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Research Article

Influence of Drying Techniques on Antioxidants and Functional Properties of Bighead Carp (*Hypophthalmichthys nobilis*) Fillets

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Abstract

Bighead carp fish (*Hypophthalmichthys nobilis*) under different drying processes was evaluated for heavy metals, proximate compositions with functional properties, and free radical scavenging activity to identify different nutritious components that can be applied in food industries. Protein, ash, and fat were higher under microwave drying compared with the air-drying process. Protein content was 64.89% using microwave technique, while oven drying was 64.57%. WHC value was higher under oven drying technique while the raw fish was recorded the lowest value of WHC. Antioxidant activity (DPPH) was showed a significant difference among all treated samples. Farming and breeding bighead carp in fish farms can be a good resource for protein and other nutritional components in some poor countries to reduce the nutritional gap in food products.

Keywords: Bighead carp; Drying techniques; Heavy metals; Functional properties

Introduction

Bighead carp (*Hypophthalmichthys nobilis*) can be found in many locations across the globe. They are generally found in freshwater regions, especially big lakes and rivers [1]. These species are very hardy and can survive in huge different freshwater environments, promoting the ability to live in different parts of the globe. Bighead carp prefer water temperatures ranging from 4 to 26 °C. The bighead carp's original area is Asia, particularly China and southeastern China [2]. The bighead carp can be survived in many countries in other parts of Asia like Thailand, Myanmar, and Vietnam, different countries in Europe and Northern America, and some parts of the Arab area. Nowadays, most freshwater areas in the United States are comparable bighead carp habitats to the original one in China so that this fish can be found all across freshwater environments in North America from rivers like Missouri and Mississippi rivers to different lakes in many areas like Wisconsin and magician great lakes [3].

Bighead is adopted to feed by zooplankton with larger phytoplankton. Bighead carp used to have fine and comb rakers to fatigue little animals and planktons from the water [4]. Biologically, bigheaded carps can rival freshwater fishes during planktivore phases (from larvae normal fish) [5,6]. Carp species could cause a real threat to fishers in some areas in Asia or North America because of their huge body and the possibility to leap above the water when terrified by ships or boats [5,7]. Carp species affect the ecosystem, which could provide momentum and thrust for governmental plans, including some regions in North America around the Mississippi River to control and oriented carp species, including bighead carp [6,8]. As we know, fish is a huge source of protein due to enriched nutritional values with good protein quality containing efficient amounts of amino acids, fish contain considerable amounts of fatty acids and vitamins, in addition to, freshwater fish provide important minerals such as phosphorus, calcium, magnesium [9,10]. Different species of fish have different protein content.

However, protein ranges from 8% to 22%, leading to around 60-80% of the energy produced in various carp fish species. In hot regions, High temperature causes a quick spoiled and degradation of tropical fish species. Therefore, fast preservation is very important to stop or reduce undesirable changes of fish species, which contains traditional techniques such as salting, solar drying, hot air drying, and smoking preservation [11]. One tasty and suitable food is dried fish, which can be used in various food applications [12]. Commonly, a very traditional and cheap drying method of fish was occurred under the solar system, which was very low-cost technique to preserve fish products used from ancient time [10]. The other technique is Hot air method, which could control the air velocity and humidity of the experiments conditions, but consume high energy due to high-applied temperature, could be contaminated by bacteria and the quality change potentially [13]. Microwave technique provides a high heating average and does not affect changes on the surface of the food, hence no crust is formed, therefore this technique is efficient with meat and fish due to needs less time with low energy and can provide a high quality of fish products [14]. With some improvement in advanced drying technologies, different conditions can be monitored under the drying process, such as temperature, power, odor, or samples shape [15]. In this present study, we used two drying techniques to investigate drying effects on minerals, amino acids, heavy metals, and functional properties of bighead carp (*Hypophthalmichthys nobilis*). However, the target of the present study is to provide new results regarding different drying methods and use our results to develop new food of aquatic products in the future.

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Materials and Methods

Materials

Fresh bighead carp (*Hypophthalmichthys nobilis*) was obtained from Yangtze River aquatic products science and technology industry Co., Ltd in Wuxi, Jiangsu, China. Then the fish was transported to an ice Styrofoam container directly to the school of food science and technology (Jiangnan University), food processing, and aquatic products laboratory. Solutions, acids, and alkaloids were purchased from Sinopharm chemical reagents Co, Ltd (Shanghai, China). Other reagents and chemicals in this research were under analytical grade.

