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ORIGINAL PAPER

EVALUATION OF THE NUTRITIONAL VALUE OF EDIBLE FINE BONE POWDER, RICH IN BIOELEMENTS EXTRACTED USING A LONG-TERM HYDROTHERMAL METHOD, FOR NUTRITION AND HUMAN CALCIUM SUPPLEMENTATION*

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Abstract

Although bone is rich in vital proteins and minerals, it is inedible, and its use in nutrition and nutritional supplements is hampered by its strength as well as the risk of disease transmission and lead contamination. Since bones are difficult to grind, high-quality fine bone powder (FBP) without additives is still needed. The study was designed to extract FBP and evaluate its physicochemical properties and value for human nutrition based on its chemical composition, including the mineral and toxic metal content. The applied long-term hydrothermal treatment sterilized and softened hard bones, which were then ground into FBP that inherited bone properties. The physicochemical properties of the extracted FBP were studied, including the structure, composition, thermal behavior, morphology, elemental analysis, and proximate composition analyses. The high-quality FBP has indeed inherited bone properties; it has micro-crystalline particles with sizes less than 100 µm and is characterized by a higher content of biological hydroxyapatite, 65% by weight of FBP with a calcium/phosphorous ratio of 1.57, and it is rich in protein and vital bioelements, such as Na, Mg, Fe, Si, Al, K, Ba, Cr, Cu and Zn. The powder is free from lead and cadmium, which are well below detection limits. The Recommended Dietary Allowances (RDAs) for calcium are 700-800 mg per day, which equates to about 2800-3300 mg of FBP per day. Given the beneficial protein and biomineral composition of FBP, it may be a promising choice in the development and enrichment of nutritional products and calcium supplements.

Keywords: fine bone powder, bioelements, trace elements, hydrothermal treatment, nutrition, calcium supplements.

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INTRODUCTION

Bones contain large amounts of proteins, biological apatite and biominerals, but their strength and rigidity make them inedible and unusable for nutrition and nutritional supplements (Hendriks et al. 2002, Hendriks et al, 2004, Khalil et al. 2017). Bone is a complex, physically strong, highly organized, and specialized connective tissue (Berendsen, Olsen 2015) composed of two parts: proteins and biological HAp, which account for about 35% and 65% of the total bone weight, respectively (Vallet-Regi, Navarrete 2008). Bones are also rich in vital biominerals that are important in metabolism and cellular functions (Danilchenko et al. 2019). The biominerals which are substituted in the bone structure are main minerals (Ca and P in the molar ratio 1.93), macro-elements (Mg, Na, and K), and trace elements (Sr, Zn, Fe and Si), as well as the carbonate group minerals (Basle 1990, Prentice, Bates 1994, Martınez-Valverde et al. 2000).

Bone powder is used as a dietary supplement for calcium and phosphorous, which are essential for improving the function and health of bones, teeth, muscles, and nervous system (Hintz, Schryver 1972, Piste et al. 2013). However, there are many challenges and drawbacks when using bones either as an edible bone meal or for medicinal uses. The challenge of preparing fine bone powder (FBP) is attributed to the high strength and hardness of the bone, which requires heavy mechanical work to grind the hard bone into fine particles (Ogedengbe, Abadariki 2014). Also, possible transmission of infections and diseases, such as bovine spongiform encephalopathy or Salmonella pseudomonas, remains a problem (Johnson et al. 2011). In addition, bone powder may cause side effects resulting from the high level of toxic elements, such as lead, that can be incorporated into bones (Fox 1987). Several production processes are applied to prepare bone powder, including sterilization, combustion, and alkaline hydrolysis, but none of them can prepare sterile and pure FBP (Chaala Roy 2003, Conesa et al. 2003, Deydier et al. 2005, Cascarosa et al. 2012, Krička et al. 2014).

