya et al. (2021) used distinct varieties of fish bones to extract HAp using different methods. The routes most commonly used for the synthesis of HAp are sol–gel, precipitation, co-precipitation, and hydrothermal (Malla et al., 2020). Malla et al. (2020) confirmed the formation of HAp extracted from ostrich bone; followed by alkaline and thermal treatment methods at 650°C in a muffle furnace for consecutive 6 h. Ur Rehman et al. (2021) used caprine bones to extract HAp by hydrothermal method. In addition, as the most stable and used form of calcium phosphate, HAp has a non-toxic and non-inflammatory effect (Jiang et al., 2022).

Furthermore, HAp was characterized using various spectroscopy techniques such as scanning electron microscopy (SEM) and laser-induced breakdown spectroscopy (LIBs) that elucidate the elemental mapping of biological substances. Several studies reported the Ca/P ratio of HAp by SEM EDX lay within the range of 1.5 to 2.13 (Akram et al., 2014; Pu'ad et al., 2019). From the findings of preliminary literature, only minimal data was found to calculate the Ca:P ratio of HAp by LIBS (Mazalan et al., 2018). Herein, the current study followed the protocol of Ca:P ratio by LIBS.

The flattened and circular tortilla with a thickness of about 2–6 mm and 160–300 mm in diameter is a traditional food that is exclusively consumed by the population. According to statistical data, the annual per capita consumption of tortillas was recorded as 7.4 million tons (Valderrama-Bravo et al., 2018). Sensory attributes analysis is also noteworthy in the tortilla industry that significantly affects the quality of tortillas (Valderrama-Bravo et al., 2020).

The current research has, therefore, taken into account the preparation and characterization of nano-sized-biogenic HAp using fish bones of the most abundantly consumed fish in our country named Rohu (Labeo rohita). HAp fortified tortillas were also developed as a final bioactive food commodity and were physically characterized and sensory evaluated. Tortilla one of the iconic foods, highly valued by consumers is an excellent vehicle for fortification. Globalization has resulted in an increasing demand for tortillas (Subiria-Cueto et al., 2019). It is, therefore, interesting to make calcium-fortified tortillas to help nourish those whose bone health is at risk.

2 | MATERIAL AND METHOD

2.1 | Procurement and preparation of fish-bone hydroxyapatite (HAp)

The bones of Rohu fish were collected from restaurants in different localities around Rawalpindi and Islamabad, Pakistan. The collected sample was cleaned and alkalinized to free from residual particles and the process was followed by drying, grinding, and sieving with mesh (95 μm). The calcination of the resultant powder was done at 850°C in a muffle furnace (Dentsply Ceramco; Neycraft JFF-2000). The annealed powder was further used to prepare nano-sized-biogenic fish bone hydroxyapatite (HAp) slurry in 0.1 g/ml distilled water with continuous magnetic stirring involving the process of ultra-sonication. Cole Parmer's Sonicator, (Power 220–230 VAC 50/60 HZ Cat No. C2-04711-45 S/N. 535,529 Y, USA) was used for sonication. The time of 20 min, frequency of 20 kHz, amplitude of 20%, and probe temperature of around 45°C were opted. The resulted slurry was dried after placing overnight to settle down for the removal of surfactant. Further analysis of dried slurry was carried out accordingly.

2.2 | Characterization of nano-sized-biogenic fish bone HAp

The annealed and ultra-sonicated nano-sized-biogenic HAp was characterized using different machine analyses and techniques including particle size distribution (PSD), Fourier transform infrared spectroscopy (FTIR), and laser-induced breakdown spectroscopy (LIBS), Scanning transmission electron microscopy (STEM) and X ray-diffraction (XRD). The calcium content of nano-sized-biogenic HAp was determined by atomic absorption spectroscopy (AAS).

2.2.1 | Fourier transform infrared spectroscopy

The FTIR spectra of nano-sized-biogenic fish bone HAp were obtained using the KBr method and recorded on the Bruker instrument. The powdered sample was palletized with KBr (1:10), pressed, and recorded in a region of 4000–400 cm^{-1} with optimum resolution (4 cm^{-1}).

2.2.2 | Particle size distribution

The measured amount of nano-sized-biogenic fish bone HAp specimen (1 mg) was suspended in 0.02% water to determine particle size distribution (PSD) using Malvern Instrument (Zetasizer Ltd, Spectris) followed by 1 h as post suspension time.

2.2.3 | Atomic absorption spectroscopy

The calcium content of nano-sized-biogenic fish bone HAp was assessed using the atomic absorption spectroscopy (AAS) technique following the method, described by Uddin et al. (2016). The specimen (0.1 g) was digested in 20 ml of HCl and HNO_3 (1:3) solution. All measurements were performed in triplicate.

2.2.4 | Laser induced breakdown spectroscopy

Laser-induced breakdown spectroscopy (LIBs) is a tool that illustrates the elemental mapping of biomaterials. The experiment of LIBS was carried out using a Q-Switched Nd:YAG laser (Quantal-Brilliant) beam, having a wavelength of 1064 nm, frequency of 10 Hz, a pulse width of 10 ns, and pulse energy of 40 mJ, coupled with a spectrometer. The spectral lines of the interface were processed with associated software (Ava Soft).

