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Investigating the Nutritional Value, Microbial, and Sensory Properties of Cultured Silver Carp Fish Bone Powder (*Hipophthalmichthys molitrix*)

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Abstract

The present study aimed to prepare powder from silver carp fish bone applying alkaline method and investigate its nutritional, sensory, and microbial characteristics. In this research, calcium carbonate was used as a control. The amounts of protein (18.51%), fat (5.11%), moisture (5.58%) and ash (70.82%) were determined in the fish bone powder. The efficiency of powder preparation was determined to be 66.98%. Unlike fish bone powder, the mineral elements silicon, aluminum, barium, and chromium were not observed in the control sample. Phosphorus in bone powder (81580 mg/kg) was more than the control (310 mg/kg) (P<0.05). However, calcium in the experimental treatment was lower (325000 mg/kg) compared to the control (388000 mg/kg) (P<0.05). In terms of overall acceptance and color no significant difference was observed between experimental and control treatments (P<0.05). Among saturated fatty acids, monounsaturated and polyunsaturated fatty acids, respectively, palmitic acid (22.73%), elaidic acid (43.74%) and linoleic acid (7.35%) had the highest amounts in bone powder. Also, the total amount of essential amino acids including threonine, valine, lysine, isoleucine, methionine, histidine, and phenylalanine was 218.29%. The no microorganisms such as fungi, *Escherichia coli*, and *Salmonella* in the treatments were observed. Since bone powder can be produced in large quantities from different types of fish and is rich in nutritional compounds, it is suggested to the food industry for enriching food products.

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Introduction

In Iran during the years 1394-1399 and in the world during the years 2010-2018, the production of warm water fish increased from 184064-221090 t and from 3972-4788.5 thousand t, therefore, by acquiring 8.8% of the world's aquaculture production, it ranked second to produce farmed fish (FAO, 2020; IFO, 2021). Considering the large volume of carp production, the consumption of a large amount of this fish as fresh food, and the fact that silver carp is currently used as a raw material for the production of paste products in some seafood processing factories, which produces a large amount of solid waste, including bones. Also, based on the fact that bone is known as the main residue of fish processing products, which constitutes a large amount of fish weight (10 to 15%), (Asikin *et al.*, 2019), and the studies conducted by other researchers have also proven that about half of the

weight of aquatic animals is made up of raw waste such as skin, head, viscera, bones, and fins (Amitha, 2019), and taking into account the volume of waste production, the economic use of aquatic waste, the production of value-added products, and the reduction of environmental pollution, and that the structure and composition of bones in all aquatic animals are almost the same, and the product produced from the remains of bone species can be extended to other species. In this regard, it is requested pay attention to the production of fish bone powder from the wastes of aquatic processing factories as the raw material for the production of products for the enrichment of food products and human consumption and plan in this field.

Usually, a large amount of processing residues are thrown away and lead to adverse environmental effects as well as loss of resources. Currently, waste management and their use as raw materials for human food preparation are a big challenge for the economic use of products from the aquatic processing industry. Fishbone is considered a potential and natural source of calcium (Asikin *et al.*, 2019). It is also rich in phosphorus and calcium phosphate. Among the other components of fish bone is hydroxyapatite, we can mention Its role is to help repair bones after major trauma or surgery. Although researchers have proven that fish bone calcium has high bioavailability. However, most of the fish bones are still used as raw material for the production of fish meal, which has a low economic value. But fish bones may serve as a raw material to increase income from fishing and produce value-added products. In this way, it is possible to achieve stable conditions in the environment (Malde *et al.*, 2010).

Studies conducted by other researchers have proven that fish bones contain significant amounts of minerals (21 to 57%), organic substances (20 to 30%), and peptides, and the amount of carbohydrates in them is negligible (Pyz-Łukasik & Paszkiewicz, 2018). Therefore, they may have more benefits for maintaining bone health compared to artificial calcium. Therefore, the enrichment of the main foods consumed by humans can be accepted as an efficient method to provide daily food needs due to the use of many vitamins and minerals. Products enriched with bone meal can be useful in increasing the amount of calcium intake, especially in people with lactose intolerance who do not consume enough milk and dairy products (Murillo *et al.*, 2022; Pyz-Łukasik & Paszkiewicz, 2018).

Milk and its products, wheat and corn flour, salt, sugar, fats, and oils are common food products in developing countries that have been successfully fortified using commercial calcium salts such as calcium carbonate, calcium citrate, and tricalcium phosphate. While using natural sources of calcium such as fish bone powder due to the presence of other compounds such as some amino acids that may lead to an increase in calcium metabolism, compared to pure and artificial calcium carbonate, it is more effective for human bone health and can be accepted by consumers. Also, due to having calcium phosphate similar to the composition of human bones, it may have more effects. Researchers have shown that biological access to the mineral part of fish bone is possible. Therefore, this part has the ability to provide benefits for human health. In addition, the consumption of calcium-rich foods during life is effective and recommended to prevent or delay osteoporosis in the elderly (women and men), (Bhenjapaipong, 2021; Desai *et al.*, 2018).

The constituents of fish bones, including (inorganic) minerals, collagen, and gelatin proteins, can be considered useful for the preparation of useful products and human health. Therefore, the increased interest in benefiting from the health benefits of fish waste led to the use of aquatic by-products in the food industry and the production of fish bone powder (Nuraeni, 2020; Ratnamanjari Senapati, 2014).