Here, the current study demonstrated the use of long-term hydrothermal treatment to sterilize and soften bones in order to facilitate grinding it into fine, edible bone powder for nutrition and dairy supplementation. Hydrothermal treatment can sterilize and soften bones, enhance their nutritional and medicinal uses, as well as extract biological apatite, rich in trace elements. Hydrothermal treatment of bone in water at high temperatures and pressures is an effective method for causing an abrupt decrease in bone strength and stiffness due to collagen denaturation and damage (Morsy et al. 2018.). Also, the hydrothermal treatment at temp. above 100°C and pressure at 1.5 atm for 20 min can cause cell death and provide complete sterilization of samples (Garibaldi et al. 2017). There is still a need to explore and use solid bones to meet the current and future demand for food. Given the beneficial protein and biomineral composition of FBP, it may be a promising

choice for development and enrichment nutritional products and calcium supplements. Therefore, the main objective of this study is to extract fine bone powder (FBP) that inherits bone properties by long-term hydrothermal treatment at high temperatures and pressures for many hours applied to soften the bone and to ensure that all potential pathogens are eliminated. The physicochemical properties of the extracted FBP were studied, which include structure, composition, thermal behavior, morphology, elemental analysis, and proximate composition analyses.

MATERIALS AND METHODS

Healthy femur bones from an animal's species (domestic cow: *Bos Taurs*), from 3-year-old specimens without any apparent pathologies, were collected from a local meat market located in El-Mahalla City, Egypt. Fine bone powder (FBP) was extracted from the raw bone using a long-term hydrothermal approach to bone pretreatment and bone powder extraction. Bone pretreatment included washing and boiling the bones with water to ensure decontamination. Three kilograms of bones were cleaned, chopped, and washed with fresh water. Samples were boiled in water at a ratio of 3:1 water/ /sample for 1 h to remove contamination. Bone powder extraction involved the hydrothermal treatment of filtered bone in a commercial autoclave (130°C and 1.5 atm for 4 h) to soften the bone and ensure that all potential pathogens are eliminated. The treated soft bone is filtered, washed, dried, ground in a blender, and sieved to obtain FBP.

The phase analysis and crystal structure of fine bone powder (FBP) were investigated using X-ray diffraction analysis (XRD: GNR-APD 20000 pro, H423-vertical diffractometer) in the range (2θ from 20° to 70° with a step size of 0.05°). The infrared active covalent functional groups were investigated with a Fourier transform infrared spectrometer (FTIR: Bruker Tensor 27 Spectrometer) with a spectral resolution of 4 cm^{-1} in the region from 400 cm^{-1} to 4000 cm^{-1}. The thermal transformation process of FBP to biological apatite was studied using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), performed with SDT Q600DSC-TGA equipment (TA Instruments, USA). The analysis was made at a heating rate of 10°C min^{-1} within the heating range from 21°C to 880°C in a stream of nitrogen. The surface microstructure and morphology of FBP were investigated using scanning electron microscopy (SEM: JEOL JSM 6510 lv) operating at an accelerating voltage of 30 kV.

Proximate composition analyses (moisture, protein, and ash contents) of FBP were performed. The moisture content of the samples was evaluated gravimetrically after drying at 105°C in an electric oven until reaching constant weight. The Kjeldahl procedure was used to measure the crude protein of FBP. Total ash content was assessed by burning the samples in a muffle

furnace at 700°C for 2 h to remove all organic matter (Udall, McCay 1953, Benedict 1987).

For elemental analysis, samples were digested with nitric acid at 90°C until a clear solution was obtained, which was filtered and diluted with deionized water. The measurements were performed using inductively coupled plasma optical emission spectrometry (ICP-OES). Phosphorous was measured using UV-visible spectrophotometry using the ammonium phosphomolybdate method (Bartels, Roijers 1975, Chaube, Gupta 1983).

Proximity composition analysis and element analysis tests were performed in triplicate and data were analyzed using SPSS software (version 20, SPSS Inc., USA).

RESULTS AND DISCUSSION

Structural characterizations of FBP

The XRD patterns of fine bone powder (FBP) – Figure 1*a*, shows wider diffraction peaks at 2θ values of 25.85°, 31.79°, 32.91°, 39.83°, and 49.54° for the diffraction peaks (002), (211), (300), (310) and (213) respectively. For phase identification, the XRD pattern of FBP was compared with standard diffraction data for hydroxyapatite (ICDD No. ICDD 9-432). The results showed that FBP contains a crystalline hydroxyapatite phase. The broad diffraction peaks of FBP can be mainly attributed to the presence of organic matter (Morsy 2015).