2.2.5 | Scanning transmission electron microscopy

Scanning Transmission Electron Microscopy (STEM) is a useful tool to explore the surface morphology and size of nano material (Rojas-Molina et al., 2020). Scanning Transmission Electron Microscopy (STEM) was operated on Nova-Nano-SEM at 10 kV to investigate the morphological and chemical nature of crystallinity of the tested specimen at a nano level. The tested specimen was spread onto Cu stab and images were obtained using an electron beam probe.

2.3 | X-ray diffraction

XRD spectra were used to determine the sample crystalline nature using a monochromatic CuKa (Carleton) radiation source at 0.02°, 40 mA current, and 40 kV voltage. The spectra were recorded at 2θ.

2.4 | Preparation of calcium-based tortilla

Non-fortified refined wheat flour tortillas (control) were prepared according to the method described by Topete-Betancourt et al. (2020) with some modifications. The standardized formulation was as follows; a measured amount of 100 g of refined wheat flour, salt (5 g), and butter (5 g) were mixed and kneaded with distilled water to reach 45% of the moisture of total weight. The varied amounts of HAp (nano-sized-biogenic HAp) used for fortification were in the range of 0.2%, 0.4%, and 0.6%, whereas formulation not containing HAp served as control. The HAp weight, cutting down the same amount of all-purpose flour from the specified formulation, has been shown in Figure 1. Tortilla dough was prepared by adding all the dry weighed ingredients in a sanitized bowl, hand mixed, kneaded, and set for 15 min to rise. Triplicate tortillas samples were prepared from each concentration. All the ingredients were properly mixed and blended. The dough was proportioned into a 35 g ball each, pressed with a rolling pin, and then heated tortilla on a nonstick hot griddle (preheated to 221.1°C) by turning over each side for 30 s. Pressing of tortillas tends to remove air pockets. The prepared nano-sized-biogenic HAp fortified calcium tortillas were then cooled down to room temperature, packed, and stored for snack time.

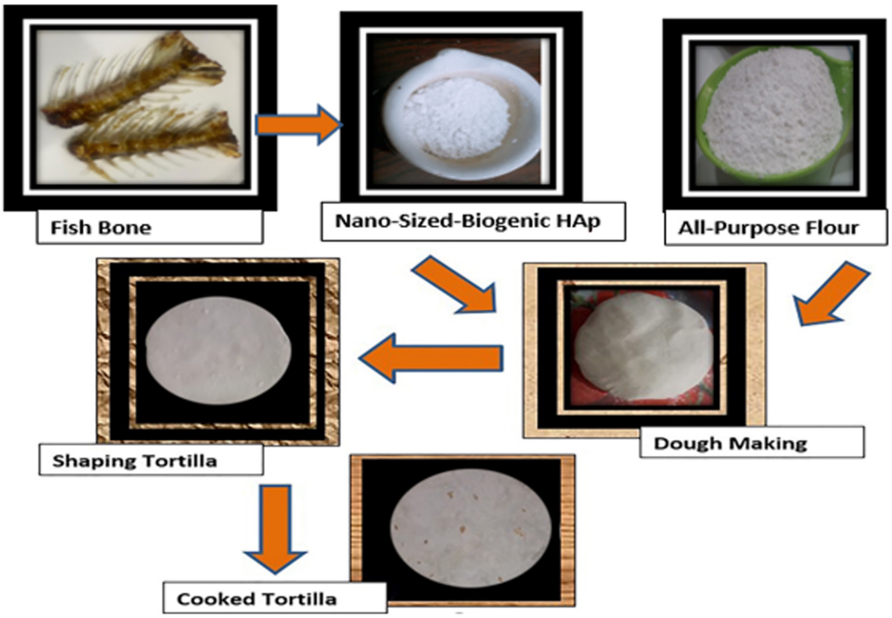

FIGURE 1 Development of tortilla with nano-sized-biogenic HAp of fish bone.

2.5 | Physical analysis of HAp fortified tortilla

2.5.1 | Moisture (%)

The moisture content of freshly prepared tortillas was determined by drying the tortillas for 24h at a temperature (25 ± 2°C). The moisture content (%) was calculated as follows

$$\text{Moisture}\,(\%) = \frac{(\text{Initial Weight} - \text{Dried weight})}{\text{Initial Weight}} \times 100$$

2.5.2 | Spread ratio

The spread ratio of the tested specimen was measured with its thickness (mm) and diameter (cm) using Vernier caliper (Mitutoyo, Japan) and manual perpendicular measurements, respectively as prescribed by (Winstone, 2010) with some modifications. The formula to measure the spread ratio is as follows

$$\text{Spread Ratio} = \frac{\text{Tortilla diameter (cm)}}{\text{Tortilla Thickness (mm)}}$$

2.6 | Sensory evaluation and consumer acceptance test

Forty trained sensory panelists (male = 23, female = 17) with mean age (30.23 ± 0.13 years) took part in sensorial test aspects of calcium-fortified tortillas. The sensory panelists signed consent forms and indicated food allergies to the ingredients used in the calcium-fortified tortillas before participation. All panelists were food scientists and well-trained. Though having expertise in assessing the taste of different commodities including cereals, meat, vegetables, fruits, etc., all panelists practiced for 6–7 h to train their palate on a daily basis so that they can easily distinguish all the sensory variables of tortilla. Each sensory panelist followed a hedonic scale ranking; (9) like extremely to slightly, (5) neither like nor dislike, and (1) dislike extremely to slightly, to evaluate each tortilla for its appearance, taste, aftertaste, mouthfeel, and overall acceptability (Nilsson, 2018; Subiria-Cueto et al., 2019). During the test session, the participants used distilled water to clean their palates before testing the next sample as described by Heymann and Lawless (2013).

