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Changes in nutrient profiling and antioxidant activities of different fish soups, before and after simulated gastrointestinal digestion

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Abstract

Different kinds of freshwater fish soups show a diverse range of health functions, due to their different nutritional substances and corresponding bioactivities. Crucian carp soup and snakehead soup have different dietotherapy functions, crucian carp soup is suitable for lactating women and snakehead soup is suitable for postoperative patients. In the current study, the changes of nutrient profiles in the different fish soups, such as chemical composition, free amino acids, mineral and fatty acid contents, were investigated. The antioxidant activities of the fish soups were evaluated by using the DPPH radical scavenging activity, the ferrous ion chelating activity, the hydroxyl radical-scavenging activity and the reducing power effect. In order to learn the theoretical basis of the potential role fish soup plays in diet therapy functions after being digested by the human body, the nutrient profiling and bioactivities of the fish soup samples after simulated gastrointestinal digestion were also explored. The intensive profiles of nutritional composition and antioxidant activities of these two kinds of fish soups were expected to partly provide the theoretical basis of therapeutic effects.

Keywords nutrient profiling; simulated gastrointestinal digestion; antioxidant activity; fish soup

Introduction

Soup is a liquid food that is cooked with different ingredients such as meat, vegetables, and beans in hot water until the flavor is extracted. The broth usually has strong flavors, and becomes a principal source of energy-yielding dietary fluids. Usually, soup has different properties and nutrients based on the different materials used during the cooking procedure, including chicken [1], beef [2], pork [3], and fish [4], *etc*. It plays an essential role in physical growth, maintenance of normal bodily functions, and good health. In addition, drinking soup could increase satiety and lower the incidence of obesity by helping people keep fit [5]. Therefore, various soups have become more and more important for people who consume them frequently, especially nutritious and healthy soups.

Freshwater fish is rich in nutrition, and it is a source of high-quality proteins, minerals [6] and essential fatty acids, particularly polyunsaturated fatty acids – docosahexaenoic acid (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3), which are good for one's health [7-9] and preventing many coronary artery diseases [7]. Therefore, freshwater fish have the potential to become raw materials for a health preserving soup.

Snakehead (*Channa argus)* and crucian carp (*Carassius auratus*) are two kinds of popular freshwater fish in Hubei province (China) for health-care soup ingredients. Snakehead soup is nutritious, and it is commonly used during adjuvant therapy for people with a weak body and a poor nutritional situation, especially for the healing of wounds and burns [10]. Snakehead soup may help muscle growth, blood regeneration, post-operative pain reduction, improve microcirculation and so on. Crucian carp soup has an attractive milky white color and a great taste, it can invigorate the spleen and prompt milk secretion. This suggests that it's consumption is suitable for the elderly and for lactating women[11]. The nutritional profiles of the fish soups may

56 determine the different dietotherapy functions; therefore the investigation of nutrient components
57 may provide relevant information.

58 Furthermore, the biological activity of food is further enhanced by the release of bioactive
59 compounds and peptides during the digestive procedure, thermal pre-treatment, microbial
60 fermentation or other technological processing [12]. Studies of the pharmacological effects of fish
61 peptides or the other metabolites hydrolyzed by digestion, no matter in-vitro and in-vivo, have
62 revealed their different functions, such as antihypertensive, immunomodulatory, antioxidant,
63 antitumor, and antimicrobial activities [13]. Antioxidant activities as a representative biological
64 activity were closely to related to the function of the food, it is commonly used to assess or
65 explain the effect of adjuvant therapy of food, even for fish or fish products [14].

66 In this study, the intensive profiles of nutritional compositions of these two homemade fish
67 soups (crucian carp soup and snakehead soup) showed different therapeutic effects. They were
68 investigated systematically, including the chemical components, free amino acid, fatty acids, and
69 mineral contents. In addition, the antioxidant activities of the fish soups, before and after
70 simulated gastrointestinal digestion, were explored by 1,1-diphenyl-2-picrylhydrazyl (DPPH)
71 radical-scavenging activity, hydroxyl radical-scavenging activity, ferrous ion chelating activity and
72 reducing power. This study could provide the theoretical basis of diet composition and properties
73 for understanding the reason of various medicinal tonic functions of different nourishing soups.

74

75 **Materials and Methods**

76 **Materials and reagents**

77 Fresh snakehead (*Channa argus)* (~750g) and crucian carp (*Carassius auratus*) (~250g) were

purchased from a local market in Huazhong Agricultural University, Hubei, China. Each specimen was gutted and cleaned. All chemicals used in this work were analytical grade.

Preparation of fish soup samples

According to the method of Tang [15], the handled fish was cooked at a suitable mince/solution ratio of 1:4 (w/v) adopting stew soup with Induction Cooker (RT2134, Midea, China) for 1.5 h. At the beginning the power was set 500W to simmer the soup for 20 min, then the power was kept at 300W and the soup maintained boiling.

Simulated gastrointestinal digestion

A two-step process was used to simulate the gastric and intestinal digestion of fish soup using the *in-vitro* enzymatic digestion protocol described in Lin et al. [16] with slight modification. The pH of the samples was adjusted to 2.0 with 1 M HCl，then pepsin was added (4%, w/w, protein basis). The mixture was incubated at 37 °C for 2 h in a shaking water bath. Subsequently, the pH value was adjusted back to 5.3 with 0.9 M $NaHCO_3$ and further to 7.5 with 1 M NaOH. Then pancreatin was added (5%, w/w, protein basis) and the mixture was further incubated at 37 °C for 2.5 h. After incubation, the test tubes were kept in a boiling water bath for 10 min to inactivate the enzyme.

For analyzing the antioxidant activities during the process of digestion, the samples were collected at different time intervals (0h, 0.5h, 1.0h, 1.5h, 2.0h, 2.5h, 3.0h, 3.5h, 4.0h, 4.5h) during in-vitro gastrointestinal digestion. All samples were adjusted to pH 7.0 and treated with 10% trichloroacetic acid (TCA) and centrifuged at 12000 rpm for 20 min. After centrifugation, the supernatant (protein hydrolysate) was stored at -20 °C for further usage.