In addition, in 2019, the per capita consumption of seafood in Iran reached 13.38 kg and the protein consumption reached 6.92 g, which is lower than the global per capita consumption by 20.3 kg

(IFO, 2021). Enriching food products with fish bone powder can be considered an effective step to increase the consumption of aquatic protein (Gupta, 2018). However, limited studies have been conducted on the nutritional value of fish bone powder and the beneficial effects of its use in the human diet, and no study has been reported on the effects of fish bone consumption on human health. Therefore, the present study was conducted with the aim of preparing silver carp bone powder and evaluating its nutritional value compared to calcium carbonate as a commercial source of calcium.

Materials and methods

Processing of silver carp bone powder

10 kg of silver carp was caught in the autumn season. To carry out this study, 2 treatments were considered, which were performed in 3 repetitions. The treatments included bone powder and control. The alkaline method was used to prepare bone powder. Bones were washed after meat removal. Then they were boiled in water at 100 °C for 30 min. The remains of meat and muscle were separated from the backbone of the fish. They were boiled again in water at 100 °C for 2 h. Then the samples were placed in an autoclave (RT model, Iran) for 1 h at a temperature of 121 °C. In the next step, they were boiled in water mixed with 1 N hydroxide sodium for 30 min. Then the treatments were dried at 50 °C for 24 h. Then, they were turned into powder using an electric mill (Moulinex, France). The obtained powder was packed in 200 g using polyethylene plastic and stored at room temperature for 6 232.13 g kg^{-1}. Commercial calcium carbonate powder (Merck, Germany) was used as a control sample (Wulandari & Kusumasari, 2019). The nutritional value of the experimental treatment and its sensory and microbial characteristics compared to the control treatment were evaluated through the following tests. It should be noted that in this research, all the chemicals needed to perform chemical experiments as well as microbial culture media were obtained from Merck.

Sampling

Sampling to perform chemical, sensory, and microbial tests during storage was done once a month at a specific time for 6 months. Also, after production at zero time, samples were taken from experimental and control treatments to conduct experiments.

Protein

Macro Kjeldahl (Gerhardt-Behr, Germany) was used for protein measurement. For the digestion of the samples, 2 g of the sample plus 8 g of catalyst and 25 mL of concentrated sulfuric acid were transferred to the digestion balloon. In the next step, the clear and greenish liquid was distilled along with two-thirds of the volume of the distilled water flask and some boiling stones. The vapors were collected in an Erlenmeyer flask containing 50 mL of 2% boric acid with 3 to 4 drops of bromocrosol as a reagent, and titration was performed with 0.1 N sulfuric acid (AOAC, 2005).

Fat

Fat was measured by the acid hydrolysis method. To determine fat, 50 mL of 4 N hydrochloric acid was added to 5 g of homogenized fish sample. After being placed for 1 h in a bain-marie at a temperature of 80 °C, it was smoothed using Whatman paper. In the next step, the filter paper was transferred to the Soxhlet extractor (Behr-AK5, Germany). After connecting the balloon with a certain weight, two-thirds of its volume was filled with solvent (hexane) and hydrolysis took place for 6 to 8 h (AOAC, 2005).

Ash

Ash was measured by the gravimetric determination method. To measure ash, a clean cruise was placed in a furnace (Fine tech, Korea) with a temperature of 600 °C for 1 h. The cooled cruise was transferred to a desiccator and cooled to room temperature. Then, they were weighed quickly and with 10 g of wet sample placed for 12 to 18 h at a temperature of 550 °C in an electric oven (Fine tech, Korea). After this period and cooling, the cruise was weighed, and was calculated the ash (AOAC, 2005).

Moisture

Moisture was determined by the dry oven method. To determine the moisture content of the sample, 10 g of fish were placed in a petri dish with a certain weight and placed in an oven (Memmert, Germany) at a temperature of 100 °C. It was placed in a desiccator for 1 h (AOAC, 2005).

Fatty acid profile

50 mg of the sample was transferred to a marked volumetric container. A few boiling stones and 2 mL of sodium methoxide solution in methanol (a concentration of 0.2 mol L^{-1}) were added to it. Refrigerant was attached to it, after boiling, it was mixed until it became clear. After turning off the heat and stopping stirring, 2 mL of methanolic phenolphthalein solution was added to it. To decolorize, sulfuric acid solution in methanol (1 mol L^{-1}) was added to it and boiled for 5 min. In the next step, it was placed under cold water for cooling and mixed with 4 mL of 40% sodium chloride solution. Then 1 mL of isooctane was added to it, vigorously stirred for 15 s, and the solution was kept at room temperature until it changed into two phases. To reach the aqueous phase at the lower end of the neck of the container, sodium chloride solution was added again, and the upper phase containing isooctane was separated from other phases to measure the profile of fatty acids. Gas chromatography (2010Pro, Japan) with a 60 mm capillary column and a flame ion detector was used to measure the profile of fatty acids (Iran National Standards Organization (INSO), 2022).

Amino acid profile

Amounts of amino acids were measured by electrospray ionization method. In this order, 0.5 g of the sample was acid hydrolyzed with 4 mL of hydrochloric acid solution for 24 h at 110 °C. When the temperature of the sample reached 24 °C, it was centrifuged using a centrifuge (Hettich Universal 320, Germany) at a speed of 4000 rpm (gram force: 3756.48) for 5 min. In the next step, 10 µL of supernatant along with 1 mL of distilled water was transferred to the sample. Except for isoleucine and histidine, which included IS leucine and 3-methylhistidine, stable isotope mixtures of each amino acid were considered as internal standards for calibration. After hydrolysis, standards and sample calibration were prepared by transferring 50 µL of the hydrolyzed or diluted standard to the sample. Then 50 µL of labeled stable isotope mixture as internal standard and 700 µL of centrifuged reagent were added to the sample for 5 s. 3 µL of the prepared sample, which was stored at 30 °C, were injected into the C18 column of HPLC (HP 1100, Germany) for amino acid analysis. Jasem A and B mobile phases and gradient elution with a flow rate of 0.7 mL/min were used for separation by chromatography and amino acids were determined at a wavelength of 254 nm (Iran National Standards Organization (INSO), 2016a; Zhao *et al.*, 2010).