Sample preparation

Targeted Fish were cleaned with tap water, removed the head with bones, and then chipped into small slices about 1 to 2cm to get small fish fillets. Raw samples were grinded, homogenized, and then kept frozen at -20°C until needed for further experiments. Drying treatment was carried out in two different methods. The first part was dried using a lab hot air-drying oven (Oven DHG-9030A: 490×500×625mm, Suzhou Qiandong, China) at 90°C for 150min. In contrast, the second part of fish fillets was dried using a microwave oven (ORW1.0S-5Z, 100-1000W, Nanjing Aorun Microwave Technology Co., Ltd.) at 2450MHz, 360W for 10min. The dried bighead carp fish fillets were blended and homogenized using a high-speed blender (25000/min).

Proximate composition

The raw bighead carp sample and the treated sample under drying techniques were analyzed individually. The composition contents of (protein, fat, moisture, and ash) were determined utilizing (AOAC) standard methods 925.09, 932.06, 985.2912, and 923.03, respectively [16]. The content of ash was determined using the standard method of association of official analytical chemists. In short, 3g of each sample was weighed in the furnace crucible then burned at 550°C to 600°C for 5h in the muffle furnace until constant weight and the reddish ash. Lipid content was estimated by extract (3g) of the sample utilizing a solvent (petroleum ether) in the Soxhlet extraction instrument (Auto fat determiner, Xian Jian Instruments Co, Shanghai, China) for 4.5h. Kjeldahl analyzer (DK-3400/FOSS, Hilleroed, Denmark) evaluated the Crude protein of fish samples by multiplying determining total nitrogen by 6.25 (standard factor). The moisture content was evaluated using 3g of the sample, then drying for 2 to 3 h at 105°C until the constant weight was obtained.

Heavy metals concentration analysis

Dried fish samples (0.5g) with the raw sample were put in a 50mL beaker, then 8mL of nitric acid (68%) with 4mL of perchloric acid (72%) were mixed and added to the samples. A transparent light-colored mixture was obtained after being heated lightly for two periods, at 40°C for 30min, then increased to 70°C for 40. Then the samples were washed using high purity water two times, and the contents were filtered and transferred into a 50mL flask. Before dilution to the mark of 50ml, the filtered mixture was cooled at room temperature. Atomic absorption spectroscopy instrument (Spectra AA-220FS; Varian Co, CA, USA) was used to measure the concentration of heavy metals. The concentration of heavy metals was expressed as (µg/g) of the sample weight.

Determination of functional properties

Bulk density (BD): The BD value was determined using a slight modification of the method presented by [17]. About (10g) of each sample were gently placed in a 15mL graduated cylinder, then gently tapping the graduated cylinder several times on a laboratory bench until no decrease in the sample volume was observed. The BD value was calculated based on sample weight per unit volume of sample (g/mL).

Water absorption capacity and oil absorption capacity: The WAC and OAC of fish samples were measured according to the method described by Wasswa and colleagues [18] with slight modification. Fish samples (1g) were placed in pre-weighed centrifuge tubes then 10ml of distilled water was added and dispersed into the subjected samples. Samples were vortexed for 60s. The samples were left to stand at room temperature for 3h, then were subjected to centrifuging at 5000rpm for 25min. The supernatant of the dispersion solution was filtered utilizing a filter of 0.45µm, and then the volume recovered was measured and recorded accurately. The initial volume of purified water added to the samples subtracted the volume of the supernatant was determined. The value of WAC was obtained as milliliters of water absorbed per gram of fish sample. To determine the oil absorption capacity, 10ml of sunflower oil was used to measure OAC. The value of OAC was measured and calculated as the gram of oil absorbed per gram of fish sample [18].