Proximate chemical composition

The proximate composition was performed on samples using the standard methods (AOAC 1996). The moisture content of samples was determined by drying samples in an oven at 105 °C for 16–18 h (AOAC Method 950.46). The total protein was determined by the Kjeldahl method (AOAC Method 940.25). During the analysis, an automated distillation unit (Bűchi 339, Switzerland) was utilized, and the factor of 6.25 was used to convert the nitrogen content into the protein content. Ash was determined by the incineration of samples in a muffle furnace at 550 °C for 18 h (AOAC Method 938.08). The extraction of total fat was performed according to the method of Folch et al [17].

Mineral element analysis

Sample preparation and determination of the mineral contents were according to Jiang et al [18]. The soup sample (20g) was weighed and placed into a crucible, and then carbonized at 250 °C on an electrothermal plate until the sample was fully black. The crucibles with samples were dry-ashed by heating them in a muffle furnace at 550 °C (about 10−12h). Then the sample was incinerated, a white residue was obtained, which was carefully transferred into a 50 mL volumetric flask, dissolved with 5mL of 6 M HCl and then diluted to 50 mL with water. The mineral contents (K, Ca, Na, Mg, Fe, Zn) of the fish soups were detected by Atomic Absorption Spectrophotometer (TAS-990F, Fairburn, Shanghai, China).

Free amino acid analysis

Free amino acids (FAA) were extracted from the fish soups according to Tanimoto's method[19] with minor modifications. The fish samples were mixed in the same volume of sulfosalicylic acid and the mixture were centrifuged at 3000r/min for 15 min, then the supernatant was collected. 1mL supernatant solution was mixed with 6 mL TCA, and centrifuged for 15 min

and the supernatant was transferred to the round bottom flask. The rotary evaporator was used, 10mL distilled water was added, and this was repeated several times to completely remove TCA. In the end, the extracted solution was mixed with 0.02 M HCl and the pH value was adjusted to 2.0. The prepared samples were analyzed using L-8900 Amino Acid Analyzer (Hitachi, Tokyo, Japan).

Fatty acid analysis

Fatty acid methyl esters (FAMEs) were prepared according to the method presented by Bligh and Dyer [20] with slight modifications. The detailed steps were as follows: the sample (~5 mL) was mixed with 30 mL chloroform-methanol (2:1, v/v) and was homogenized at 8000 rpm for 20s twice using T18 digital Ultra-turrax (IKA, German). Then the mixture was put under stationary conditions for 1h, and then filtered after adding 0.2 times the volume of physiological saline. The suspension was centrifuged (3000 rpm, 15 min), and the upper liquid was removed and the remaining liquid was concentrated using nitrogen purging. Afterwards, 14% BF_3-methanol reagent (2 mL) was added, and the mixture was kept at 60 °C for 60 min. After cooling, 2 mL of hexane was added and the mixture was shaken for about 15s. The hexane solution of methyl-esters at the top was extracted and transferred into a tube to preserve at -20 °C.

A gas chromatography-mass spectrometer (QP2010，Shimadzu, Kyoto, Japan) equipped with a 30 m column (Rtx-5MS column 30 m × 0.25 mm × 0.25 μm column) was used. The GC conditions were set as follows: the oven temperature was initially set at 40 °C for 2 min and then increased to 100 °C at 10 °C/min. The temperature was then increased to 290 °C at 5 °C/min and held for 10 min. Split injection was conducted with a split ratio of 100: 1, the flow-rate 1 mL/min; Helium was used as a carrier gas; injector temperature was 250 °C. The MS detection conditions

were as follows: Ionization mode, EI^+; electron energy, 70 eV; full scan acquisition mode; mass range, 45-450 amu.

Measurement of antioxidant activity

DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical-scavenging activity

The DPPH scavenging activities of samples were measured as previously described with slight modifications [21]. In the current study, a 4.0 mL sample was mixed with 1.0 mL DPPH· solution (0.1 mM in 99.7% methanol). The mixture was shaken and left for 30 min at room temperature, and the absorbance of the resulting solution was measured at 517 nm using UV-1750 spectrophotometer (Shimadzu, Kyoto, Japan). Ethanol instead of DPPH was used as the blank, while distilled water was used for the control. The DPPH radical scavenging activity was calculated with the following equation:

$$\text{DPPH scavenging activity (\%)} = [1- (A_{sample}- A_{blank})/ A_{control}]\times 100 \quad (1)$$

where A_{sample}, A_{blank} and $A_{control}$ are the absorbance of sample, blank and control, respectively.

Hydroxyl radical-scavenging activity

The hydroxyl radical scavenging assay was carried out according to the method described previously [22] with some modifications. A reaction mixture solution containing 1,10-phenanthroline (0.75mM, 1mL), $FeSO_4$ (0.75mM, 1mL) and phosphate buffer (pH 7.4, 2mL) was mixed with a 2mL sample (2mL distilled water was used as the control). H_2O_2 (1mL, 0.12%) was added to the mixture and incubated at 37 °C for 60 min, and the absorbance was measured at 536 nm. The results were determined using the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = [(As- A_1)/ (A_0- A_1)]\times 100 \quad (2)$$

where As is the absorbance of the sample mixture; A_1 is the absorbance of the control (distilled

166 water instead of sample); and A_0 is the absorbance of the blank solution containing
167 1,10-phenanthroline and $FeSO_4$.

168 ***Chelating activity of Fe^{2+}***

169 The chelation activity on Fe^{2+} was measured based on the following method. An aliquot of
170 1.0 mL of sample was mixed with 3.7 mL of distilled water, 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of
171 5 mM ferrozine. The mixture reacted for 20 min at room temperature. Then the absorbance was
172 measured at 562 nm. The distilled water was used as the control, while the distilled water instead
173 of $FeCl_2$ and ferrozine was used for the blank. The chelating activity was calculated as follows:

174 $$\text{Metal ion chelating activity (\%)} = [A_{control} - (A_{sample} - A_{blank})] / A_{control} \times 100 \quad (3)$$

175 where A_{sample}, A_{blank} and $A_{control}$ are the absorbance of sample, blank and control, respectively.

176 ***Reducing power***

177 The reducing power was measured by using a modified version of Oyaizu's method [23]. 2
178 mL sample and 2 mL phosphate buffer (0.2 M, pH 6.6) were mixed with 2 mL 1% $K_3Fe(CN)_6$.
179 The mixture was incubated at 50 °C for 20 min and mixed with 2 mL 10% TCA, followed by
180 centrifugation at 3000 rpm for 10 min. Then, 2 mL of the supernatant was drawn and mixed with 2
181 mL distilled water, followed by the addition of 0.4 mL of 0.1% $FeCl_3$. After 10 min of incubation
182 at room temperature, the absorbance of the resulting Prussian blue solution was read at 700 nm.

