

# Comparison the Chemical and Functional Properties of Protein Hydrolysates from Different Mature Degree Hawk Teas

Xuejing Jia<sup>1</sup>, Chunbang Ding<sup>1</sup>, Lihua Dong<sup>1</sup>, Shu Yuan<sup>2</sup>, Zhongwei Zhang<sup>2</sup>, Yanger Chen<sup>1</sup>, Ming Yuan<sup>1,\*</sup>

<sup>1</sup>College of Life and Basic Sciences, Sichuan Agricultural University, Ya'an, China

<sup>2</sup>College of Resources and Environmental Science, Sichuan Agricultural University, Chengdu, China

\*Corresponding author: [yuanmingsicau@126.com](mailto:yuanmingsicau@126.com)

Received October 29, 2013; Revised November 22, 2013; Accepted December 05, 2013

**Abstract** Hawk tea is one of the most popular beverage in southwest of China. In the present study, the effects of alkaline protease on the formation and characteristics of protein hydrolysates from different mature degree Hawk teas were investigated. For antioxidant activity in vitro, Hawk mature leaf tea protein hydrolysates (MPH) had the strongest 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity and reducing power. And MPH also showed the highest iron chelating activity among the three samples. With regard to functional property, MPH and Hawk primary leaf tea protein hydrolysates (PPH) had higher solubility than that of Hawk bud tea protein hydrolysates (BPH). And the same trend was found in emulsifying property. The amino acid composition of MPH, PPH and BPH was very dissimilar. MPH and PPH mainly contained low molecular weight peptides, while BPH majorly included high molecular weight peptides. MPH may have potential applications in food industries as natural antioxidants.

**Keywords:** Hawk tea, antioxidant activity, metal chelating activity, functional property, amino acid composition

**Cite This Article:** Xuejing Jia, Chunbang Ding, Lihua Dong, Shu Yuan, Zhongwei Zhang, Yanger Chen, and Ming Yuan, "Comparison the Chemical and Functional Properties of Protein Hydrolysates from Different Mature Degree Hawk Teas." *Journal of Food and Nutrition Research* 1, no. 6 (2013): 138-144. doi: 10.12691/jfnr-1-6-5.

## 1. Introduction

All cells generate free radicals during their normal cellular metabolic process [1]. However, producing excess free radical can induce damages to cell and tissue, and cause various diseases [2]. Fortunately, the normal biological system can be kept by antioxidants in the case of excessive free radicals. Therefore, it is beneficial for human being to develop and utilize effective antioxidants. Recently, bioactive peptides from plants as a natural antioxidant have attracted researchers' great interest [3].

Generally, bioactive peptides are derived from plant protein by enzymatic hydrolysis method. To avoid the negative impacts of the toxic chemicals or organic solvents residues, protein hydrolytic enzyme is applied widely during food processing [4]. Bioactive peptide is specific protein fragment, and it can exert various functions in the food industries and human health after it is released by proteolytic enzyme. However, it is inactive when it lies in the protein that did not pass enzymatic hydrolyze former [5]. This kind of peptide consists of 2-20 amino acids, and its bio-activity can be affected greatly by its amino acid composition and sequence [6]. These bio-peptides can act various roles in antimicrobial, antioxidative, immunomodulatory and multifunctional properties in accordance with their amino acid composition and structural properties [7,8].

It had been found that peptides which were produced by alkaline protease had various biological activities, such as antioxidant activities and functional properties. In comparison to other proteases, alkaline protease produced shorter peptides [9] and the antioxidant activity of hydrolysates produced by alkaline protease derived was higher than that of other hydrolysates [10].

Hawk tea (*Litsea coreana* var. *lanuginosa*), a medicinal and edible plant, is a herbal tea and one of the most popular beverage in southwest of China. Hawk tea is cultivated widely in southwest of China. It is also one of the most favorite beverages and attracts a great quantity of consumers who live in the mountainous area and the number of consumers amounts to ≈30 million. Based on the mature degree of the raw materials, Hawk tea can be divided into three types: Hawk bud tea (HB, made from the most tender shoots), Hawk primary leaf tea (HP, made from new leaves), and Hawk mature leaf tea (HM, made from mature leaves) [11]. Their prices vary greatly on account of their yields. The price of HB is about \$50 per kilogram in the local retail market, and the price of HP and HM is about \$20 and \$3 per kilogram respectively.