A consumer acceptance test was also done on choosing 25 consumers to evaluate their liking and disliking of tortilla consumption. They scored each sample on considering the hedonic scale. Fresh water was also provided to them to rinse their palate before testing each sample.

2.7 | Statistical analysis

Descriptive analysis including mean and standard deviation was subjected to measure the responses of all triplicate samples for sensory attributes and physical characteristics of experimental tortillas using statistical software Minitab version 20.3. Tukey's test was performed to determine statistically significant differences among samples. Meanwhile, an inferential statistic test was applied to assess the differences among variables by one-way ANOVA with probability < 0.05. For PCA analysis, biplot, scree, score, and loading plots were determined. Graphs were designed on OriginLab (version 19) software.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of nano-sized-biogenic HAp

3.1.1 | Fourier transform infrared spectroscopy

The FTIR spectra (4000–400 cm^{-1}) of nano-sized-biogenic and unheated (raw) fish-bone HAp, have been presented in Figure 2. The prominent absorption peaks of calcined HAp have suggested the presence of organic group (C-H) at 2929.46 cm^{-1} and 2857.14 cm^{-1} which was not seen in nano-sized-biogenic HAp. The recorded peak at 3282.16 cm^{-1} indicated the existence of the O-H group in heat unheated HAp. Similarly, the 1741.47 cm^{-1} absorption peak was the indication of the organic amide group (C-N) in unheated HAp. Peaks at 1031.77 cm^{-1}, 690.43 cm^{-1} and 659.39 cm^{-1} were of great importance which justified the presence of inorganic phosphate functional group in nano-sized-biogenic HAp. Whereas similar peaks were recorded at 1031.29 cm^{-1}, 876.37 cm^{-1}, and 692.14 cm^{-1} in unheated HAp. In favor of the previous scientific literature, reported by Nawaz et al. (2020), the findings indicated the presence of phosphate group (PO_4^{3-}) at 565 cm^{-1}, 605 cm^{-1}, 875 cm^{-1}, and 1030 cm^{-1} were in close agreement with the findings of the current study.

3.1.2 | Particle size distribution

Particle size distribution (PSD) of nano-sized-biogenic HAp of fish bone was determined by Zetasizer Ver 7.11 standard (Malvern

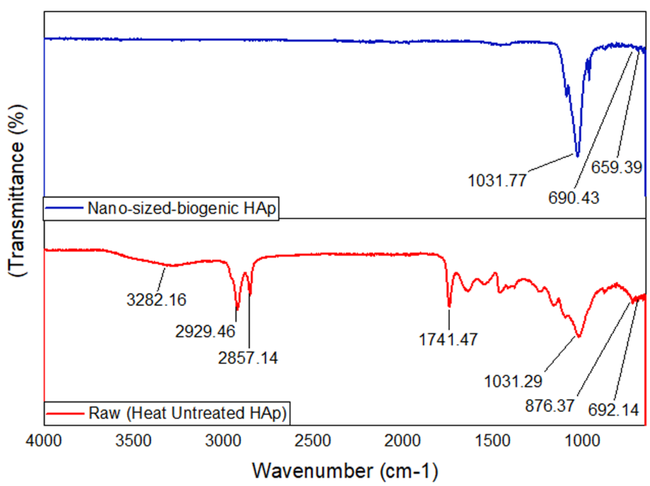

FIGURE 2 FTIR Spectra of raw and nano-sized-biogenic HAp.

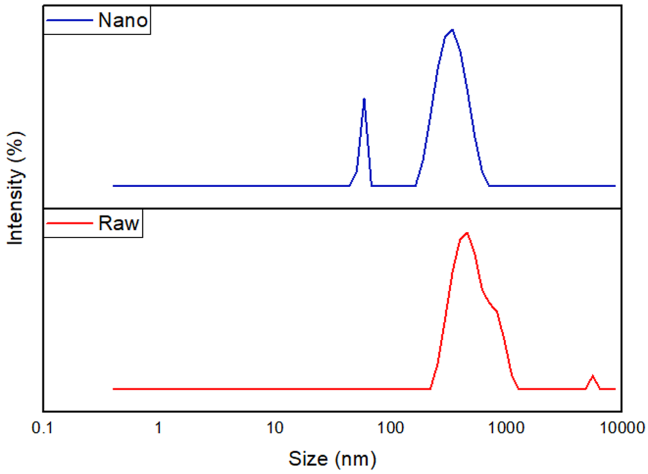

FIGURE 3 Particle size distribution of nano-sized-biogenic HAp in comparison with the raw form.