183 **Statistical analysis**

184 All experiments were taken in triplicate, and all analyses were also conducted in triplicate.
185 Data was expressed as the mean ± standard deviation (SD). Statistical analysis was performed
186 with MS Excel (Microsoft Windows 2010) and SAS version 9.2 (SAS Institute Inc., Cary, NC,
187 USA), a significant difference in means between the samples was determined at 5% confidence

188 level ($p<0.05$).

189

190 **Results and Discussion**

191 **Proximate chemical composition**

192 The chemical compositions of the crucian carp soup and snakehead soup were shown in
193 Table 1. The results showed that water was the major component in both freshwater fish soups
194 before and after simulated gastrointestinal digestion due to the soup materials issue. There was
195 almost no difference in water, ash and fat contents that were observed in both of the fish soups,
196 except the protein content. In the beginning, the crucian carp soup had twice the soluble protein
197 content compared to the snakehead soup, however, the protein content of the snakehead soup had
198 a large increase after in-vitro gastrointestinal digestion. The protein content increased from 0.82%,
199 0.41% to 0.91%, 0.70% for the crucian carp soup and the snakehead soup, respectively.
200 Furthermore, the fat content of both fish soups represented a slight decrease after simulated
201 gastrointestinal digestion, the reason was pancreatin is a digestive enzyme which helped the
202 digestion of fat, ultimately leading to a decrease of the content of total fat[24].

203 **Mineral elements analysis**

204 The mineral element contents of the crucian carp soup and snakehead soup before and after
205 *in-vitro* digestion were collected in Table 2. From the mineral element analysis, the results of
206 major minerals (Na, K, Ca,) and trace minerals (Mg Zn, Fe) indicated that there were high
207 concentrations of K and Mg in both fish soups. Comparing the different fish species, the
208 snakehead soup appears to have a higher mineral content, especially for Ca, Zn and Fe. Usually,
209 Zn and Fe are involved in the composition of multiple enzyme active centers and have direct or

210 indirect effects on the synthesis of nucleic acids, proteins and the immune process [25]. Research
211 shows that Fe is involved in hematopoiesis, which is an important component of heme iron in
212 erythrocyte, and easily leads to anemia and other symptoms [26]. Zn deficiency can cause
213 metabolic dysfunction, decrease immune function, cause infection by bacteria, viruses and fungi
214 and growth retardation, premature and poor wound healing [27, 28]. These reasons may be why
215 Snakehead soup is suitable for patients after surgery during the time their wound is healing. After
216 the process of digestion, the contents of Na, Zn and Fe were significantly increased ($p<0.05$).
217 During the pancreatin digestion, $NaHCO_3$ and NaOH were used to adjust pH, which perhaps was
218 the major reason leading to the increase of Na. Protein degradation was lower than that of
219 snakehead soup (6.28mg/100mL) promoted by chelating metal groups exposure, thus the content
220 values of Zn and Fe went up.

221 **Free Amino acids contents**

222 The mean values (with their standard errors) of the amino acids were set out in Table 3,
223 including amino acid (AA) compositions, the concentrations of total amino acids (TAA), total
224 essential amino acids (TEAA) and flavor amino acids for all kinds of fish soup. This was done
225 regardless of the status of the fish soup samples, being before and after digestion processing.

226 From Table 3, it can be seen that the content of flavor amino acids for both fish soups were
227 different, in particular the contents of Asp, Glu, Gly, Ala and Arg in the crucian carp soup was
228 successively 0.4mg, 1.88mg, 35.48mg, 1.43mg and 0.1mg in each 100mL fish soup, but for the
229 snakehead soup it was 0.33mg, 0.82mg, 22.92mg, 7.04mg and 0.34mg, respectively. Flavor amino
230 acids, which belong to the (umami)-taste active amino acids, have been considered as one of the
231 main contributors to the flavor of a soup [29]. The total content of flavor amino acids in crucian

carp soup (38.93mg/100mL) were higher than that of snakehead soup (31.45 mg/100mL), that is the reason that crucian carp soup has a more attractive taste than snakehead soup.

The content of essential amino acids and non-essential amino acids TAA of crucian carp soup (82.51mg/100mL) were much higher than that of snakehead soup (47.54mg/100mL) ($P<0.05$), while the TEAA of crucian carp soup (4.44mg/100mL) was lower than that of snakehead soup (6.28mg/100mL) ($P<0.05$). After in-vitro gastrointestinal digestion, the variation tendency of most TAA and EAA contained in the two kinds of soups sharply increased ($P<0.05$) except His, Gly, Thr and Glu in the crucian carp soup. In the gastrointestinal tract, the soluble proteins degraded into small peptides and free amino acids under the action of pepsin and pancreatin, which are more beneficial to human absorption. The data obtained for the contents of EAA showed that the content of Thr in the crucian carp soup (5.01mg/100mL) was four times larger compared to the snakehead soup (1.05mg/100mL) after digestion. Thr is involved in a variety of human metabolism functions known as second or third restricted amino acids [30]. The contents of Val, Ile and Leu in the crucian carp soup were higher than that of snakehead soup whether it was digested or not. The Leu, Ile and Val compose the Branched-Chain Amino Acids (BCAAs) which have special physiological functions, such as strengthening immunity [31]. That means crucian carp soup has more potential abilities to enhance immunity than snakehead soup.