However, up to now, few works have been made to assess the effects of the raw materials' mature degree on the chemical and functional properties of their protein hydrolysates. For this study, the relationship between the mature degree of Hawk teas raw material and the chemical and functional properties of protein hydrolysates was

studied, by considering their antioxidant activities, amino acid compositions, the distribution of molecular weight, and the metal chelating activities. Three kinds of Hawk teas' proteins were enzymatically hydrolysed with alkaline protease to prepare bioactive peptides.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

Three kinds of Hawk teas (*Litsea coreana* var. *lanuginosa*) were purchased from local retail shops (Yucheng District, Sichuan Province, China). Ferrozine, pyrocatechol violet, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and TNBS (2,4,6-trinitrobenzenesulphonic acid solution, 5% in H<sub>2</sub>O) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Alkaline protease was purchased from KaYon Biological Technology Co. (ShangHai, China). All other reagents were the highest commercially available grade.

### 2.2. Production of Hawk Tea Protein Isolates

Hawk tea proteins were isolated according to the previously published protocol [12] with slight modification. All samples were ground to powder and passed through 40 mesh sieve. Then, the flour was extracted five times with petroleum ether and anhydrous ethanol for 4 h each in order to remove colored ingredients, polyphenols and small molecular impurities. Proteins were extracted by suspending the flour in distilled water (1 : 10, w/v), adjusted to pH 10 with 1 mol/L NaOH, and agitating for 4h at 50°C. The residues were re-extracted three times. Then all of them were centrifuged at 5 000 g for 20 min, and in order to precipitate the proteins the supernatant was adjusted to pH 4.0 with 1 mol/L HCl, and followed by centrifugation at 8 000 g for 20 min.

### 2.3. Enzymatic Protein Hydrolysis

Subsequently, the sample proteins were hydrolysed with alkaline protease for 270 min at 55°C. The ratio of enzyme to substrate (E/S) was at 1 : 100 (w/w), while the pH of the reaction system was 8.5 and constantly maintained during the entire hydrolytic process with 1 mol/L NaOH. Then the solutions were adjusted to pH 7.0, heated in boiling water for 20 min, and centrifuged at 5 000 g for 20. The supernatants (protein hydrolysates) were lyophilized and stored at 20°C for further use. After lyophilizing, the peptides were obtained and named BPH (Hawk bud tea protein hydrolysates), PPH (Hawk primary leaf tea protein hydrolysates), and MPH (Hawk mature leaf tea protein hydrolysates) according to their leaf mature degree, respectively.

### 2.4. Degree of Hydrolysis

The degree of hydrolysis (DH) of BPH, PPH and MPH by alkaline protease was determined via the TNBS reaction according to a previously established protocol [13]. The protein was fully hydrolyzed with 6 mol/L HCl at 110°C for 24 h.

### 2.5. Determination of Molecular Weight Distribution

Molecular weight distribution of BPH, PPH and MPH was determined by a Sephadex G-50 (20 mm×600 mm) gel filtration and eluted by distilled water with a flow rate of 1 mL/min, and detected at 280 nm [10]. The reference curve was made by using Cytochrome C (12 500 Da), insulin (5 807 Da), glutathione (307 Da) and arginine (174 Da).

### 2.6. Amino acid Analysis

Protein was fully hydrolyzed and determined by automatic amino acid analyser (L-8800, Hitachi, Japan). The amino acid composition for BPH, PPH and MPH was expressed as g/100 g. Nutritional value was measured by the proportions of essential amino acids (E) to total amino acids (T).