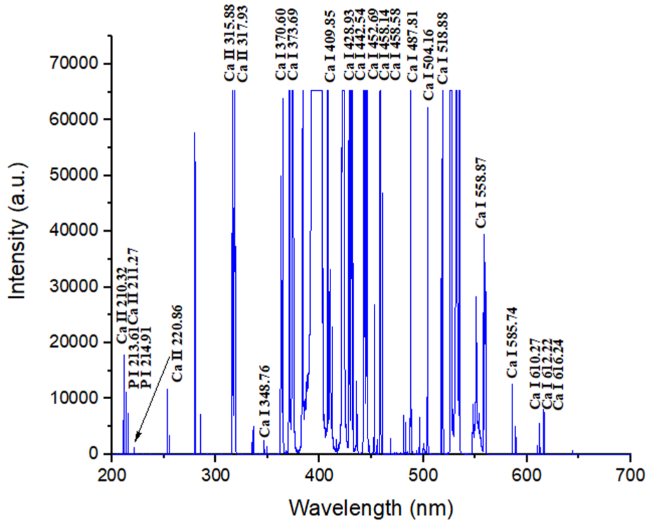

FIGURE 4 LIBS spectra of nano-sized-biogenic HAp.

Instrument Ltd., Grovewood, Worcestershire, UK) and distribution spectra have been shown in Figure 3. The tested specimen was dispersed in water, having a refractive index (RI) of 1.33 and viscosity of 1.736 cP. The particle size of nano-sized-biogenic HAp of fish bone was observed at conspicuous peaks of 58.8 nm and 342 nm with a PDI value of 0.407 when compared to the raw form of HAp at 459 nm and 5560 nm with a PDI value of 0.443. The mono-homogeneity level was linked with a polydispersity index (PDI) that expressed the excellent stability of particles. Nawaz et al. (2020), determined the release and retention of reduced-size inorganic calcium owing to the breakage of the bond in fish bone. Moreover, ultra-sonicated treatment is the novelty of the current study to reduce the particle size. Furthermore, Jiang et al. (2020) determined nano-scaled fish bone with a particle size of 150 nm.

3.1.3 | Atomic absorption spectroscopy

The calcium content in the nano-sized-biogenic HAp sample of fish bone was confirmed by atomic absorption spectroscopy (AAS). The findings of the current study indicated that calcium content was 128.04 ± 0.19 ppm. The findings of the current study were affirmed by Florentina et al. (2018); who estimated calcium content that ranged from 6.76 to 7.78 ppm in seawater shrimp by Atomic Absorption Spectroscopy.

3.1.4 | Laser-induced breakdown spectroscopy

The spectra of LIBS for the nano-sized-biogenic HAp of fish bone have been depicted in Figure 4. Several characteristics emission lines determined by Calcium-I have been recorded on 348.76 nm, 370.60 nm, 373.69 nm, 409.85 nm, 428.93 nm, 442.54 nm, 452.69 nm, 458.14 nm, 458.58 nm, 487.81 nm, 504.16 nm, 518.88 nm, 558.87 nm, 585.74 nm, 610.27 nm, 612.22 nm, and 616.24 nm as described in Table 1 and Figure 4. Calcium-II emission lines have been recorded as 210.32 nm, 211.27 nm, 220.87 nm, 315.88 nm, and 317.93 nm. The emission lines for P were observed at 213.61 nm and 214.91 nm. The emission lines of spectra are identified by matching the wavelengths with NIST atomic absorption database (https://www.nist.gov/pml/atomic-spectra-database) and published scientific literature (Tariq et al., 2017). The emission lines of LIBS spectra and intensities of Ca and P species have been shown in Table 1.

The basic principle of elemental concentration refers to the intensity of spectral emission lines in tested HAp. The best possible match for the Ca/P ratio was recorded on the Ca emission line at 585.74 (12,592.91 a.u.) and with the P emission line at 214.91(7451 a.u.). The Ca/P ratio in the current study was 1.69, which was higher than the reference value, that is, 1.67 (Ebrahimi & Sipaut, 2022; Tariq et al., 2018).

3.1.5 | X-ray diffraction

The X-ray diffraction (XRD) technique is applicable to elucidate the crystalline nature of biomaterials. Figure 5 expresses the crystalline nature and phase composition of resulted nano-sized-biogenic HAp of fish bone by XRD pattern at $2\theta = 10°$ to 70°. Phase analysis was done using ICCD standard HAp 00–009-0432. The hexagonal nano-crystalline nature of extracted nano-sized-biogenic HAp peaks was observed at 2θ values of 31.74°, 32.88°, and 33.85° corresponding to 256, 185, and 300 plane phases. The deviated peaks from the standard may be due to the presence of other ions (Mg^+, Na^+, K^+). Maximum intensity may lead to a higher degree of crystalline lattice in the composition of HAp. From the literature, it was ensured that the calcination process also enhanced the crystallinity and substituted the Ca, PO_4^{3-} with CO_3^{2-} (Bano et al., 2019). In this regard, the affirming literature was presented by Hammood et al. (2019) who highlighted major defined peaks planes at 112, 211, and 300 at 2θ near 31.8°, 32.2°, and 32.9° in fish bone powder.