Fatty acids profile

Fatty acids profiles（% weight of methyl esters）, the sum of total saturated fatty acids（SFA）, unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) inherent in the crucian carp soup and the snakehead soup before and after digestion are presented in Table 4. Thirty-two kinds of fatty acids including twenty SFAs, five MUFAs and

seven PUFAs, were characterized in the different kinds of original fish soup samples. Twenty-three of them were detected in the crucian carp soup, except Caprylic acid, Pelargonic acid, 12- methyl tridecanoic acid, Heneicosanoic acid, Tricosanoic acid, Lignoceric acid, Nervonic acid, Linoleic acid and Cis-11,14-Eicosadienoic acid. Twenty-six fatty acids were detected in the snakehead soup except for Caprylic acid, Pelargonic acid, Arachidic acid, Tricosanoic acid, Cis-8,11,14-Eicosatrienoic acid and Cis-11,14-Eicosadienoic acid. After simulated gastrointestinal digestion, six fatty acids, which were not monitored in the original crucian carp soup, appeared. However, two fatty acids 14- methyl palmitic acid and Lignoceric acid disappeared in the snakehead soup. Comparing the fatty acid profiles of fish soup samples before and after simulated gastrointestinal digestion, the sum of SFA and UFA showed almost no change in general, the content of MUFA increased a little bit, the content PUFA went down a lot. The content of UFA for the crucian carp soup and the snakehead soup were 62.26% and 59.35%, respectively. The two major fatty acids were oleic acid and palmitic acid, accounting for 44.08%, 23.56%, 38.42% and 25.31% of the total fatty acids in the crucian carp soup and the snakehead soup, respectively. Usually, oleic acid has the capability of lowering the content of total cholesterol and low-density lipoprotein cholesterol, so it has the reputation of ‘safe fatty acid’ [32]. Palmitic acid is one of the most important dietary fatty acids, which plays a crucial role in the cellular biological functions. It is equipped with functions to provide energy for humans or transmitting into other fatty acids through metabolism[33]. Snakehead soup contained EPA 3.02% and DHA 5.59% equal to 8.61% of the total fatty acids, which was higher than that of crucian carp soup 5.36%. Eicosapentaenoic acid (EPA) is an important and necessary nutritional element which can increase cell viability and neuroprotective effects[34]. Commonly known as ‘brain gold’, DHA is crucial to the development

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of the nervous and visual systems and also for its deficiency during gestation, lactation and early stages of life[35]. In addition, nervonic acid was found in snakehead soup before and after digestion, but it was not detected in crucian carp soup. Generally, nervonic acid is a major long-chain MUFA which was found in the white matter of mammalian brains, it plays an important role in the treatment of psychotic disorders and neurological development[36]. Therefore, snakehead soup may have a better capability in accelerating cell growth and dramatic impacts on brain functions and mental health compared to crucian carp soup.

In order to investigate the contribution of fatty acids contained in SFA, MUFA and PUFA in different fish soup samples, the distribution plot of different fatty acid types was shown in Fig. 1. On the whole, fatty acids existed in both of the freshwater fish soups and rose after simulated gastrointestinal digestion. More specifically, there were 14 SFA (37.6%), 4 MUFA (54.34%) and 5 PUFA (7.92%) detected in crucian carp soup. However, after digestion, 20 SFA (37.17%), 5 MUFA (57.12%) and 6 PUFA (5.64%) were found in crucian carp soup. For snakehead soup, there were 16 SFA (40.73%), 5 MUFA (48.34%) and 5 PUFA (11.01%) identified, after pepsin-pancreatin digestion, there were 17 SFA (40.21%), 5 MUFA (53.29%) and 5 PUFA (6.23%). It is clear to see that the changes of fatty acids contained in the SFA and MUFA increased but the trend of PUFA types decreased. The reason may be that long-chain polyunsaturated fatty acids were cut off and converted to short-chain monounsaturated fatty acids and saturated fatty acids after digestion. Based on Table 4, the highest content of saturated fatty acids was palmitic acid which reached 25.13% and 23.59% for crucian carp soup and snakehead soup, respectively.

Antioxidant activities

DPPH radical-scavenging activity

298 DPPH was a wildly used stable free radical scavenger or hydrogen donor for assessing
299 antioxidant activities of bioactive compounds and food itself [37]. In this study, The DPPH free
300 radical scavenging activity of two kinds of freshwater fish soups with time during in-vivo
301 digestion are shown in Fig. 2. Before *in-vitro* digestion, the DPPH· radical scavenging activity of
302 snakehead soup was about 75.24%, which was higher than that of crucian carp soup (65.31%) that
303 means the fish soup had DPPH radical-scavenging activity to some extent without any digestion
304 processing. The reason may be some bioactive compounds existed in the fish soup, which
305 contained hydrophobic groups and it as easy to capture the DPPH radical. During pepsin digestion,
306 the DPPH radical scavenging activity showed a significant increasing trend in both fish soups,
307 crucian carp soup and snakehead soup; they separately rose by 32% and 21%. Such results may
308 be attributed to higher exposure to hydrophobic amino acid residues in the peptide chains, which
309 can increase antioxidative properties when some soluble proteins existed in the fish soup, were
310 further hydrolyzed by the peptin. However, from Fig. 2 it was clear to see that both of fish soup
311 samples exhibited dramatic decreases in DPPH radical scavenging activities during pancreatin
312 digestion ($P<0.05$). During the pancreatin digestion stage, the original soluble peptides contained
313 in fish soup were thoroughly hydrolyzed into shorter peptides (three peptides or four peptides) and
314 even amino acids, which had strong hydrophilicity to decrease the DPPH free radical trapping
315 capability, this phenomenon is agreed on in former research [38]..

316 **Hydroxyl radical-scavenging activity**

317 Oxidation and metabolism continuously produce various reactive oxygen radicals in the
318 process of an organism's life activities. However, the hydroxyl radical has a high toxicity to the
319 organism [38]. Reactive oxygen species inducing DNA damage are considered to be one of the

major reasons for aging [39]. Therefore, the hydroxyl radical scavenging capacity is usually used to measure the antioxidant activity of the product. As illustrated in Fig. 3, there was no significant difference in the hydroxyl radical scavenging activity between crucian carp soup and snakehead soup during *in-vitro* simulating gastrointestinal digestion. Generally, the ability of the hydroxyl radical scavenging activity showed in both fish soups kept rising during digestion, although the hydroxyl radical scavenging activity of the snakehead soup was higher than that of crucian carp soup. After finishing the simulated Gastrointestinal digestion, the value of the hydroxyl radical scavenging activity reached 3.6 and 2.7 times versus the original values for crucian carp and snakehead soup, respectively. The reason may be more peptides were generated in fish soups during the simulated gastrointestinal digestion process, this lead to more effective hydrogen or electron donors to capture hydroxyl radical, which supported former published work [40].