Determination of heavy metals

The acid chemical digestion determined the amounts of heavy metals lead and cadmium. To the ash (20 g) was added 50 mL of 6 M hydrochloric acid. To evaporate the acid, the flask was placed in a water bath. Then 30 mL of 0.1 M nitric acid was added to this. Cruise was placed in a water bath (Memmert, Germany) for 15 min. This step was followed by covering the sample with aluminum foil and was placed in the environment temperature for 2 h. Then, the contents of the flask were mixed using a glass rod. After filtration, the cooled sample was transferred to another flask and filled with deionized 2-fold distilled water. It was shaken to homogenize. An optical atomic absorption spectrometer with a graphite furnace (ZA3700, Japan) was used to measure the light absorption of heavy metals. To measure the amount of light absorption, a wavelength of 228.8 nm for cadmium and a wavelength of 283.3 nm for lead were determined for standard solutions, and the concentration of each element in the sample was determined based on the calibration curve. The measuring range of the device for lead metal is 5-10-30 ppm and the recycling

percentage is 107%, for cadmium metal, it is 0.5-1-1.5 ppm and the recycling percentage of the device is 80%. The recycling values of the device are 0.02 mg/kg for lead and 0.01 mg/kg for cadmium (AOAC, 2000b; Iran National Standards Organization (INSO), 2016b).

Phosphorus measurement

A spectrophotometer (LAMBDA 365, USA) was used to measure phosphorus. 5.2 g of the sample was mixed with 1 g of calcium carbonate. This mixture was placed in an Electric oven (temperature of 550 °C) to obtain ash from the samples. 20 mL of hydrochloric acid and 10 mL of nitric acid were added to the ash and boiled for 2 to 5 min. The obtained solution was brought to a volume of 500 mL and then filtered. A specific amount of filtered solution was diluted with distilled water until the phosphorus concentration per mL was less than 40 µg/mL. 10 mL of molybdenum vanadate reagent was added to 10 mL of the obtained solution, and after mixing, it was placed in 20 °C for 10 min. The absorbance of the resulting solution was read using 4 cells with a diameter of 10 mm and a spectrophotometer at a wavelength of 430 nm. 10 mL of molybdenum vanadate reagent and 10 mL of distilled water were used as controls. To prepare a standard phosphorus solution that contains 1 mg/mL, in a 1 L volumetric flask, 394.4 g of potassium dihydrogen phosphate, which was dried at 103 °C before weighing, was dissolved in some distilled water and its volume was increased to 1 L (AOAC, 1990).

Measuring mineral elements

Atomic absorption spectrometry (PU9100X, Netherlands) was used to determine mineral elements. After turning into white powder, the samples were dissolved in 1 mL of nitric acid solution using an electric furnace. The contents of the flask were transferred to a marked 250 mL flask by washing with water and the volume was brought to 250 mL. After mixing, the dilution process continued. Lanthanum trichloride solution was added to the amount of 0.1 volume of the balloon. The solution was made up to volume using water in a volumetric flask. All steps were used with the same method and amount of materials and added reagents as controls. A flame spectrometer with an acetylene air torch (PinAAcle900F, USA), which is suitable for measuring at different wavelengths (400 to 800 nm), was used to determine the amounts of mineral elements. Calibration solutions for each element were prepared by dissolving 1000 g of the pure element in 1 L of deionized water. Using a micropipette, the volumes of zero, 1, 2, 3, 4, and 5 mL of the solution were transferred to 6 100 mL volumetric flasks, and 10 lanthanum trichloride solutions were added to each flask and then the volume was brought to volume with water and mixed (AOAC, 2000a; Iran National Standards Organization (INSO), 2008).

Colorimetric

The Huntlab device (Colorflex model, USA) determined the samples' color. Color intensity was expressed using Hunter's parameters in terms of brightness (L), red-green (a), and yellow-blue (b), (Gilbert, 2013).

Sensory evaluation

Smell, texture, color, taste, appearance, and overall acceptance were used to determine the sensory characteristics using the 5-point hedonic method. Sensory characteristics were performed by 30 male and female evaluators in the age range of 30 to 40 years, where the numbers 5, 4, 3, 2, and 1 indicated excellent, very good, good, average, and poor quality, respectively (Gilbert, 2013).

Mold and yeast

The surface culture method was used to check the contamination of produced bone powder with mold and yeast. For this purpose, first, 25 g of the sample was homogenized using 225 mL of physiological serum. Then successive dilution of prepared suspension was done up to 10^{-3} dilution. Then, 1 mL of each

dilution was cultured on Sabord dextrose chloramphenicol agar medium, the lid of the plate was attached to it using paper glue and kept for 1 week at a temperature of 25 °C. It should be noted that 0.1 g of chloramphenicol was dissolved in 40 mL of distilled water to add antibiotics to the culture medium. The antibiotic solution was transferred to 960 mL of the culture medium before the sterilization process (Tournas *et al.*, 2001).