TABLE 1 Emission lines of LIBS spectra and intensities of Ca and P species in nano-sized-biogenic HAp of fish bone

Emission lines	Wavelength (nm)
Ca-I	348.76, 370.60, 373.69, 409.85, 428.93, 442.54, 452.69, 458.14, 458.58, 487.81, 504.16, 518.88, 558.87, 585.74, 610.27, 612.22, 616.24
Ca-II	210.32, 211.27, 220.87, 315.88, 317.93
P-I	213.61, 214.91
Ca/P Ratio	1.69

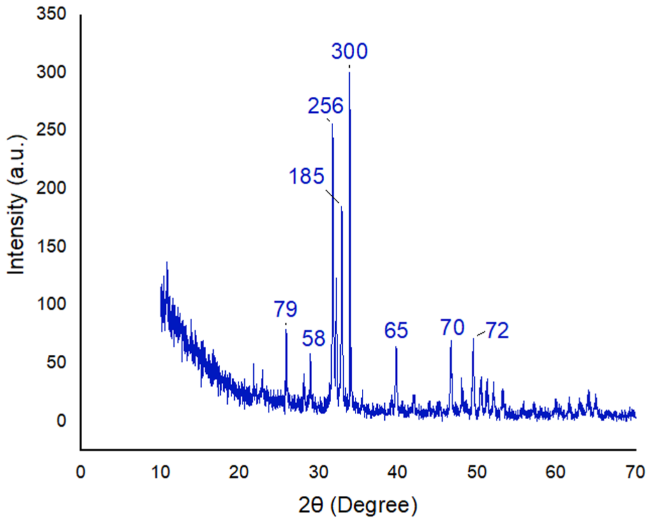

FIGURE 5 XRD pattern of nano-sized-biogenic HAp.

3.1.6 | Scanning transmission electron microscopy

A significant difference in structural morphology and crystal lattice images of nano-sized-biogenic HAp of fish bone has been confirmed by STEM at the nano level with different magnifications as shown in Figure 6. The particle size and surface of nano-sized-biogenic HAp powder micrograph at magnification 5000× (10 μm) and 10,000× (5 μm) showed a continuous layer of HAp with rough, porous, and crystalline agglomerated pattern (Figure 6a,b). At 25,000×, it seems that branched-chain biomaterial of about 2 μm size has been formed (Figure 6c). When biomaterial was observed at 50,000× and 100,000×, it seemed that HAp was composed of small granules with a smooth surface having almost uniform sizes ranging from 500 nm to 1 μm (Figure 6d,e). Similarly, the morphological examination confirms particle structure as smooth branched chain globules which is a good indication of the development of nano-sized and agglomerated crystal lattice patterns. High temperature plays a significant role in the formation of circular crystals; a similar trend was observed in heat-treated nano-sized-biogenic HAp. Rafeek et al. (2018) reported a similar morphological behavior pattern of calcium phosphate ceramics powder with grain sizes up to 200–250 nm.

3.2 | Physical properties of nano-sized-biogenic HAp-fortified tortillas

The physical properties of tortillas prepared from nano-sized-biogenic HAp of fish bone are presented in Table 2. Thickness (mm), diameter (cm), spread ratio, and weight (g) of the non-fortified and