Ferrous ion chelating activity

Fe^{2+} chelation might render important antioxidative effects by impeding metal catalyzed oxidation[41]. Therefore, Fe^{2+} chelating activity was also measured in this study to assess the free radical scavenging activity of fish soup samples comprehensively. The Fe^{2+} chelating activity of the two kinds of freshwater fish soups during *in-vivo* digestion was shown in Fig. 4. It can be seen that there was a significant change to the metal chelating activity of the two kinds of fish soups ($P < 0.05$) within the first half hour of the digestion procedure. For crucian carp soup, the Fe^{2+} chelating activity rose to 53.58 ± 1.33% from 44.43 ± 5.2% (20% increase) in the pepsin stage, and went up to 60.05 ± 1.04% from 54.3 ± 0.11% (11% increase) in the pancreatin stage. For snakehead soup, the value of metal chelating activity increased from 26.32±3.3% to 33.29±4.07% (27% increase) in the pepsin stage, and from 34.37±4.2% to 43.48±1.98% (27% increase) in the

pancreatin stage. It could be inferred that during the whole digestion, more free amino acids were released, so more metal chelating groups exposed or generated to bind $Fe^{2+,}$ which lead to the increase of the antioxidant activity of fish soup.

Reducing power

The reducing power activity, which may serve as a significant reflection of antioxidant activity, was determined using a modified Fe (III) to Fe (II) reduction assay; the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of the samples. The presence of antioxidants in the samples causes the reduction of the Fe^{3+}/ Ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring of the formation of Perls Prussian blue at 700 nm [42]. The reducing power of the two kinds of fish soups was shown in Fig. 5, it was very clear that the reducing power of crucian carp soup is much stronger than that of snakehead soup, whether it was processed by simulated gastrointestinal digestion or not. Particularly, reducing power of both fish soups increased within the first hour ($P < 0.05$), then it kept the invariant mode from one hour to two hours during the pepsin digestion step. In the simulated intestinal digestion step, there appeared to be a dramatic increase in reducing power within half an hour after 2h of the whole digestion time. After that, the reducing power of fish soups seemed to stay stable for the remaining time. Generally, the tendency of reducing power in these two kinds of fish soups was consistent with the result of the Fe^{2+} chelating activity.

Conclusion

Various foods usually show dietary therapy functions closely related to their individual

364 unique profiles of chemical components, nutrient compositions and bioactivities. In this study, the
365 nutritional compositions and antioxidant activities of two kinds of freshwater fish soup (crucian
366 carp soup and snakehead soup) with different Dietotherapy functions were investigated. In order
367 to explore their intensive nutritional profiling, the nutritional composition changes of the fish soup
368 samples after simulated gastrointestinal digestion (pepsin and pancreatin hydrolysis) were also
369 considered. It can be concluded that the content of the total free amino acids of crucian carp soup
370 (82.51 mg/100mL) were higher than that of snakehead soup (47.54 mg/100mL) ($P < 0.05$),
371 especially for the flavor or umami amino acids content, the dominant amino acids were Glutamine
372 and Glycine. After simulated gastrointestinal digestion, the number of fatty acids existed in both
373 fish soups increased, the number of saturated fatty acids, unsaturated fatty acids and
374 polyunsaturated fatty acids were 20, 5, 6 and 17, 5, 5, for the crucian carp soup and the snakehead
375 soup, respectively. However, snakehead soup had more mineral contents especially for Ca
376 (0.81μg/g), Fe (0.35μg/g) and Zn (0.25μg/mg) than that of the crucian carp soup Ca (0.13μg/g), Fe
377 (0.12μg/g) and Zn (0.07μg/mg). Minerals usually play a key role in biological processes and
378 metabolism and are considered as nutrient minerals related to specific health benefits [43], so
379 snakehead soup may have unique therapeutic use in dietary supplements. Furthermore, crucian
380 carp soup showed a better antioxidant capacity compared to snakehead soup in the hydroxyl
381 radical-scavenging activity, ferrous ion chelating activity and reducing power.
382
383 **Ethics**
384 All animal (fish) procedures were performed in accordance with the Guidelines for Care and Use
385 of Laboratory Animals of Huazhong Agricultural University and Experiment were approved by

the Animal Care and Use Committee of Huazhong Agricultural University.

Data Availability

All data associated with this paper are available in the supplementary materials.

Authors' Contributions

H.D. was involved in study design, data analysis and writing the manuscript; G.Z. performed all the experiments, including the sample preparation, data analysis and writing the manuscript; Y.F. and S.X were participated in data analysis, S.Z. did the gas chromatography-mass spectrometer experiment, and G.S. prepared the samples. All authors gave final approval for publication.

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523 **Table 1** Proximate chemical compositions of different fish soup samples before and after
524 simulated gastrointestinal digestion (g/100g) (mean ± SD)

Component	Crucian carp soup		Snakehead soup	
	before	after	before	after
Moisture	98.82±0.10^{b}	98.2±0.25^{c}	99.16±0.12^{a}	98.64±0.28^{b}
Ash	0.14±0.01^{c}	0.095±0.00^{d}	0.095±0.26^{b}	0.52±0.02^{a}
Protein	0.82±0.11^{b}	0.91±0.05^{a}	0.41±0.02^{d}	0.70±0.01^{c}
Fat	0.18±0.06^{a}	0.17±0.01^{b}	0.15±0.09^{c}	0.13±0.01^{d}

525 Note: Different lowercase letters in the same line indicate significant difference ($p<0.05$).

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528 **Table 2** Mineral contents of different fish soup samples before and after simulated gastrointestinal
529 digestion (mean ± SD)

Mineral element	Crucian carp soup		Snakehead soup	
	before	after	before	after
Na(mg/g)	0.13±0.03^{c}	0.69±0.01^{a}	0.21±0.09^{b}	0.69±0.00^{a}
K(mg/g)	0.79±0.05^{a}	0.79±0.04^{a}	0.75±0.01^{a}	0.87±0.04^{a}
Ca(μg/g)	0.13±0.01^{b}	0.14±0.01^{b}	0.81±0.01^{a}	0.82±0.00^{a}
Mg(μg/g)	6.12±0.497^{b}	6.12±0.21^{b}	6.45±0.165^{a}	6.01±0.11^{c}
Zn(μg/g)	0.07±0.01^{d}	0.16±0.00^{c}	0.25±0.12^{b}	0.37±0.01^{a}
Fe(μg/g)	0.12±0.01^{d}	0.17±0.00^{c}	0.35±0.02^{b}	0.39±0.01^{a}

530 Note: Different lowercase letters in the same line indicate significant difference ($p<0.05$).

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Table 3 Free amino acid compositions of different fish soup samples before and after simulated gastrointestinal digestion (mg/100mL) (mean ± SD)