Salmonella

Salmonella bacteria were cultured in 2 stages including pre-enrichment and enrichment. In the first step, 25 g of the sample was mixed with 225 mL of 0.5% lactose broth. This suspension was kept for 24 h at a temperature of 35 °C. In the enrichment step, the sample was mixed slowly, and 1 mL of it was transferred to 10 mL of Selenite F broth and Tetrathionate broth, and it was left for 24 to 48 h at a temperature of 37 °C. In the final stage, 0.3 mL of Selenite F broth suspension and Tetrathionate broth were cultured on *Salmonella Shigella* agar and bismuth sulfite agar. The culture mediums were placed in an incubator with a temperature of 37 °C for 24 h (Andrews *et al.*, 2022).

E. coli

To detect the presence of coliform and *E. coli* bacteria, 50 g of the sample was homogenized using 450 mL of physiological serum. Consecutive dilutions continued until 10^{-3}. 1 mL of each dilution was cultured on McConkey agar and McConkey sorbitol, cefixime tellurite agar, and kept in an incubator at 37 °C for 48 h. To prepare the McConkey agar containing different compounds, first, 0.05 g of 4-methyllumbeliferyl B-D-galactopyranoside was mixed in 495 mL of distilled water. 5 g of sorbitol, 5 g of salicin, and 20 g of McConkey basic agar were added to it. The medium was sterilized at 121 °C for 15 min. In the next step, when the ambient temperature reached 50 to 55 °C, 5 mL of commercial Cefixime Tellurite (CT) suspension was added to it (Feng *et al.*, 2002).

Efficiency measurement

The yield was calculated using the formula r=P/C, where P is the amount of product produced per raw material (C). 500 g of raw material was used to determine the yield. By dividing the amount of powder produced by the amount of raw material (500 g) and multiplying the obtained number by 100, the yield was determined in terms of percentage (Demerjian, 2018).

Statistical analysis

In the present study, the results obtained from different experiments were analyzed using SPSS version 25 software. The results were analyzed at a significant level of 95%. The non-parametric Kruskal-Wallis method and, if necessary, the Mann-Whitney test were used to compare the mean between the treatments and evaluate the statistical difference between the sensory analysis results. The t-test was used to compare the averages of the color test results of the experimental and control samples at a significance level of 5%. The results were expressed as mean ± standard deviation.

Results and discussion

In the present study, an autoclave was used to prepare bone powder. For effective use and preparation of bone powder, fish bones must be softened. Bones may be softened by autoclaving or hydroxide sodium treatment during cooking, and softening increases with cooking time. The softening of fish bone cooked in water can be explained by the leaching of a small amount of bone proteins in water and the resulting change in bone tissue (Benjakul *et al.*, 2017). Nawaz *et al.* (2020) prepared bone powder from Grass carp (*Ctenopharyngodon idella*) using an autoclave process. These researchers suggested the possibility of using an autoclave as an alternative to heat treatment for the preparation of fish bone powder, which is consistent with the results of the present study (Nawaz *et al.*, 2020). In the present study, bone powder was produced by the alkaline method, which is in line with the opinion of many researchers who stated that the alkaline method can be

used to prepare powder from fish processing residues (Amitha, 2019).

In this study, although the packaging was aerobic and mold and yeast are obligate aerobic microorganisms and have the ability to grow in the studied conditions, they were not observed in bone powder. In addition to aerobic conditions, humidity is also one of the other factors required for the growth of mold and yeast, which was not observed due to the decrease of humidity below the level required for the growth of mold and yeast. *Salmonella* are also bacteria that can grow in aerobic conditions, but the amount of moisture in the experimental treatment was not suitable for the growth of these bacteria, so the desired microorganisms were not observed in the experimental treatment. In addition, *E. coli* and *Salmonella* are among the microorganisms that are secondary contamination and are transferred to the product as a result of contamination with water contaminated with sewage. It is also possible that *E. coli* is transferred to the product under the influence of mechanical processing. However, in the current study, silver carp is a type of farmed fish that was fed with underground water, as well as the use of drinking water for processing and hygiene caused this bacterium not to be observed (Odeyemi *et al.*, 2018; Wang *et al.*, 2017). No report has been published regarding the investigation of the microbial characteristics of fish bone powder in terms of the presence of mold and yeast, *Salmonella*, and *E. coli* in previous studies.

In several studies, the digestibility of commercial calcium carbonate powder is accepted and used as a source of calcium, and considering that fish meal is not produced for human consumption in Iran, therefore, in the present study, calcium carbonate powder was used as a control sample (Murillo *et al.*, 2022). Based on the results (Table 1), unlike calcium carbonate powder, fish bone powder contains fat (5.11%) and protein (18.51%) and has high nutritional value as a raw material for the production of edible powder. In the alkaline extraction method, boiling with hydroxide sodium solution removes protein content from fish bone powder. Also, in addition to protein, other organic molecules such as fat in calcium powders prepared using alkaline treatment are significantly reduced, so this method was investigated for the processing of bone powder (Idowu *et al.*, 2020). However, the amount of ash in the experimental treatment was determined to be 70.82%, which was lower compared to artificial calcium (99.50%), which is due to the powder extraction method. Wulandari and Kusumasari (2019) investigated the effect of alkaline extraction on the nutritional value of powder prepared from milkfish (*Chanos chanos*) bones. The amounts of protein (27.88%), fat (7.85%), and ash (1.42%) were observed in milkfish powder, compared to the present study, the amount of protein and fat was higher, but the amount of ash was lower. Considering that the same method was used to prepare the powder, the resulting difference can be related to the type of fish, fishing season, fish feeding, and other factors (Wulandari & Kusumasari, 2019). Yin *et al.* (2016) prepared silver carp bone powder using grinding. These researchers proposed ash (63.72%) and protein (52.52%) as the main components of the powder, which is consistent with the results of the present study. In this study, protein and ash were identified as important components of the powder. The chemical composition of fish species, age, sex, environment, and seasonal changes are factors that can lead to differences in the nutritional value of the powder obtained in this study compared to studies conducted by other researchers (Nuraeni, 2020).