### 2.7. Antioxidant Activity

#### 2.7.1. DPPH Radical Scavenging Activity

DPPH radical scavenging activity of BPH, PPH and MPH was evaluated according to precisely reported method [14]. Dissolving the samples and diluting it to the concentration of 0.3, 0.6, 1, 3 and 5 mg/mL, respectively. Shaken and incubated in the dark for 20 min after mixing 0.15 mL samples and 3 mL 0.05 mmol/L DPPH methanol solutions. Then, the absorbance was measured at 517 nm. The calculated equation was following:

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \right] \times 100$$

Where  $A_{\text{DPPH}}$  is the absorbance of the DPPH radical solution without sample and  $A_{\text{sample}}$  is the absorbance of the DPPH radical solution with tested samples.

#### 2.7.2. Reducing Power

Reducing power was evaluated by previous method [15]. Dissolving the samples and diluting it to the concentration of 0.3, 0.6, 1, 3 and 5 mg/mL, respectively. Incubated at 50°C for 20 min after mixing 50  $\mu$ L samples, 50  $\mu$ L 0.2 mol/L phosphate buffered saline (pH 6.6) and 50  $\mu$ L 1% potassium ferrocyanate (w/v). Then, 50  $\mu$ L 10% trichloroacetic acid, 10  $\mu$ L 0.1% ferric chloride and 400  $\mu$ L distilled water were added to the mixture. The absorbance was read at 700 nm after 20 min.

### 2.8. Metal Chelating Activity

#### 2.8.1. Iron Chelating Activity

Iron chelating activity was measured according to the former method [16]. Dissolving the samples and diluting it to the concentration of 0.3, 0.6, 1, 3 and 5 mg/mL, respectively. Incubated for 30 min at room temperature after mixing 50  $\mu$ L samples, 250  $\mu$ L 100 mmol/L Na acetate buffer (pH 4.9), and 30  $\mu$ L 0.01% FeCl<sub>2</sub> (w/v). Then, 15  $\mu$ L 40 mmol/L Ferrozine was added to the mixture. The absorbance was measured at 562 nm. The calculated equation was following:

$$\text{Iron chelating activity (\%)} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

### 2.8.2. Copper Chelating Activity

Copper chelating activity was measured by previous method [13]. Dissolving the samples and diluting it to the concentration of 0.3, 0.6, 1, 3 and 5 mg/mL, respectively. Incubated for 10 min at room temperature after mixing 50  $\mu$ L sample, 290  $\mu$ L 50 mmol/L Na acetate buffer (pH 6.0), 6  $\mu$ L 4 mmol/L pyrocatechol violet and 10  $\mu$ L 0.1 mg/mL  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . Then, the absorbance was read at 632 nm. The calculated equation was following:

$$\text{Copper chelating activity (\%)} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

## 2.9. Determination of Functional Properties

### 2.9.1. Solubility

The solubility was tested by previous method [17]. Dispersed samples to the concentration of 5 mg protein/mL and adjusted it to different pHs (3.0, 5.0, 7.0, 9.0, 11.0) by using 1 mol/L HCl and 1 mol/L NaOH. Then, stirred for 10 min and centrifuged at 5 000 g for 20 min at room temperature. The protein content of supernatant was measured by coomassie brilliant blue reaction.

### 2.9.2. Emulsifying Properties

The emulsion activity index (EAI) and emulsion stability index (ESI) were measured by previous method [8]. Homogenized 3 mL sample solution (5 mg protein/mL) and 1 mL camellia oil for 1 min at 20 000 rpm. Then, 3 mL 0.1% SDS was added to 30  $\mu$ L emulsion and mixed for 10 s. The absorbance was read at 500 nm for 0 min ( $A_0$ ) and 10 min ( $A_{10}$ ). The calculated equation of EAI and ESI was following:

$$\text{EAI (m}^2/\text{g)} = \frac{2 \times 2.303 \times A_0 \times \text{dilution}}{C \times (1 - \phi) \times 10^4}$$

$$\text{ESI (min)} = \frac{A_0 \times t}{A_0 - A_{10}}$$

where Dilution=100, C represents the initial concentration of BPH, PPH and MPH (g/mL),  $\phi$  is the emulsion oil volume (mL,  $\phi=0.3$ ), and  $t=10$  min.

## 2.10. Statistical Analysis

The data were presented as mean  $\pm$  standard deviation and analysed using SPSS 19.0 statistics programme. The  $P$  values were set at  $P < 0.05$  to assess the statistically significant.