Compositional analysis by FTIR

In the FTIR spectrum of FBP (Figure 1*b*), a broadband is observed at 1640 cm^{-1}, 2852 cm^{-1}, and 2921 cm^{-1}, indicating the presence of proteins and the organic phase (Barbara et al. 2021). FTIR shows that FBP contains many bands in agreement with the bands that characterize biological hydroxyapatite, namely the phosphate, hydroxide, and carbonyl group. The phosphate group exhibits four distinct internal vibration modes at 608 cm^{-1}, 868 cm^{-1}, 987 cm^{-1}, and 1088 cm^{-1} (Morsy 2016, Ashokan et al. 2021). The carbonate phase ($MgCO_3$ or $CaCO_3$) exhibits two vibration modes at 1406 cm^{-1} and 1462 cm^{-1}, while the hydroxyl group has a vibration mode at 3450 cm^{-1} (Morsy 2016, Ginalska et al. 2021, Ashokan et al. 2021). The carbonate ion vibration band indicates the presence of small amounts of carbonate in the sample. The FTIR data for FBP is consistent with the XRD results, and both the FTIR and XRD data confirmed that the extracted fine bone powder contains mainly a crystalline hydroxyapatite phase.

Microstructure analysis of FBP

The SEM image (Figure 2*a*) shows the morphological characteristics of FBP. The image reveals irregularly shaped microparticles with sizes less

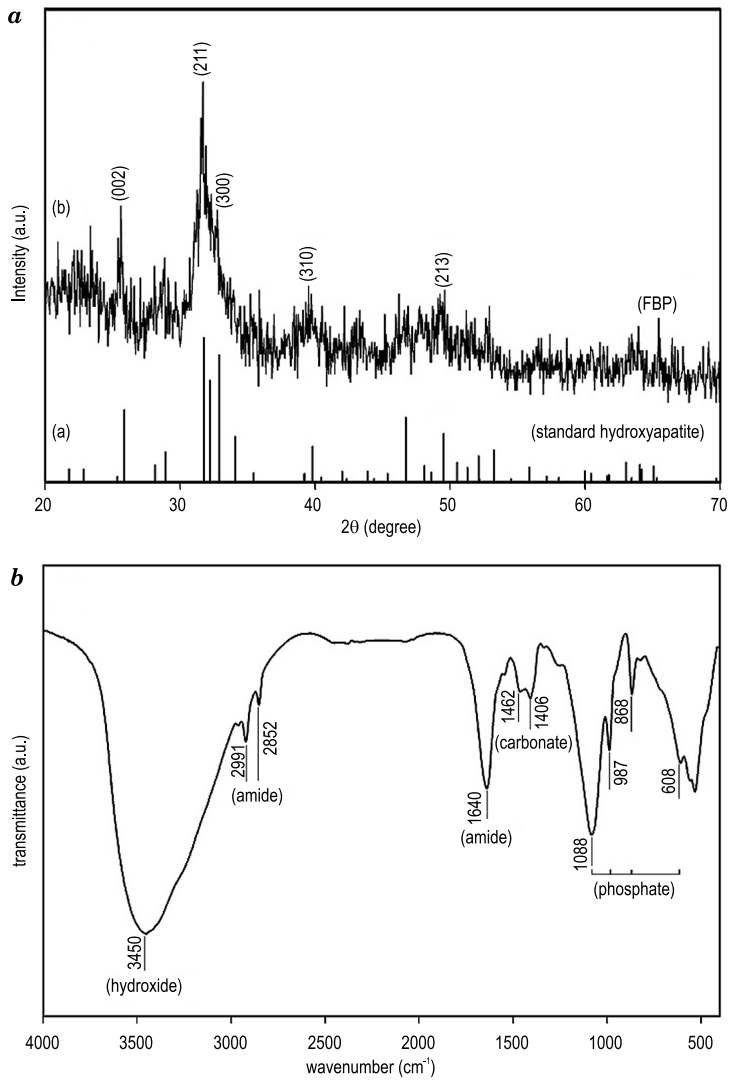

Fig. 1. XRD patterns of Standard hydroxyapatite and FBP (*a*), and FT-IR spectrum of FBP (*b*)

than 100 µm, and the magnified surfaces of the microparticles show that biological hydroxyapatite has been embedded within the organic matrix. The extracted FBP microparticles formed are composed of the homogeneous distribution of biological hydroxyapatite within the organic matrix.