Table 1. Evaluation of nutritional value and efficiency of silver carp bone powder (%)

Sample	Specifications				
	Protein	Fat	Moisture	Ash	Efficiency
Fish bone	18.51±1.56	5.11±1.12	5.38±1.36	70.82±1.95	66.98±1.75

The results are expressed as mean ± standard deviation.

Table 2. The results of the mineral elements of the powder obtained from the cultured silver carp bone compared to the control (calcium carbonate), (mg/kg)

Mineral composition	Treatments	
	Fish bone powder	Control (Calcium carbonate)
Sodium	6630.00^{A}	650.00^{B}
Phosphorus	81580.00^{A}	310.00^{B}
Calcium	325000.00^{B}	388000.00^{A}
Potassium	4.58^{A}	0.41^{B}
Silicium	3.35	-
Aluminum	3.53	-
Barium	0.28	-
Arsenic	0.20^{A}	0.12^{A}
Chrome	5.85	-
Copper	0.92^{B}	2496.00^{A}
Iron	57.00^{B}	1701.00^{A}
Manganese	0.26^{B}	107.00^{A}
Zinc	147.00^{A}	26.00^{B}
Cadmium	0.01>^{A}	0.09^{A}

Dissimilar letters in a row indicate a significant difference at the 5% level (P<0.05).

Checking the presence of mineral elements in the produced powder showed that lead and cadmium were not detected in the experimental treatment (Table 2). Cadmium accumulates in different organs of fish, but the kidney and liver are the main sites of cadmium accumulation, therefore, it can be expected that it is not observed in fish bones. But since the bones are considered the main place of accumulation of lead in fish, its presence in this part is not far from expected, but several factors affect the bioavailability and absorption of this element in fish. Also, most fish farms are located in places that are surrounded by plants, and their role cannot be ignored. This coating affected reducing the concentration of heavy metals in the water entering the breeding ponds, but it was not able to completely remove heavy elements from the water, and considering that the absorption of lead metal through food is small and the largest amount of it is absorbed by the fish through water, and considering that Silver carp is a cultured fish and underground water is used as a water source for fish breeding ponds, the absence of lead in the bones can be justified (Seifzadeh *et al.*, 2018). Malde *et al.* (2010) did not report the presence of cadmium in salmon (*Salmo salar*) bones and reported lower lead (0.11 mg/kg) compared to reference standards. Nemati *et al.* (2017) produced powder from yellowfin tuna (*Thunnus albacares*) waste using the alkaline method, and their report showed that lead and cadmium were not observed in the powder. Calcium, phosphorus, zinc, and iron occupied the first to fourth positions in terms of quantity in bone powder. Compared to bone powder, silicon, aluminum, barium, and chromium elements were not found in calcium carbonate. Barium, manganese, and arsenic were identified as the elements that had the lowest amounts in bone powder. Although the amounts of elements such as phosphorus, arsenic, and sodium were determined more in the experimental treatment compared to the control, the amounts of iron, manganese, copper, and calcium in calcium carbonate were reported more (Table 2).

Calcium carbonate is a calcium salt that has been widely accepted by consumers as a food supplement due to its high calcium content (approximately 40%). Based on studies conducted by other researchers, the amount of calcium in fish bones is slightly lower compared to calcium carbonate (Murillo *et al.*, 2022). As shown in Table (2), the bone powder contained 325000 mg/kg and calcium carbonate contained 388000 mg/kg of calcium, and bone powder contained 81580 mg/kg and calcium carbonate contained 310 mg/kg of phosphorus. As can be seen in Table (2), these elements have the highest amounts in the powder structure. Nemati *et al.* (2017) in the review of yellowfin tuna waste powder prepared by alkaline method, introduced calcium (38.16%) as the most important element of the powder. Yin *et al.* (2016) reported a total calcium content of 236.90 mg/g in silver carp bone powder produced by a mill. Savlak *et al.* (2020) in the study of the effect of different compounds such as pure hydroxide sodium and hydroxide sodium in combination with citric acid, sodium hypochlorite, ethanol, hydrogen chloride, and drinking water on the mineral compounds of gilt-head bream (*Sparus aurata*) powder found that the

powder prepared with 21.46% hydroxide sodium has the most compounds. It was mineral and contained high amounts of calcium (232.13 g/kg) and phosphorus (111.63 g/kg). These researchers determined the ratio of calcium to phosphorus as 2.07. Njoroge and Lokuruka (2020) prepared tilapia bone powder using 2% sodium (5 times skeleton ratio) and showed that tilapia bone powder contained approximately 100 mg/g of calcium. Nemati *et al.* (2016) produced tuna bone powder by alkaline method and found that the powder contained 38.16 and 23.31 g/100 g of calcium and phosphorus. They also calculated the ratio of calcium to phosphorus of the product as 1.25, while in the present study, this ratio was 3.93, which is due to the higher ratio of calcium and phosphorus in bone powder compared to calcium carbonate. Chemical conditions and thermal processes were among the factors that caused the change in the levels of calcium and phosphorus as well as their ratio in recent studies compared to the results of the present study. The effects of alkaline compounds as well as the use of their different concentrations for powder production cannot be ignored (Nemati *et al.*, 2016).