## 3. Results and Discussion

### 3.1. Enzymatic Hydrolysis

The degree of hydrolysis (DH) was evaluated by the level of the free  $\alpha$ - $\text{NH}_2$  groups by TNBS [18]. In this study, the alkaline protease worked in the optimal condition which was recommended by the manufacturer. When the alkaline protease was added, HB, HP and HM proteins at 1/100 (w/w) E/S were hydrolyzed at a rapid rate within the initial 90 min, then the hydrolytic rate slowed down (Figure 1). These results indicated that the main

hydrolysis of HB, HP and HM protein occurred within 90 min. This hydrolysis curve was similar to the previous one in the rice protein [10]. According to the hydrolytic efficiencies, there was not significant different between MPH and PPH. DH increased up to 13.79, 18.10 and 17.67%, respectively, after 270 min of incubation (Figure 1). The results implied that the susceptibility of BPH, PPH and MPH was related to the materials' mature degree, and the tender material exhibited a lower DH value.

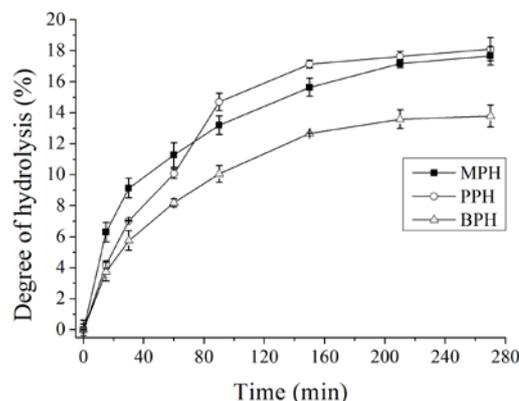


Figure 1. The degree of hydrolysis of BPH, PPH and MPH was prepared by alkaline protease

### 3.2. Distribution of Molecular Weight

Distribution of molecular weight was changed during the process of protein hydrolysis, and some hydrophobic groups which were internal within the parental protein molecule generally exposed to the aqueous phase. This phenomenon was related to bio-peptides' structure and functional property [19]. The protein samples were hydrolyzed by alkaline protease for 270 min and the molecular weight of protein hydrolysates was determined by gel permeation chromatography method. These protein hydrolysates originated from different mature degree raw material and were subjected to alkaline protease, and their molecular weight distributions were noticeably distinct. There were notable difference between the distribution of large peptide moleculars (>10 kDa) and small peptide moleculars (5~10 kDa and <1 kDa) for MPH, PPH and BPH. Table 1 showed that PPH and MPH were mainly composed of low molecular weight peptides (<10 kDa), but BPH mainly contained high molecular weight peptides (>10 kDa). There were notable difference between the distribution of high molecular weight peptides (>10 kDa) and low molecular weight peptides (5~10 kDa and <1 kDa) for MPH, PPH and BPH. This result, considering of their DH values suggested that PPH and MPH were much more effective in producing smaller peptides than BPH.

Table 1. Molecular weight distribution of BPH, PPH and MPH<sup>a</sup>

	>10 kDa	5-10 kDa	1-5 kDa	<1 kDa
BPH	50.14 $\pm$ 1.51 <sup>a</sup>	9.34 $\pm$ 0.06 <sup>c</sup>	20.39 $\pm$ 0.11 <sup>a</sup>	20.13 $\pm$ 0.10 <sup>a</sup>
PPH	21.62 $\pm$ 0.18 <sup>c</sup>	56.20 $\pm$ 1.81 <sup>a</sup>	13.28 $\pm$ 0.06 <sup>b</sup>	8.90 $\pm$ 0.04 <sup>c</sup>
MPH	26.55 $\pm$ 0.32 <sup>b</sup>	39.64 $\pm$ 0.98 <sup>b</sup>	21.37 $\pm$ 0.68 <sup>a</sup>	12.45 $\pm$ 0.12 <sup>b</sup>

<sup>a</sup> Results are presented as mean  $\pm$  standard deviation (n = 3) and different superscript letters in the same column indicate significant difference ( $P < 0.05$ ).