Amino acid	Crucian carp soup		Snakehead soup	
	before	after	before	after
Asp#	0.04±0.01^{d}	1.13±0.01^{a}	0.33±0.01^{c}	0.81±0.01^{b}
Thr*	1.10±0.02^{c}	1.05±0.05^{c}	2.04±0.03^{b}	5.01±0.04^{a}
Ser	0.66±0.02^{c}	1.43±0.06^{b}	0.54±0.01^{c}	1.87±0.01^{a}
Glu#	1.88±0.01^{a}	2.00±0.08^{a}	0.82±0.06^{b}	1.99±0.01^{a}
Gly#	35.48±0.40^{a}	28.75±1.18^{b}	22.92±0.44^{c}	21.44±0.15^{c}
Ala#	1.43±0.01^{d}	4±0.18^{c}	7.04±0.18^{b}	9.58±0.22^{a}
Cys	0.66±0.06^{c}	1.3±0.07^{b}	0.3±0.22^{d}	1.63±0.10^{a}
Val*	0.62±0.01^{b}	2.7±0.13^{a}	0.45±0.01^{b}	3.07±0.01^{a}
Met	0.52±0.01^{c}	1.16±0.07^{b}	0.27±0.01^{d}	1.57±0.02^{a}
Ile*	0.27±0.01^{c}	1.45±0.07^{a}	0.16±0.02^{c}	1.27±0.01^{a}
Leu*	0.96±0.05^{c}	14.42±0.60^{a}	0.58±0.01^{d}	10.69±0.10^{b}
Tyr	1.88±0.04^{b}	5.33±0.23^{a}	0.64±0.21^{c}	5.83±0.23^{a}
Phe*	0.68±0.09^{b}	15.69±0.67^{a}	0.82±0.12^{b}	15.53±0.08^{a}
Lys*	0.81±0.01^{c}	22.07±0.88^{a}	2.23±0.06^{b}	20.31±0.12^{a}
Pro	0.84±0.03^{b}	0.83±0.21^{b}	1.16±0.01^{a}	1.07±0.13^{a}
His	34.58±0.45^{a}	29.09±1.22^{b}	6.9±0.09^{c}	6.58±0.04^{c}
Arg#	0.10±0.02^{d}	18.68±0.72^{b}	0.34±0.01^{c}	25.45±0.21^{a}
Total Amino acid(TAA)	82.51±1.12^{c}	146.87±0.00^{a}	47.54±1.63^{d}	133.13±0.00^{b}
Total Essential Amino acid(TEAA)	4.44±0.03^{c}	58.52±2.49^{a}	6.28±0.02^{b}	57.42±0.37^{a}
EAA/TAA	5.38%	39.84%	13.21%	41.96%

Note: Different lowercase letters in the same line indicate significant difference ($p<0.05$). * Essential amino acid. # Flavor amino acid.

538 **Table 4** Fatty acid compositions of different fish soup samples before and after simulated
539 gastrointestinal digestion (relation percentage/%) (mean ± SD)

Fatty acid	**Molecular formula**	crucian carp soup		snakehead fish soup	
		Before	after	before	after
2- methyl butyric acid	C4:0	0.02±0.00^{b}	0.03±0.00^{b}	0.03±0.00^{b}	0.05±0.01^{a}
Caproic acid	C6:0	0.01±0.00^{a}	0.05±0.01^{b}	0.01±0.00^{b}	0.01±0.00^{b}
Caprylic acid	C8:0	/	0.03±0.01	/	/
Pelargonic acid	C9:0	/	0.06±0.01	/	/
Anchoic acid	C9:0	0.10±0.01^{b}	0.37±0.02^{a}	0.12±0.02^{b}	0.04±0.00^{b}
Lauric acid	C12:0	0.15±0.02^{b}	0.4±0.02^{a}	0.16±0.04^{b}	0.14±0.01^{b}
Tridecanoic acid	C13:0	0.05±0.01^{b}	0.08±0.01^{a}	0.10±0.03^{a}	0.09±0.01^{a}
12- methyl tridecanoic acid	C13:0	/	0.06±0.00^{a}	0.10±0.02^{a}	0.07±0.01^{a}
Myristic acid	C14:0	0.13±0.01^{c}	2.57±0.21^{b}	0.15±0.04^{c}	4.19±0.29^{a}
Pentadecanoic acid	C15:0	0.61±0.03^{b}	0.85±0.03^{a}	0.62±0.05^{b}	0.92±0.05^{a}
Palmitic acid	C16:0	23.56±0.43^{b}	21.47±0.54^{c}	25.31±0.64^{a}	23.42±1.48^{b}
14- methyl palmitic acid	C16:0	0.13±0.02^{b}	1.3±0.1^{a}	0.20±0.02^{b}	/
Heptadecanoic acid	C17:0	0.46±0.05^{c}	0.69±0.01^{b}	0.73±0.07^{b}	0.8±0.02^{a}
Stearic acid	C18:0	7.22±0.05^{c}	8.15±0.15^{b}	8.61±0.36^{b}	9.48±0.72^{a}
Nonadecanoic acid	C19:0	0.15±0.01^{b}	0.14±0.01^{b}	0.12±0.03^{b}	0.22±0.01^{a}
Arachidic acid	C20:0	0.36±0.04^{b}	0.35±0.01^{b}	/	0.46±0.01^{a}
Heneicosanoic acid	C21:0	/	0.4±0.02 ^{a}	0.18±0.04^{b}	0.04±0.01^{c}
Behenic acid	C22:0	0.10±0.01^{b}	0.13±0.02^{b}	0.06±0.01^{c}	0.18±0.01^{a}
Tricosanoic acid	C23:0	/	0.02±0.01^{a}	/	0.03±0.00^{a}
Lignoceric acid	C24:0	/	0.03±0.01^{a}	0.06±0.01^{a}	0.07±0.01^{a}
Saturated fatty acid（SFA）		37.60±0.77^{b}	37.17±0.79^{b}	40.73±0.43^{a}	40.21±0.5^{a}
Myristoleic acid	C14:1	0.13±0.01^{b}	0.11±0.02^{c}	0.15±0.04^{b}	0.18±0.02^{a}
Palmitoleic acid	C16:1	9.39±0.15^{a}	7.59±0.24^{b}	9.57±0.44^{a}	9.07±0.24^{a}