Based on the results of Table (3), essential and non-essential amino acids were not found in the control treatment. In the experimental treatment, leucine, lysine, and valine, which are in the group of essential amino acids, occupied the first to third positions, respectively. In this treatment, glutamic and aspartic acids and arginine, which are classified in the non-essential amino acids section, were introduced as the first to third-ranked elements. Glutamic acid was the highest among essential and non-essential amino acids. Non-essential amino acids had a higher position compared to essential amino acids. Unlike essential amino acids, all of which have been identified in silver carp bone powder, Glutamine, and asparagine, which are non-essential amino acids, were not found in this treatment.

Table 3. The results of the profile of amino acids of the powder obtained from the bone of cultured silver carp (mg/kg)

Amino acid	Silver carp bone powder
Aspartic acid	55.69±2.12
Serine	26.49±2.87
Glutamic acid	100.16±2.35
Glycine	27.78±1.24
Cysteine	7.52±1.96
Arginine	44.99±1.56
Proline	18.67±1.79
Alanine	23.53±1.86
Tyrosine	27.68±2.18
Valine	28.39±2.67
Isoleucine	24.82±2.75
Histidine	13.31±2.38
Lysine	45.80±1.82
Lucien	45.87±1.69
Methionine	11.31±1.74
Phenylalanine	23.58±2.90
Threonine	25.21±2.16
Total amino acid	551.80
Total essential amino acids	218.29
Total non-essential amino acids	333.51
The ratio of total essential amino acids to total amino acid	0.39
The ratio of total non-essential amino acids to total amino acid	0.60

The results are expressed as mean ± standard deviation.

Based on the profile of essential amino acids, the experimental treatment is described (Table 3). The use of an autoclave in the processing stages of the powder, in addition to reducing its particle size, also leads to changes in the structure of the essential amino acids of the powder. In addition, the change may be caused by the effects of using a mill to produce a powder that facilitates the breakdown and isolation of collagen peptides. Collagen is one of the main constituents of bone, and the presence of certain amino acids in it (for example, glycine, leucine, proline, hydroxyproline, and lysine) and monosaccharides increase the absorption of calcium through the intestine. Therefore, calcium from bone powder prepared from silver carp has a greater ability to be absorbed through the intestinal wall compared to calcium consumed through calcium carbonate powder (Murillo *et al.*, 2022; Nemati *et al.*, 2016). The steam pressure of the autoclave can also be another reason that opens the

peptide bonds of the powder proteins and increases their bioavailability. In addition, processing using an autoclave enables further aggregation and change in protein structure (Nawaz *et al.*, 2020). Nemati *et al.* (2017) investigated yellowfin tuna waste powder prepared by alkaline treatment method, and they realized the presence of amino acids such as lysine, valine, leucine, isoleucine, methionine, threonine, histidine, phenylalanine and tryptophan (essential amino acids) in the composition of the powder. These researchers reported higher levels of non-essential amino acids, including glutamic acid, arginine, alanine, aspartic acid, and serine, compared to non-essential amino acids such as glycine and proline, as well as hydroxyproline, which were effective in collagen production (Nemati *et al.*, 2017). In the present study, all types of amino acids from the essential group were found in bone powder. Glycine and proline were also identified, and glutamic and aspartic acids were present in high levels in bone meal. Following them, arginine, alanine, and serine accounted for the highest amount compared to other amino acids. However, hydroxyproline was not observed in the experimental treatment. Based on the opinion of other researchers, the alkaline method is a factor that has significant effects in reducing the organic compounds of calcium-containing powders, which can justify this phenomenon.

According to the results, the presence of fatty acids in the control treatment (calcium carbonate) was not reported (Table 4). In the present study, palmitic acid followed by stearic acid, which is classified in the category of saturated fatty acids, compared to other fatty acids, had the highest amount in the experimental treatment. Eicosanoic and palmitoleic acids (cis) and linoleic and α-linolenic acids are from the group of monounsaturated and polyunsaturated fatty acids, respectively, which occupied the highest position in these categories. Based on the results of Table (4), essential fatty acids have been observed in the experimental treatment. The presence of these fatty acids in the powder can be considered as influenced by the use of raw material of aquatic origin to produce the powder and that aquatics are rich in unsaturated fatty acids. However, according to the studies of other researchers, fatty acids are sensitive to heat. During powder processing, heat was used in different ways such as autoclaving and boiling bone in water and water with hydroxide sodium, but fatty acids were abundant in bone powder (Kandyliari *et al.*, 2020).

Table 4. Fatty acid profile of powder obtained from cultured silver carp bone (%)

Fatty acids	Silver carp bone powder
Myristic acid	2.65
Lauric acid	0.11
Tridecanoic acid	0.09
Pentadecyclic acid	0.85
Arachidic acid	0.24
Lignoceric acid	0.03
Margaric acid	0.75
Palmitic acid	22.73
Stearic acid	5.12
Behenic acid	0.09
Total saturated fatty acids	32.66
Vaccenic acid	5.65
Elaidic acid	43.74
Eicosanoic acid	0.14
Myristoleic acid	0.09
Ginkgolic acid	0.07
Palmitoleic acid (trans)	0.73
Palmitoleic acid (cis)	11.23
Jing cholic acid	0.99
Total monounsaturated fatty acids	62.64
Linoleic acid	7.35
Eicosadienoic acid	0.35
α-linolenic acid	5.97
γ-linolenic acid	0.27
Dihomo-γ-linolenic acid	0.56
Arachidonic acid	1.96
Docosapentaenoic acid	0.75
Total polyunsaturated fatty acids	17.21
The ratio of polyunsaturated fatty acids to saturated fatty acids	0.52
Eicosapentaenoic acid	271.95
Docosahexaenoic acid	253.67