Antioxidants activity analysis free radical scavenging assay (DPPH)

DPPH radical scavenging assay of fish samples was conducted with the method presented by Baydar [19] with minor modification. 0.1mL of each fish sample was extracted at various concentrations (10, 30, 50, 60, and 70 mg/mL) mixed with 3.5mL of DPPH methanolic solution (6×10^{-5}M/L). The above concentrations were chosen to calculate the IC50 values related to initial optimal conditions to ensure that the optimal concentrations correspond with the DPPH· method's sensitivity. The mixtures were incubated at room temperature for 30min. Then the absorbance was detected and recorded at 517nm by a spectrophotometer (model-UV-2100), and the DPPH activity was calculated using the following equation (1):

$$\%DPPH\cdot\text{-}SA = (A_0 - A_1)/A_0 \times 100 \qquad (1)$$

Where A_1 is the absorbance of the extract while A_0 is the absorbance of the control.

Sensory analysis (Odor Test)

Six experienced people carried out a sensory odor test. The raw and dried samples were presented for odor test. The experiment was designed and carried out on a seven-measuring scale point: 1 (strong taint), 2 (taint), 3 (mild taint), 4 (without taint), 5 (flat aroma), 6 (aroma), and 7 (obvious aroma). The scores were calculated, and the average for each sample was recorded [23].

Statistical analysis

All the data were presented as mean values ± standard deviation (±SD), and the samples were analyzed in triplicate (n=3). Reported results were subjected to one-way analysis of variance (ANOVA) to detect significant differences of different samples. At the same time, Duncan's test was used to perform multiple ranges between means using SPSS ver.19 (SPSS., Chicago, USA). The significance level was defined at p ≤0.05.

Results and Discussion

Proximate compositions

The chemical composition of bighead carp fish under different drying techniques (oven drying and microwave drying) is shown in Table 1. The current study results showed no significant difference in the moisture factor using different drying techniques. The moisture value ranged from 24.19% using oven drying to 23.92 under the microwave-drying technique. At the same time, it was recorded 77.69 % in the raw bighead carp. However, the study was showed no significant differences (p <0.05) in fat values using different drying methods. The protein observed significant values and recorded in (oven drying, microwave drying, and the raw bighead carp) 64.57%, 64.89%, and 17.72%, respectively. Based on dry weight, protein value showed no significant differences between oven drying and microwave drying technique. The distribution of fat based on dry weight (g/100g) in bighead carp was as follows: oven drying 5.69%, microwave drying 5.88%, and the raw sample 5.97%. The ash content in dry weight was 4.83% for oven drying, 4.79% for microwave drying, and 4.36 in the raw sample. The chemical composition of different drying methods with raw bighead carp was studied in our research. Moisture is an important parameter for food storage, and over 20% to 25% of moisture content microbes start growing in food products [20]. Moisture recorded 27.93% using the oven drying method compared with 15.62% in the same method [21,22]. Fat content ranged from 4.1% to 4.45% in different drying methods compared with 1% in the raw fish sample. These results were almost similar to fat contents reported by Tao [23]. Fats are essential compounds to provide our body with efficient energy and support cell growth to keep some functions working regularly. Fats help our bodies to absorb some nutrients and induce important compounds to produce hormones as well. Protein content as the main chemical composition of bighead carp reported different values under different drying techniques. The protein was significantly different among all samples 65% under microwave drying, while 64% under oven drying.

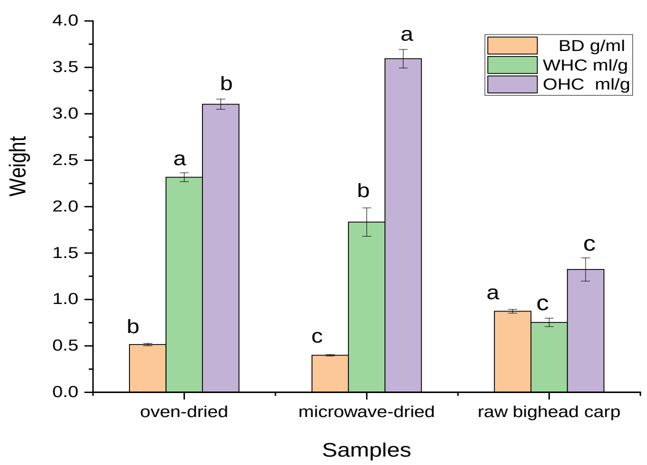

Figure 1: Water Holding Capacity (WHC), Oil Holding Capacity (OHC) and Bilk Density (BD) of different drying methods compare with raw bighead carp. Means ± SD (n=3). Mean values with different letters differed significantly (p <0.05).