Oleic acid	C18:1n9	44.08±0.08^{b}	48.42±1.22^{a}	38.42±0.33^{c}	42.81±0.57^{b}
Erucic acid	C22:1	0.62±0.03^{b}	0.98±0.06^{a}	0.14±0.02^{c}	1.15±0.07^{a}
Nervonic acid	C24:1	/	0.01±0.00^{b}	0.07±0.02^{a}	0.08±0.02^{a}
Monounsaturated fatty acid（MUFA）		54.34±0.22^{b}	57.12±1.07^{a}	48.34±0.83^{c}	53.29±0.31^{b}
Linoleic acid	C18:2n6	/	0.42±0.03^{a}	0.14±0.03^{b}	0.06±0.01^{c}
Arachidonic acid	C20:4n6	1.52±0.03^{a}	1.25±0.08^{b}	1.83±0.10^{a}	1.11±0.14^{b}
Cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n3	2.04±0.06^{b}	1.26±0.06^{c}	3.02±0.09^{a}	1.61±0.19^{c}
Cis-8,11,14-Eicosatrienoic acid	C20:3	0.45±0.03^{a}	0.66±0.09^{a}	/	/
Linolenic acid	C18:3	0.61±0.01^{a}	0.73±0.01^{a}	0.21±0.03^{b}	/
Cis-11,14-Eicosadienoic acid	C20:2	/	/	/	0.03±0.00
Docosahexenoic acid	C22:6n3	3.30±0.03^{b}	1.33±0.12^{c}	5.80±0.27^{a}	3.41±0.22^{b}
Polyunsaturated fatty acids (PUFA)		7.92±0.12^{b}	5.64±0.08^{d}	11.01±0.12^{a}	6.23±0.23^{c}
Unsaturated fatty acid (UFA)		62.26±0.34^{a}	62.76±1.01^{a}	59.35±0.71^{b}	59.52±0.30^{b}

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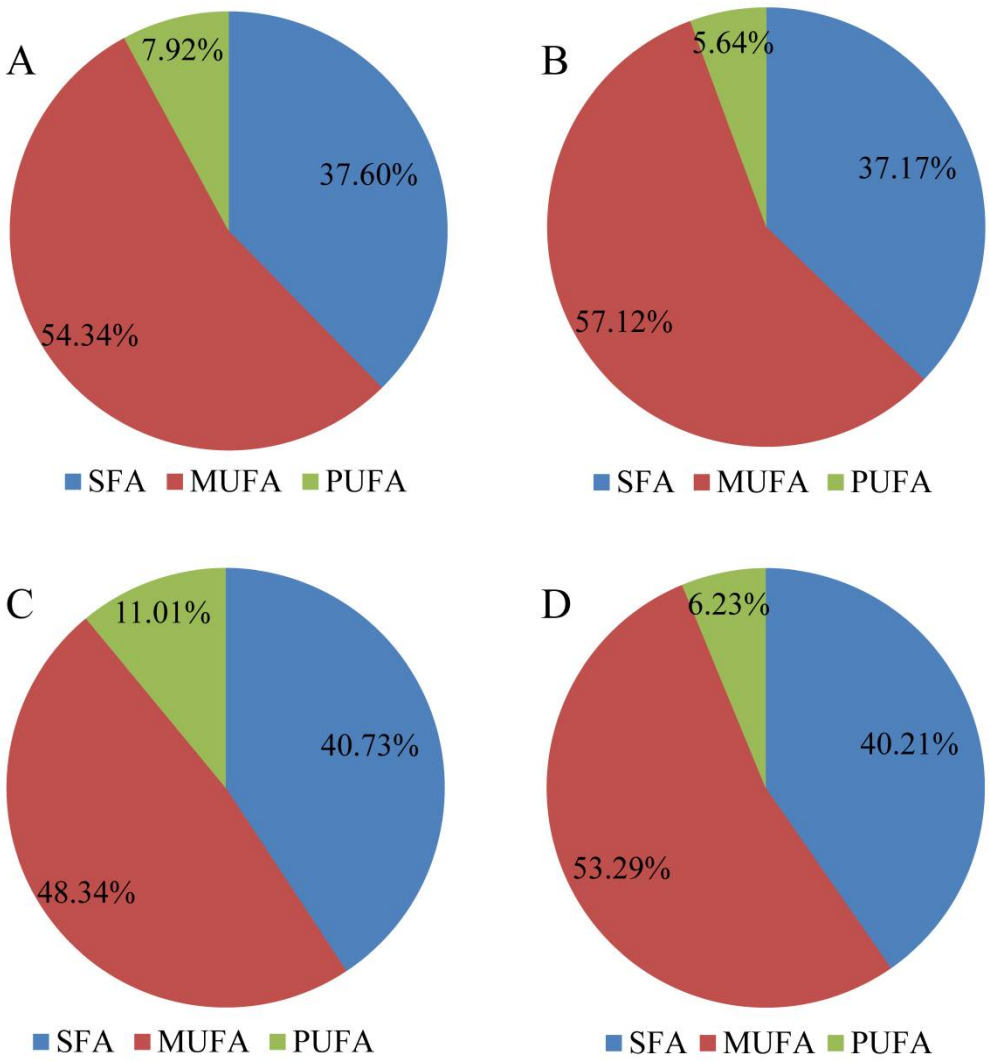
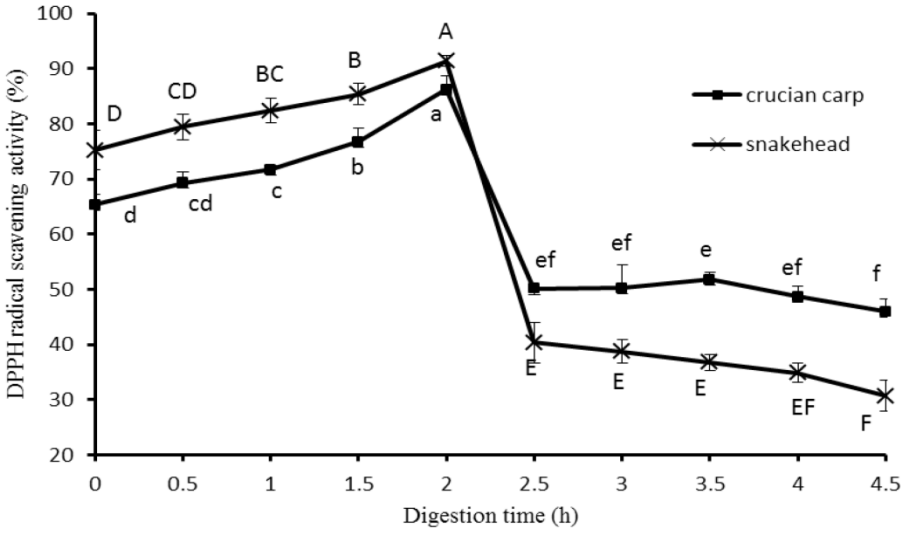


Note: SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acids.