Nemati *et al.* (2017) in the study of yellowfin tuna waste powder prepared by the alkaline method, found that the amounts of oleic, palmitic, and gondoic acids in the powder were higher compared to the amount of hexadecatrienoic, γ-linolenic, and dihomo-γ-linolenic acids. Also, these researchers

found the amount of myristic, stearic, eicosapentaenoic, and docosahexaenoic acids relatively higher compared to the mentioned acids (Nemati *et al.*, 2017). In the present study, elaidic, palmitic, palmitoleic (cis) and α-linolenic acids of the experimental powder occupied the first to fourth positions in terms of quantity. The amounts of eicosapentaenoic and docosahexaenoic acids in the experimental treatment were higher compared to other determined fatty acids. The difference between the results of the present study and other research can be explained by the type of fish used to prepare the powder, the chemical composition of the fish, the fishing season, the feeding of the fish, the temperature and time used to produce the powder, pH, processing steps, autoclave, and other possible factors (Nemati *et al.*, 2017).

According to the results of Table (5), the brightness of the color in bone powder is not significantly different compared to the control (P>0.05). The powder obtained from fish bones was white in color. Also, this feature did not change significantly during 6 months of bone powder storage (P>0.05). Bhenjapaipong (2021) when preparing fish bone powder from pink salmon (*Oncorhynchus gorbuscha*) and Atlantic salmon (*Salmo salar*), chose to dry at 70 °C for 120 min, which resulted in the production of powder with the highest white color compared to other powders (Bhenjapaipong, 2021). However, in the present study, the temperature and time of drying the powder was 50 °C for 1 h, and the resulting powder had a very favorable white color. The difference in the results of these researchers compared to the results obtained from the present study is due to different production conditions and the use of a pressure cooker.

Based on the results of Table (6), no significant difference was observed between the texture, flavor, and taste of bone powder and the control (P>0.05). Also, the sensory characteristics of fish bone powder and the control treatment did not show a significant decrease during the storage time (P>0.05). Bone powder had a small amount of fish smell, but the characteristic of the smell in bone powder did not show a significant difference compared to calcium carbonate (P>0.05). Due to the non-use of odor-reducing compounds in the preparation of bone powder, as well as the lack of effect of sodium hydroxide in removing the smell of the product, the produced powder had a small amount of fish smell (Savlak *et al.*, 2020). Considering that the sensory characteristics between the powder and the control treatment were almost the same, therefore, no significant difference was observed in the overall acceptance index between the powder and the control (P>0.05). Bhenjapaipong (2021) found that when preparing fish bone powder under optimal conditions from pink salmon and Atlantic salmon under a pressure cooker and heat at 140 °C for 60 min, the resulting powder has low hardness. In the present study, an autoclave was used for 60 min, and the resulting powder was relatively stiff, so it is consistent with the results of the present study. No report has been published about the evaluation of sensory characteristics of odor, taste, and general acceptance of fish bone powder.

Table 5. Evaluation results of color factors of powder from cultured silver carp bone during 6 months of storage at ambient temperature compared to calcium carbonate (control)

Storage period (month)	Fish bone powder			Control (Calcium carbonate)		
	L	a	b	L	a	b
Zero	93.68±0.89^{aA}	1.98±0.79^{aA}	1.86±0.93^{aA}	92.76±0.98^{aA}	1.73±0.90^{aA}	1.82±2.95^{aA}
1	93.66±0.78^{aA}	1.95±0.72^{aA}	1.85±0.42^{aA}	92.74±0.26^{aA}	1.71±0.42^{aA}	1.81±0.24^{aA}
2	93.62±0.32^{aA}	1.93±0.58^{aA}	1.82±0.77^{aA}	92.71±0.38^{aA}	1.71±0.37^{aA}	1.79±0.71^{aA}
3	93.44±0.96^{aA}	1.93±0.63^{aA}	1.72±0.88^{aA}	92.65±0.37^{aA}	1.67±0.89^{aA}	1.79±0.99^{aA}
4	93.41±0.97^{aA}	1.91±0.72^{aA}	1.70±0.86^{aA}	92.61±0.94^{aA}	1.64±0.81^{aA}	1.77±0.91^{aA}
5	93.41±0.94^{aA}	1.87±0.97^{aA}	1.70±0.83^{aA}	92.53±0.92^{aA}	1.64±0.87^{aA}	1.68±0.89^{aA}
6	93.36±0.85^{aA}	1.85±0.49^{aA}	1.69±0.78^{aA}	92.49±0.69^{aA}	1.61±0.74^{aA}	1.62±0.76^{aA}

Dissimilar letters in a column and row indicate a significant difference at the 5% level (P<0.05). The results are expressed as mean ± standard deviation.