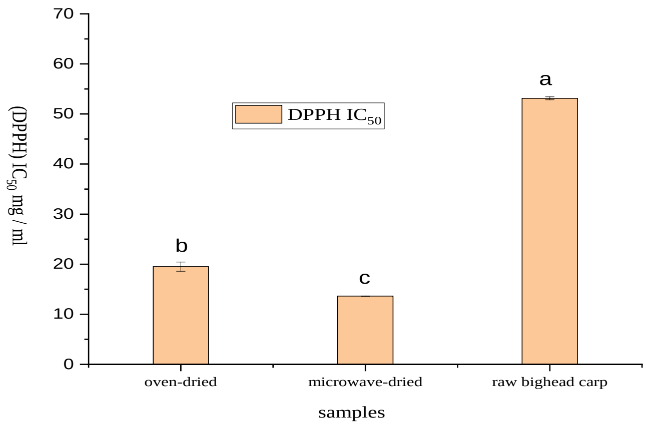

Figure 2: DPPH IC_{50} of bighead carp fish using different drying techniques. IC_{50} refers to the concentration of sample required to 50% of the free radical scavenge content; DPPH 2 2-Diphenyl-1-pic-rylhydrazyl. Values represent mean ± SD (n=3). Different letters refer that the values are significantly different (P ≤0.05).

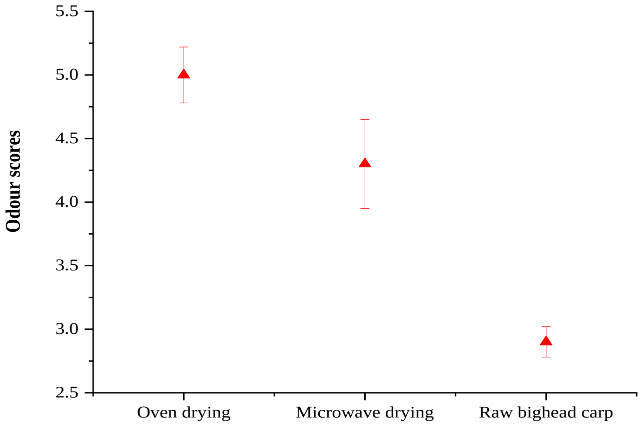

Figure 3: Odor test scores of raw, oven and microwave drying bighead carp *H. nobilis* fillets

Compared with previous studies, protein recorded 69% using oven drying [23] and showed 63% using the kiln-dried method [8]. On the other hand, in our research, protein content in raw bighead carp was 17.86%. In comparison, Ogbonnaya Chukwu reported 19.51% [21], and Tao showed that protein content in raw grass carp was 19.1% [23].

Heavy metals analysis

The concentrations of heavy metals in bighead carp fish samples are presented in Table 2.

The values were showed significant differences based on the various drying process. No significant differences were recorded among all samples related to the (Se) element. Table 2 was showed that the level of cadmium was higher in microwave treatment compared with oven treatment. However (Pb) in this study was 0.24µg/g under the oven drying process than 0.21µg/g (dry weight) in the Raw sample. The results of heavy metals in bighead carp fish grown and cultivated in china were internationally in the accepted range [24]. Kabelitz and others [25] were reported the limits of different heavy metals concentrations, 10 (Pb), 1 (Cd), 5 (As), and 0.1 (Hg) µg/g. In

Table 1: Chemical composition (mean ± SD) of bighead carp fish samples based on fresh weight and dry weight respectively.

Samples	Raw	Oven drying	Microwave drying
Moisture (%)	77.69±0.88[a]	24.19±0.91[b]	23.92±0.86[b]
Ash[1]	1.06±0.05[c]	3.48±0.06[b]	3.62±0.07[a]
Fat[1]	1.07±0.12[b]	4.10±0.16[a]	4.45±0.15[a]
Protein[1]	17.72±0.25[b]	64.57±1.47[a]	64.89±0.58[a]
Ash[2]	4.36±0.07[b]	4.83±0.06[a]	4.79±0.16[a]
Fat[2]	5.97±0.82[a]	5.69±0.22[a,b]	5.88±0.16[a]
Protein[2]	82.66±1.02[b]	84.79±0.39[a]	85.27±0.53[a]

All values represent mean of triplicate determinations, mean ± SD (n=3). Different letters refer that the values are significantly different (P ≤0.05). [1]Samples based on the fresh weight (g/100g fresh weight). [2]Samples based on dry weight (g/100g dry weight).