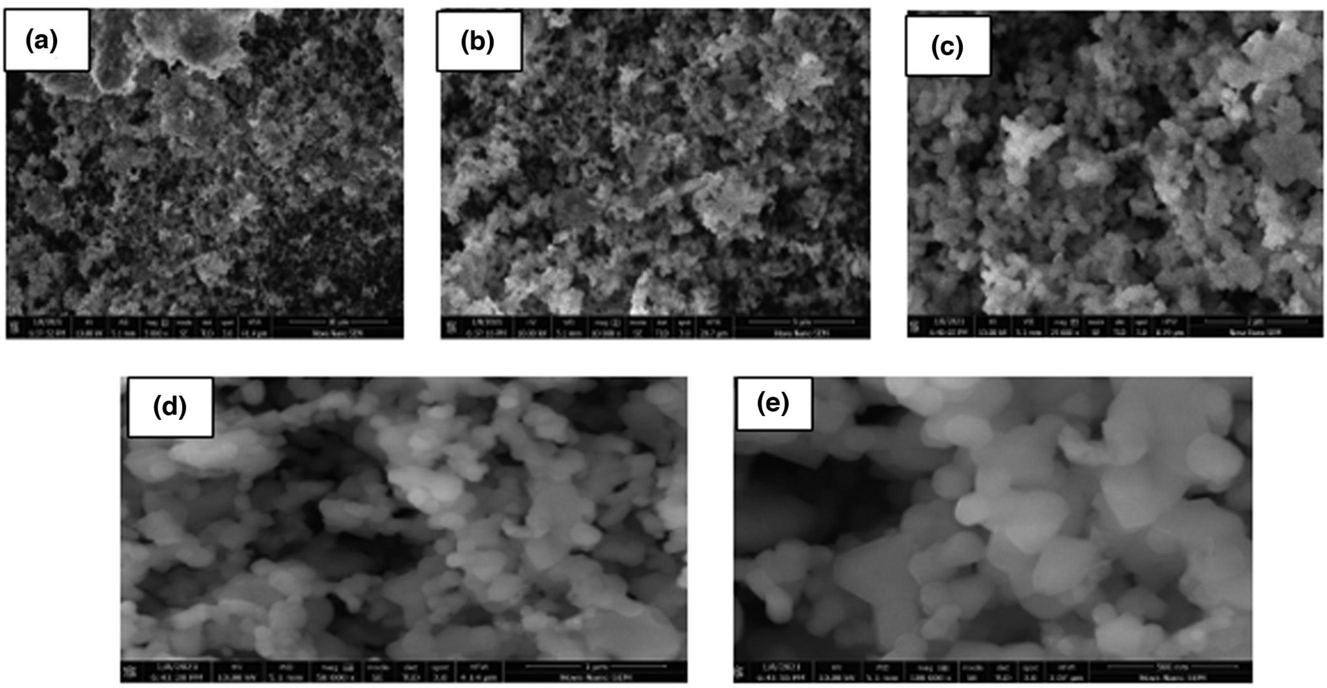

FIGURE 6 Scanning transmission electron microscopy (STEM) of nano-sized-biogenic HAp with different magnification (a) 5000× with 10 μm; (b) 10,000× with 5 μm; (c) 25,000× with 2 μm; (d) 50,000× with 1 μm; (e) 100,000× with 500 nm.

nano-sized-biogenic HAp fortified tortillas were significantly different from each other ($p \leq 0.05$). The weight of the tortilla increased as more nano-sized-biogenic HAp was added to the formulation. The initial weight ranged from 35.82 ± 0.38 to 46.18 ± 0.25 g whereas the dried weight from 32.90 ± 0.11 to 42.53 ± 0.95 g (Table 2). The moisture (%) ranged from 6.20 ± 0.05 to 8.15 ± 0.83.

The maximum thickness of the tortillas was seen in the NBio-FB3 group 2.46 ± 0.03 followed by 2.42 ± 0.01 in the NBio-FB2. From findings it was obvious that as the concentration of nano-sized-biogenic HAp increased, the thickness of the tortilla raised, and minimum expansion of diameter was observed (Table 2). The spread ratio was also kept on declining with increasing thickness and lay within the range of 5.95 ± 0.08 to 5.80 ± 0.03. In relation to previous scientific literature reported by Asiyanbi-Hammed and Simsek (2020), the findings of the current study are in favor of the reciprocal interrelationship of thickness and diameter.

3.3 | Sensory evaluation and consumer acceptance

The prepared nano-sized-biogenic HAp fortified tortilla samples were labeled as "C, NBio-FB1, NBio-FB2, and NBio-FB3". Based on consumer acceptance and better nutritional quality, three nano-biogenic calcium-fortified tortilla formulations, that is, NBio-FB1 (Tortilla with 0.2% nano-sized-biogenic HAp), NBio-FB2 (Tortilla with 0.4% nano-sized-biogenic HAp) and NBio-FB3 (Tortilla with 0.6% nano-sized-biogenic HAp) along with control (C = Tortilla with 0% nano-sized-biogenic HAp) were selected. On the basis of AAS findings, the incorporated amount of pure HAp in different formulations (NBio-FB1, NBio-FB2, and NBio-FB3) of tortillas provided approximately 20–60% of calcium/serving size (100 mg). Additionally, this calcium amount in different formulations was other than the amount of calcium available in 100 g of refined wheat flour. These findings strongly affirmed that fish bone extracted nano-biogenic HAp could be an ingredient to increase calcium content in developed products.

The mean values and standard deviation of each formulation with sensory attributes have been presented in the table. The p-value $<.05$ depicted that all formulations are significantly different from each other (Table 2). The sensory attributes depicted that the formulation NBio-FB1 is close to the control group in all aspects of sensory evaluation followed by the NBio-FB2 formulation. In the context of sensory evaluation, it is important to take into consideration the taste, aroma, aftertaste, and mouthfeel of the finished product. More calcium incorporation increases chalkiness and changes the taste of the tortilla through calcium ions (Palacios et al., 2021). Overall acceptability was 7.94 ± 0.30, seen in NBio-FB1 lay close to the control group (8.81 ± 0.17). A similar context was reported by Rodríguez-Noriega et al. (2021) who assessed the sensory profile of flour tortillas using a flash profile. The results were in close alignment with earlier findings.

The consumer acceptance test was also carried out on 25 participants using the 9-point hedonic scale. This scale was divided

TABLE 2 Physical and sensory evaluation of nano-sized-biogenic HAp fortified tortilla