Thermal analysis

Figure 2*b* shows the results of the TGA and DSC curves for the prepared fine bone powder. The graphs of the sample that were heated to 880°C showed a pattern illustrating three phases of the weight loss process.

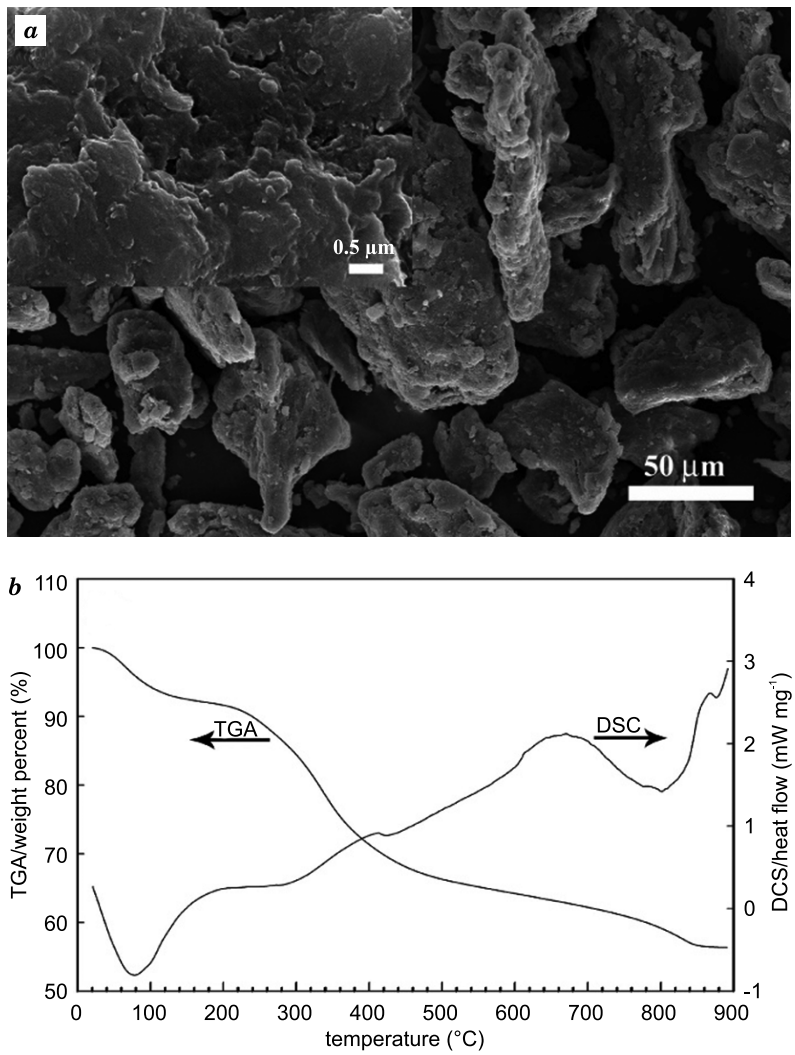

Fig. 2. SEM images of surface of FBP (*a*) and TGA/DSC analysis of extracted FBP (*b*)

The samples underwent a total weight loss of about 44% in the temp. range from 21°C to 880°C. The first stage of weight loss is 7.58% weight loss, in the temperature range of 21-169°C, which is attributed to the evaporation of adsorbed water. The weight change due to water evaporation is shown as a change in heat flux in the DSC curve due to the heat absorption process taking place at 77°C. The second stage of weight loss is a significant weight loss of 28.31% which is attributed to the decomposition of organic matter. It is characterized by a rapid slope change of the TGA curve in the temperature range of 170-576°C. The DSC thermograph shows an exothermic process around 405°C and an increase in heat flow in the temperature range from

280 to 670°C. The third stage of weight loss corresponds to a weight loss value of 7.27%, in the temperature range from 577°C to 880°C. This weight loss is attributed to the slow removal of carbonate ions in apatite bone ceramics. This observed weight change corresponds to the change in heat flow in the DSC curve due to the endothermic process occurring at 800°C due to the sample phase transformation, and the loss of the carbonate group.