Table 2: Heavy metal contents (μg/g dry weight) of bighead fish carp samples.

Samples	Raw	Oven drying	Microwave drying
Arsenic (As)	0.12±0.02[b]	0.15±0.02[a]	0.16±0.03[a]
Selenium (Se)	0.35±0.02[a]	0.35±0.02[a]	0.37±0.03[a]
Cadmium (Cd)	0.10±0.01[a]	0.10±0.01[a]	0.11±0.02[a]
Lead (Pb)	0.21±0.00[a]	0.24±0.03[b]	0.20±0.01[a]
Mercury (Hg)	nd	nd	nd

All values represent mean of triplicate determinations, mean ± SD (n=3). Different letters refer that the values are significantly different (P ≤0.05).

this study, Cd was 0.11μg/g using microwave drying compared with 0.10μg/g in the fresh sample. MacKay and others reported cadmium concentration ranged from 0.1 to 0.4 mg/kg fresh weight in blue marine [26].

Functional properties

Bulk density (BD): The BD values of bighead carp fish samples with different drying methods, shown in Figure 1, were 0.41g/ml using microwave-drying compared with 0.51g/ml under the oven drying method (p <0.05).

Water absorption capacity and oil absorption capacity: The values of WHC and OHC in treated samples are presented in Figure 1. The results showed that the oven drying sample had a high value of WHC while the sample treated under microwave drying recorded 1.83ml/g. OHC values were ranged from 1.32 to 3.59 ml/g for different samples. WHC high values indicate a potential application in the industrial production of food and fish products. OHC value refers to the potential uses of fish products in food industries because of emulsifying capacity.

Free radical scavenging (DPPH) assay

The activity of antioxidants was measured by DPPH free radical scavenging. The antioxidant activity of the extract in fish samples was evaluated using DPPH (2,2-diphenylpicrylhydrazyl) analysis method and the data were presented as milligram weight of different samples (IC50). Figure 2 was shown the results of antioxidants activity.

The antioxidant activity of bighead carp dried and raw samples were measured using DPPH (2,2-diphenylpicrylhydrazyl). The results were calculated as IC50 values. The efficiency of the antioxidant activities was related and conducted with IC50 values (the most efficient and effective sample was showed the lowest IC50 value). The microwave-drying sample had shown the highest antioxidant activity using DPPH assay (IC50 = 15mg/ml), while the raw sample had the lowest antioxidant activity (IC50 = 53mg/ml).

Odor test analysis

The odor test evaluation results are shown in Figure 3. The data presented as follows: 5, 4.3 and 2.9 using oven drying, microwave drying, and the raw sample, respectively. According to the results in the current study, the dried samples were showed a high score on the odor test compared with the raw bighead carp sample, which showed, according to the panel test committee, a mild taint. In contrast, treated samples in different drying techniques presented scores of odor ranging from no taint to flat aroma. The musty odor in freshwater fish is considered the economic problem that influences the sensory evaluation of various fish species [27]. After applying the two drying techniques, some of the undesirable compounds were removed during the drying process but were so difficult to remove all because of the oxidation and some chemical reactions that occurred during the drying process. Therefore, some tiny amounts of harmful compounds still existed after the process.

Conclusion

The results of current work presented that bighead carp fillets have an important source of nutrients that can play a significant role in the heath and metabolic process in the human body, which contain crude protein and crude fat. However, applying various drying techniques had a significant impact on the nutritional composition of bighead carp fillets. The main results of this study indicate that heavy metals and functional properties of different drying samples have differed significantly. This study demonstrated that bighead carp samples showed good functional properties that can be applied in vast areas of food industries. Freshwater bighead carp fish can be a good resource for protein, minerals, and other nutritional components. This might encourage other countries to start farming and breeding bighead carp in fish farms.

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