Physical and sensory evaluation												
	Sensory attributes						Physical characteristics					
Treatment Group	Appearance	Taste	Aroma	Aftertaste	MF	OAA	Initial Weight (g)	Dry Weight (g)	Moisture (%)	Thickness (mm)	Diameter (cm)	Spread Ratio
C	9.34 ± 0.10^{a}	8.21 ± 0.79^{a}	8.65 ± 0.08^{a}	8.88 ± 0.09^{a}	8.97 ± 0.07^{a}	8.81 ± 0.17^{a}	35.82 ± 0.38^{d}	32.90 ± 0.11^{d}	8.15 ± 0.83^{a}	2.44 ± 0.03^{d}	14.48 ± 0.02^{a}	5.95 ± 0.08^{b}
NBio-FB1	8.38 ± 0.63^{b}	7.89 ± 0.43^{b}	8.13 ± 0.17^{b}	7.25 ± 0.29^{b}	8.07 ± 0.67^{b}	7.94 ± 0.30^{b}	44.57 ± 0.4^{c}	41.81 ± 0.63^{c}	6.20 ± 0.05^{d}	2.42 ± 0.01^{b}	14.41 ± 0.01^{b}	5.93 ± 0.02^{c}
NBio-FB2	7.41 ± 0.07^{c}	7.02 ± 0.03^{c}	7.20 ± 0.12^{c}	6.97 ± 0.1^{c}	7.24 ± 0.38^{c}	7.17 ± 0.11^{c}	45.68 ± 0.21^{b}	42.04 ± 0.79^{b}	7.96 ± 0.74^{b}	2.46 ± 0.03^{a}	14.35 ± 0.02^{c}	5.83 ± 0.08^{a}
NBio-FB3	5.09 ± 0.14^{d}	4.39 ± 0.22^{d}	6.00 ± 0.55^{d}	6.07 ± 0.43^{d}	6.35 ± 0.28^{d}	5.58 ± 0.14^{d}	46.18 ± 0.25^{a}	42.53 ± 0.95^{a}	7.91 ± 0.04^{c}	2.46 ± 0.01^{b}	14.27 ± 0.08^{d}	5.80 ± 0.03^{d}
p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00

Note: p-value $< .05$ = significant.

Abbreviations: C, Control; NBio-FB1, 0.2% nano-sized-biogenic HAp of fish bone; NBio-FB2, 0.4% nano-sized-biogenic HAp of fish bone; NBio-FB3, 0.6% nano-sized-biogenic HAp of fish bone; MF, Mouthfeel; OAA, overall acceptability.

into three areas (likeness, neutral, and dislikeness) and presented in Figure 7. All the formulations were liked by consumers ($p < .02$). From findings, generally, 63.5% of consumers accepted refined wheat flour tortilla (control). Most of the consumers were in favor of the likeness of NBio-FB1 as their comments indicated the close resemblance of this formulation (56%) with the control group. Most of the consumers were unable to distinguish the taste, aroma, aftertaste, and mouthfeel between control and nbio-fb1 as compared to other formulations (NBio-FB2 and NBio-FB3). The comments of some consumers indicated that NBio-FB1 was balanced with all the ingredients added to it whereas the other two formulations were giving off flavor after eating. These remarks confirm the findings evaluated by trained panelists regarding sensory attributes, describing NBio-FB1 as good in taste (p = .00) and having a good appearance (p = .00). However, 33% of consumeres also disliked the NBio-FB1 formulation but this figure was less than the other two formulations NBio-FB2 (49.5%) and NBio-FB3 (52.2%). Furthermore, some consumers gave neutral responses that were recorded as 11–21% in all formulations.

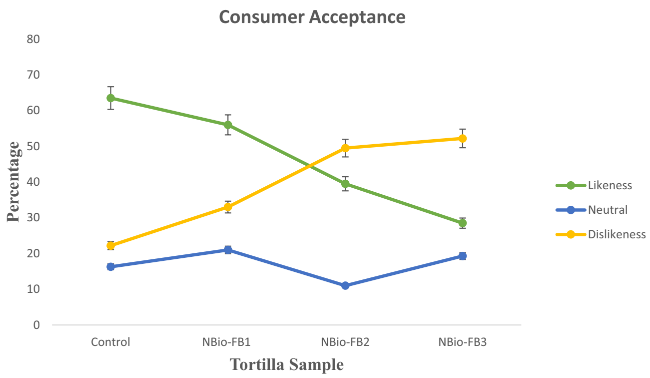

FIGURE 7 Responses of consumers on acceptance of different tortilla formulations.

3.4 | Principal component analysis

In the current study, principal component analysis (PCA) was applied for the evaluation of physical and sensory characteristics to assess their relationship with varied concentrations of nano-sized-biogenic HAp has been shown in Figure 7. The PCA analysis criteria were applied to extract the eigenvalues from original data. It was of worth-mentioning that the scree plot (Figure 8a) clearly affirmed the trend of the graph line of all studied factors. The total variance was 74.82% accounting for the first two principal components (PC) out of 12-dimensional variables. The first-dimensional component (PC1) determined 58.51% variance whereas the second dimensional