Proximate composition and elemental analysis

FBP was extracted from bones using hydrothermal treatment to soften hard bone in order to facilitate its grinding to fine powder. FBP is composed of micro-sized particles and has pale yellow color without any undesirable odor. The amount of FBP extracted from raw bones was 85%, and about 15% of bones' content was dissolved in water during the hydrothermal treatment. As a source of calcium, phosphorous, and protein, FBP can be used to develop and enrich food products and calcium supplements. Extraction of sterile FBP with proper characteristics indicated that the hydrothermal treatment is a suitable technique for extraction. The composition of the extracted FBP was not cited in the literature. The water content as a part of the proximate composition of FBP represents about 10%. The ash content was a major part of the proximate composition of FBP, which was 55.5% for FBP (wet-basis) and was 65% for FBP (dry-basis). The results shown in Table 1 represent the

Table 1

Proximate composition and elemental analysis content of FBP (given as mg 100 g^{-1} or g 100 g^{-1})

Proximate composition		
Moisture	(g 100 g^{-1})	7.01 ± 0.13
Protein	(g 100 g^{-1})	35.78 ± 1.11
Ash	(g 100 g^{-1})	55.5 ± 1.88
Elemental analysis content		
Ca	(g 100 g^{-1})	25.14 ± 1.18
P	(g 100 g^{-1})	16.01 ± 0.78
Na	(g 100 g^{-1})	0.47 ± 0.017
Mg	(g 100 g^{-1})	0.31 ± 0.012
Fe	(g 100 g^{-1})	4.70 ± 0.19
Si	(mg 100 g^{-1})	2.18 ± 0.09
Al	(mg 100 g^{-1})	1.99 ± 0.06
K	(mg 100 g^{-1})[1]	0.51 ± 0.015
Ba	(mg 100 g^{-1})	0.24 ± 0.009
Cr	(mg 100 g^{-1})	0.17 ± 0.005
Cu	(mg 100 g^{-1})	0.06 ± 0.002
Zn	(mg 100 g^{-1})	0.07 ± 0.003
Cd	(mg 100 g^{-1})	Not found
Pb	(mg 100 g^{-1})	Not found

elemental analysis content of FBP arranged in descending order. ICP analysis (OES) confirmed that major elements of FBP are calcium and phosphorous with a Ca/P ratio of 1.57, in addition to small amounts of trace elements, including Na, Mg, Fe, Si, Al, K, Ba, Cr, Cu, and Zn. The results revealed that the extracted FBP is free from contamination with cadmium and lead. Calcium (Ca) with 25.14% was the most abundant element in FBP, while the second most abundant element was phosphorus (P) at 16.01%. The Ca/P ratio was 1.57, which is near to bone mineral, biological hydroxyapatite. The recommended daily amounts (RDAs) according to the expert committee of the European Community for calcium are 700-800 mg per day (Gennari 2001). The calcium content of FBA is about 25.14 g 100 g^{-1}, which means that FBA can be used in a dose of about 2800-3300 mg per day.

CONCLUSIONS

In this study, long-term hydrothermal treatment (130°C and 1.5 bar for 4 h) was used to extract sterile fine bone powder suitable for feeding and dairy supplementation. The extracted FBP showed crystallized microparticles with sizes less than 100 µm, and biological bone apatite represented about 65% of the weight of FBP. The calcium/phosphorous ratio was 1.57, which is close to that in biological bone apatite. The RDAs for calcium are 700-800 mg per day, which equates to about 2800-3300 mg of FBP per day. Although FBP has high levels of calcium and phosphorous, which are close to amounts in biological bone apatite, it is also rich in vital elements, such as Na, Mg, Fe, Si, Al, K, Ba, Cr, Cu and Zn. The powder is pure and free from lead and cadmium, which were below detection limits. FBP is a versatile ingredient not only for nutrition, but also to expand its transformation into other forms for medicinal uses. Moreover, the use of waste bone not only provides a cost-effective and environmentally friendly source of food (proteins and hydroxyapatite), but also helps environmentally in the proper recycling of bone waste.

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