Table 6. The results of sensory characteristics of cultured silver carp bone powder compared to the control during 6 months of storage at ambient temperature

Storage period (month)	Fish bone powder				Control (Calcium carbonate)			
	Texture	Odor	Taste	Overall acceptance	Texture	Odor	Taste	Overall acceptance
Zero	4.31±1.13^{aA}	4.91±1.39^{aA}	4.56±1.35^{aA}	4.52±0.41^{aA}	4.41±1.13^{aA}	4.94±1.39^{aA}	4.68±1.35^{aA}	4.55±0.41^{aA}
1	4.31±1.41^{aA}	4.91±1.41^{aA}	4.55±1.27^{aA}	4.52±0.47^{aA}	4.41±1.41^{aA}	4.94±1.41^{aA}	4.68±1.27^{aA}	4.55±0.47^{aA}
2	4.31±1.29^{aA}	4.87±1.55^{aA}	4.46±1.21^{aA}	4.48±0.68^{aA}	4.41±1.29^{aA}	4.94±1.55^{aA}	4.68±1.21^{aA}	4.55±0.68^{aA}
3	4.27±1.32^{aA}	4.73±1.67^{aA}	4.37±1.52^{aA}	4.46±0.79^{aA}	4.35±1.32^{aA}	4.93±1.67^{aA}	4.65±1.52^{aA}	4.51±0.79^{aA}
4	4.21±1.17^{aA}	4.62±1.63^{aA}	4.25±1.57^{aA}	4.38±0.49^{aA}	4.31±1.17^{aA}	4.93±1.63^{aA}	4.65±1.57^{aA}	4.51±0.49^{aA}
5	4.15±1.28^{aA}	4.59±1.54^{aA}	4.22±1.93^{aA}	4.35±0.68^{aA}	4.30±1.28^{aA}	4.91±1.54^{aA}	4.62±1.93^{aA}	4.50±0.68^{aA}
6	4.12±1.25^{aA}	4.53±1.53^{aA}	4.20±1.98^{aA}	4.30±0.28^{aA}	4.28±1.23^{aA}	4.85±1.59^{aA}	4.55±1.90^{aA}	4.48±0.62^{aA}

Dissimilar letters in a column and row indicate a significant difference at the 5% level (P<0.05). The results are expressed as mean ± standard deviation.

Conclusion

The results of the present study showed that fish bone powder produced from silver carp bone compared to commercial calcium carbonate powder, which is known as a source of calcium, It is rich in various mineral salts and also rich in fatty acids and essential amino acids of the human body and can significantly contribute to human health needs. In addition, filleting wastes have many beneficial effects, which are used as raw materials for the production of value-added and commercial products, which may have a potential application in industry and medicine in addition to reducing the organic load caused by the fish processing industry. Therefore, to benefit more from calcium, it is recommended to enrich the food with silver carp bone powder.

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Contribution of authors

Data collection, data analysis, and interpretation, drafting of the article, data analysis, presentation of the research idea and study design, data analysis and interpretation, review, and correction of the article, supervision of the study, and approval of the final version are done by the author.

Conflict of interest

There was no conflict of interest in the implementation of this study and the writing of the article.

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بررسی ارزش غذایی، ویژگی‌های میکروبی و حسی پودر استخوان ماهی کپور نقره‌ای (*Hipophthalmichthys molitrix*) پرورشی

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چکیده

مطالعهٔ حاضر با هدف تهیهٔ پودر از استخوان ماهی کپور نقره‌ای با روش قلیایی و بررسی ویژگی‌های تغذیه‌ای، حسی و میکروبی انجام شد. در این پژوهش کربنات کلسیم به‌عنوان شاهد استفاده شد. مقادیر پروتئین (۱۸/۵۱ درصد)، چربی (۵/۱۱ درصد)، رطوبت (۵/۵۸ درصد) و خاکستر (۷۰/۸۲ درصد) در پودر استخوان ماهی بودند. راندمان تهیهٔ پودر ۶۶/۹۸ درصد تعیین شد. برخلاف پودر استخوان ماهی عناصر معدنی سیلیسیوم، آلومینیوم، باریم و کروم در نمونهٔ شاهد مشاهده نشدند. فسفر در پودر استخوان (۸۱۵۸۰ میلی‌گرم بر کیلوگرم) در مقایسه با شاهد (۳۱۰ میلی‌گرم بر کیلوگرم) بیشتر بود ($P<۰/۰۵$). اما کلسیم در تیمار آزمایشی (۳۲۵۰۰۰ میلی‌گرم بر کیلوگرم) در مقایسه با شاهد (۳۸۸۰۰۰ میلی‌گرم بر کیلوگرم) کمتر بود ($P<۰/۰۵$). ازنظر رنگ و پذیرش کلی بین تیمارهای آزمایشی و شاهد تفاوت معنی‌دار مشاهده نشد ($P<۰/۰۵$). در میان اسیدهای چرب اشباع، تک‌زنجیرهٔ غیراشباع و چند غیراشباع به‌ترتیب اسیدهای پالمیتیک (۲۲/۷۳ درصد)، الایدیک (۴۳/۷۴ درصد) و لینولئیک (۷/۳۵ درصد) بالاترین مقادیر را در پودر استخوان دارا بودند. همچنین مقادیر کل اسیدهای آمینهٔ ضروری شامل ترئونین، والین، لیزین، ایزولوسین، متیونین، هیستیدین و فنیل آلانین ۲۱۸/۲۹ میلی‌گرم بر کیلوگرم بود. هیچ‌گونه میکروارگانیسمی شامل قارچ، *اشریشیاکلی* و *سالمونلا* در تیمارها مشاهده نشد. ازآنجاکه پودر استخوان به شکل انبوه از گونه‌های مختلف ماهی قابل تولید بوده و سرشار از ترکیبات تغذیه‌ای است، ازاین‌رو برای غنی‌سازی محصولات به صنعت غذایی پیشنهاد می‌گردد.

واژه‌های کلیدی: ارزش تغذیه‌ای،استخراج قلیایی، زائدات ماهی، غنی‌سازی، ماهی پرورشی