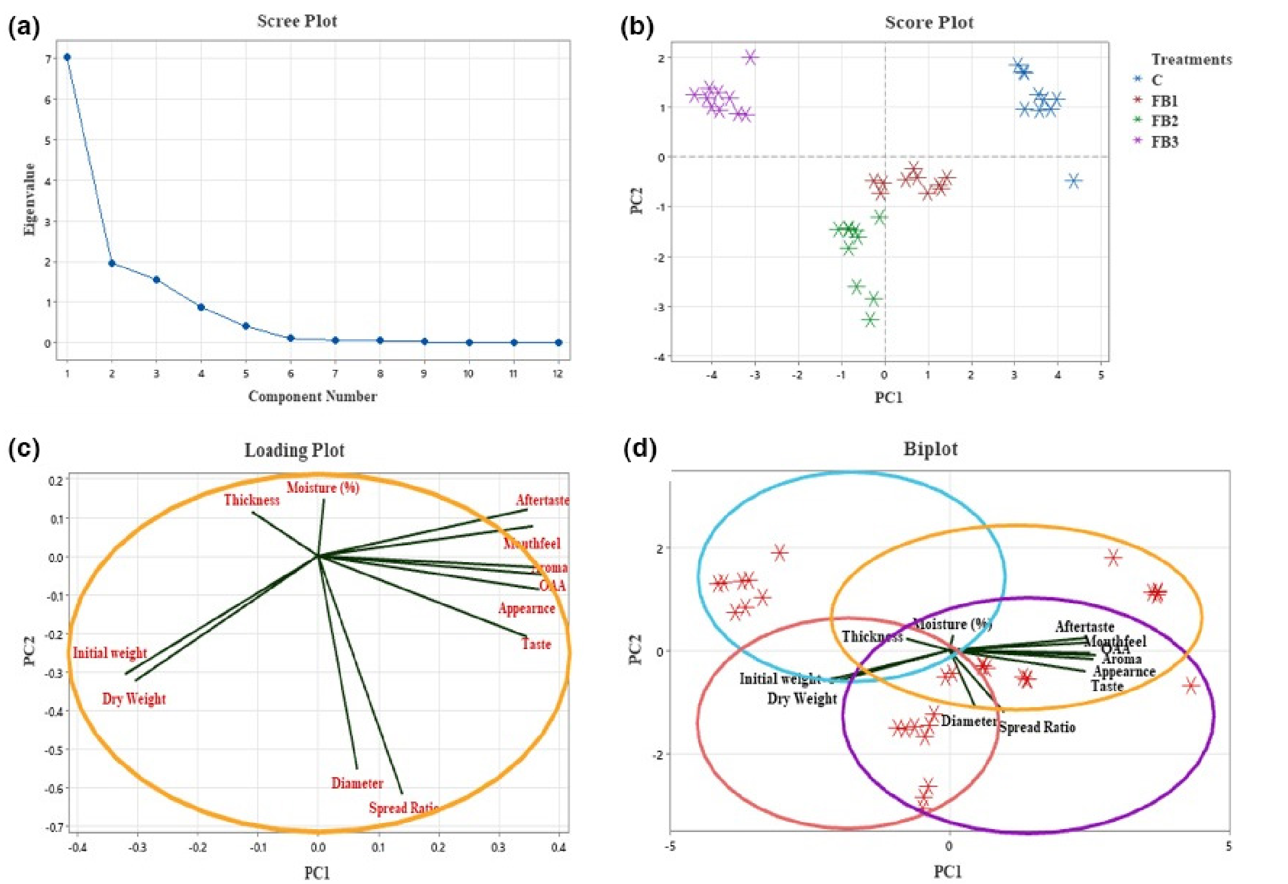

FIGURE 8 PCA plot of evaluation of sensory and physical characteristics of all the fortified formulations of tortilla (a) Scree Plot; (b) Score Plot; (c) Loading Plot; (d) Biplot.

component (PC2) determined 16.31% variation. Figure 8b presented score plot in which four formulations (Control = C, 0.2% nano-sized-biogenic HAp = NBio-FB1, 0.4% nano-sized-biogenic HAp = NBio-FB2 and 0.6% nano-sized-biogenic HAp = NBio-FB3) of tortilla and their interaction with each other has been verified. The loading plot (Figure 8c) underlined the distribution of all tested variables. In the biplot graph (Figure 8d), it was obvious from the findings that moisture (%) exhibited a negative but strong relationship with initial and dry weight. Moreover, the findings of diameter (cm) and thickness (mm) were reciprocal to each other and centralized their interlinking with spread ratio. The attributes of sensory evaluation are closely placed showing a strong correlation with each other that could be seen in the biplot (Figure 8d). It was also clear that NBio-FB1 and NBio-FB2 are strongly linked with each other. NBio-FB1 with control showed an association in terms of taste, diameter, aroma, appearance, and spread ratio, whereas NBio-FB2 was linked with NBio-FB3 in terms of moisture (%) and thickness that were sparingly cross-linked. Somewhat similar findings were reported by (Semjon et al., 2018); who studied the PCA impact on sensory evaluation and instrumental analysis in cheese. Another literature reported by Rana et al. (2021), emphasized the relationship of physical and functional properties for discriminating different cinnamomum species in averting food fraud by PCA analysis.

4 | CONCLUSION

This study is found to be successful in the synthesis of nano-sized-biogenic hydroxyapatite (HAp) sourced from fish bone to formulate functional tortillas. The incorporation of HAp enhances the calcium level of the product. The resultant HAp powder was also characterized using different spectroscopy techniques. Furthermore, the sensory attributes of each formulated sample were assessed using a hedonic scale. Moreover, PCA determined 74.82% total variance among physical and sensory attributes of the developed products. Despite these outlined attributes, with the addition of more HAp in the product, acceptability masks the chalky appearance in color and taste of the finished product which is a major concern. Besides having good acceptability and spread ratio of NBio-FB1 formulation, this developed product has lesser moisture (%) comparable to other formulations.

AUTHOR CONTRIBUTIONS

Saba Liaqat: Methodology; Writing - original draft; Investigation; Conceptualization; Writing - review & editing; Visualization; Software; Formal analysis; Data curation; Validation. **Zaheer Ahmed**: Validation; Visualization; Writing - review & editing; Project administration; Supervision; Resources. **Qasim Ali**: Formal analysis. Ali Akbar: Writing - review & editing. **Nauman Khalid**: Resources; Investigation.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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