### 3.3. Amino Acid Profiles

Composition of amino acids was one of the most important factors in protein hydrolysates' antioxidant activities. The previous report indicated that amino acids such as Asp and Glu possessed strong antioxidant activity [20]. The amino acid compositions (g/100g of protein) of BPH, PPH and MPH for 270 min hydrolysis were shown in Table 2, which revealed that they were rich in His, Tyr, Leu, Phe and Lys. Some amino acids in MPH, such as Asp and Glu, had higher content than those in BPH and PPH. There was some difference in amino acid compositions among the three samples, which might be attributed to the different raw materials, which are difference in mature degree. The nutritional qualities of BPH, PPH and MPH were evaluated by E/T. BPH with the E/T values of 45.28% was higher than MPH and PPH with the E/T values of 42.65 and 40.23%, respectively.

**Table 2. Amino acid composition (g/100 g of protein) of BPH, PPH and MPH<sup>a</sup>**

Amino acid	BPH	PPH	MPH
Asp	5.19 ± 0.03	5.72 ± 0.09	7.59 ± 0.04
Thr	2.26 ± 0.01	2.86 ± 0.01	2.26 ± 0.01
Ser	2.94 ± 0.00	3.05 ± 0.01	3.68 ± 0.02
Glu	7.65 ± 0.04	5.59 ± 0.21	10.59 ± 0.13
Gly	10.06 ± 0.05	3.00 ± 0.02	9.31 ± 0.27
Ala	3.12 ± 0.02	3.21 ± 0.11	3.30 ± 0.01
Cys	3.39 ± 0.02	2.18 ± 0.01	5.21 ± 0.03
Val	4.80 ± 0.02	4.33 ± 0.12	5.27 ± 0.03
Met	0.90 ± 0.00	0.30 ± 0.01	0.30 ± 0.00
Ile	7.87 ± 0.04	1.31 ± 0.01	3.94 ± 0.02
Leu	16.01 ± 0.08	2.62 ± 0.01	11.28 ± 0.16
Tyr	16.85 ± 0.08	15.76 ± 0.08	13.77 ± 0.19
Phe	22.14 ± 0.11	24.28 ± 0.21	18.34 ± 0.09
Lys	12.13 ± 0.06	20.91 ± 0.65	17.40 ± 0.09
His	14.43 ± 0.12	15.99 ± 0.30	10.09 ± 0.05
Arg	5.23 ± 0.03	17.42 ± 0.07	15.68 ± 0.08
Pro	3.45 ± 0.02	2.42 ± 0.01	1.50 ± 0.01
Trp		Not determined	
E/T%	45.28 ± 0.54	42.65 ± 0.48	40.23 ± 0.37

<sup>a</sup> Results are presented as mean ± standard deviation (n = 3).