Fig. 1 Distribution plots of fatty acid species determined by fish soup samples (A, B, C, D was crucian carp and snakehead soup before and after simulated gastrointestinal digestion, respectively).



548

549 *Note: different letters mean significant differences between samples ($p<0.05$).*

550 **Fig. 2** DPPH· radical scavenging activity of crucian carp soup and snakehead soup during

551 simulated gastrointestinal digestion

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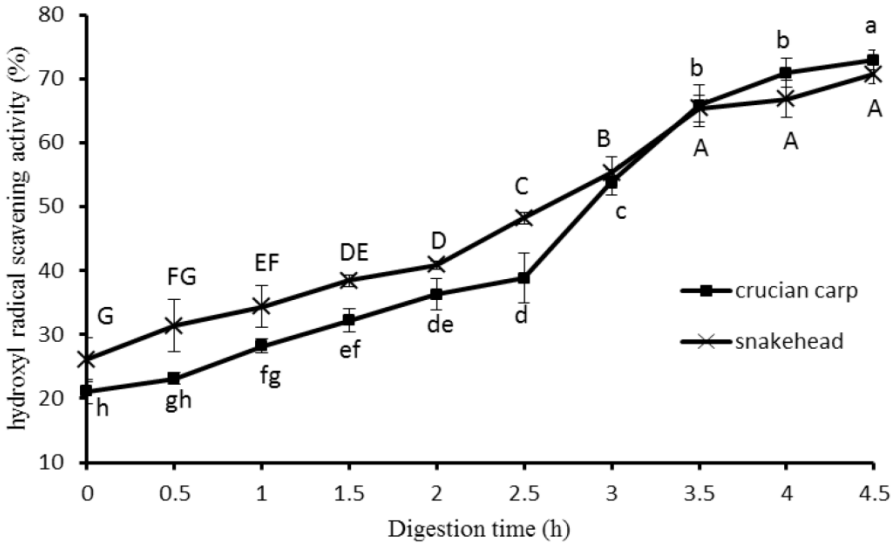
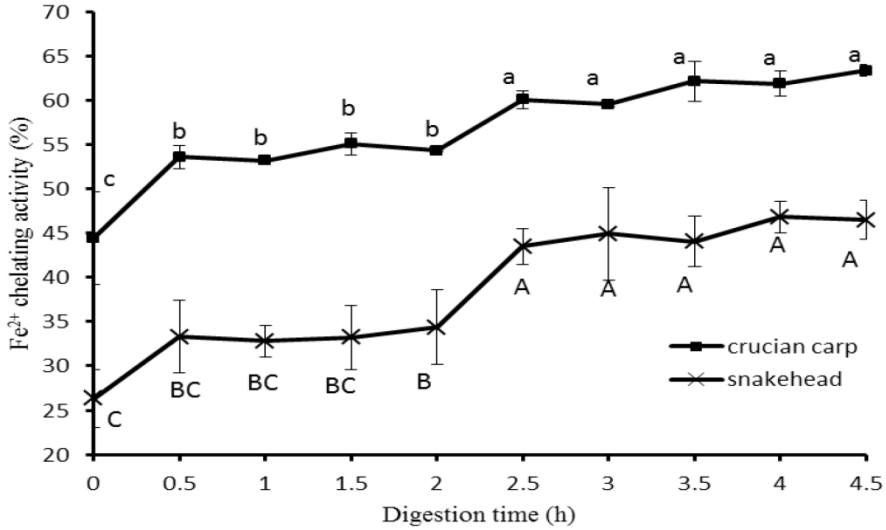


553

554 *Note: different letters mean significant differences between samples ($p<0.05$).*

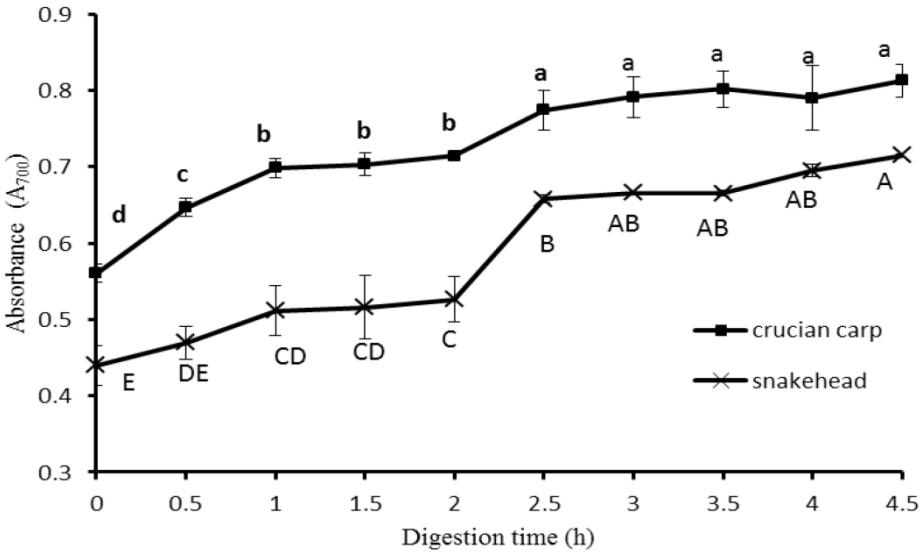
555 **Fig. 3** Hydroxyl radical-scavenging activity of crucian carp soup and snakehead soup during

556 simulated gastrointestinal digestion



Note: different letters mean significant differences between samples ($p<0.05$).

Fig. 4 Ferrous ion chelating activity of crucian carp soup and snakehead soup during simulated gastrointestinal digestion process



562

563 *Note: different letters mean significant differences between samples ($p<0.05$).*

564 **Fig. 5** Reducing power of crucian carp soup and snakehead soup during simulated gastrointestinal

565 digestion process

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