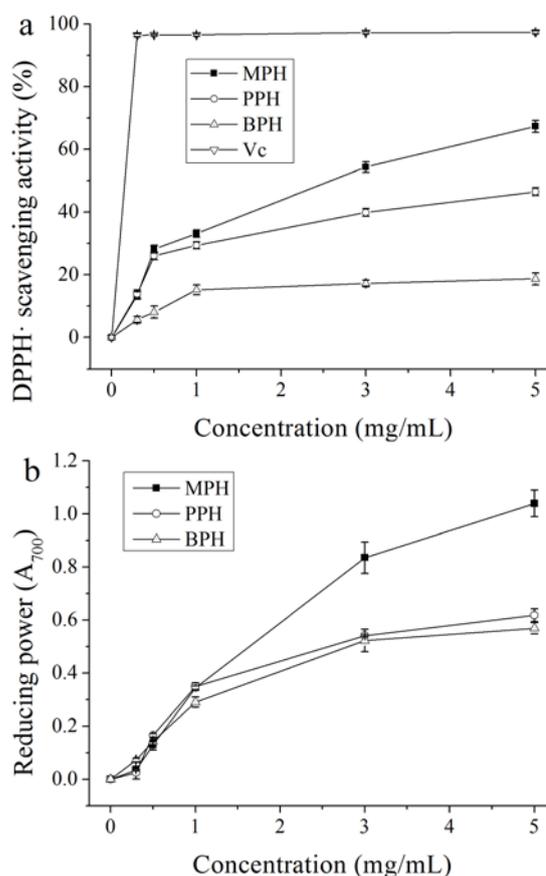
### 3.4. Antioxidant Activities

#### 3.4.1. DPPH Radical Scavenging Activity

DPPH radical will lose the absorption at 517 nm after accepting an electron, which leads to a visual color changing from purple to yellow. It also can detect active ingredients sensitively even at low concentrations [15]. In this study, the DPPH radical scavenging activities of BPH, PPH and MPH obtained by alkaline protease for 270 min at different concentrations (0-5 mg/mL) were shown in Figure 2a. Obviously, the BPH, PPH and MPH scavenging effects were increased with the concentration increasing. However, all the samples showed lower effects than Vc (control group). The scavenging effect on DPPH radical of MPH was higher than that of BPH and PPH. At 5 mg/mL, the DPPH radical scavenging activity was 67.33, 46.50, 18.68 and 97.33% for MPH, PPH, BPH and Vc, respectively. The results indicated that MPH could supply more hydrogen atoms than BPH and PPH, and it had a strong scavenging effect on DPPH radical. The differences in DPPH radical scavenging activity may be relate to their different amino acid composition (Table 2) and sequences [21], resulting from the specific mature degree of raw materials.

#### 3.4.2. Reducing Power

The reducing power of the protein hydrolysates, which is a key indicator of their antioxidant capacity, involves the transfer of electrons, and thus the color changing will be observed. The extent of this phenomenon is determined by the reducing power of the samples. The antioxidants in the samples reduce the Fe<sup>3+</sup> to the Fe<sup>2+</sup> form, which can be detected at 700 nm after formation Perl's Prussian [15]. In Figure 2b, apparently, all the samples showed electron-donating ability in a linear dose-dependent manner in a certain range. The reducing power of BPH was close to PPH at all concentration from 0 to 5 mg/mL. Depending on the mature degree of materials, the older one showed more powerful reducing activity than the tender one. The difference in the reducing power might be due to the specific peptides or amino acid composition [22]. The results suggested that MPH prepared by alkaline protease with DH 17.67% could donate more electrons to the free radicals, and thus it had more activity to reduce the free radicals and form stable products.



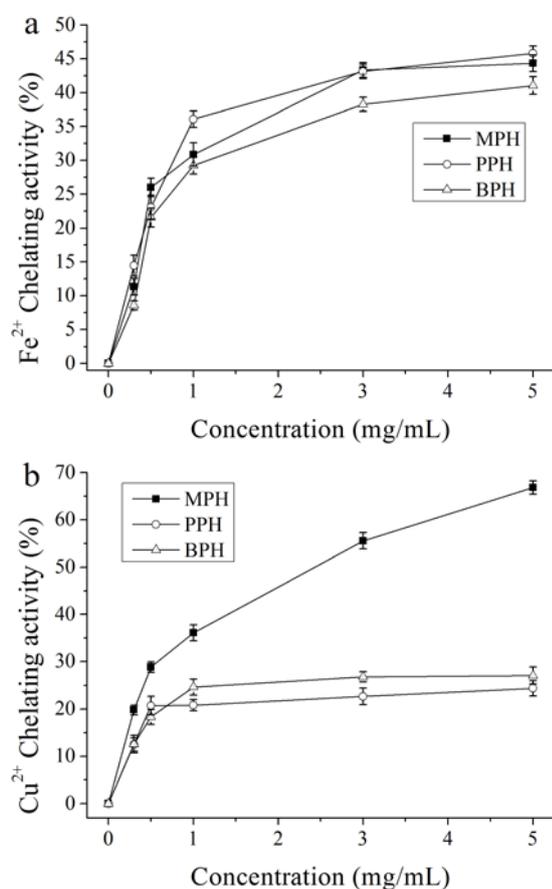
**Figure 2.** DPPH radical scavenging activity (a) and reducing power (b) of BPH, PPH and MPH prepared by alkaline protease

### 3.5. Metal Chelating Activities

Transition metals can react very rapidly with peroxides as one-electron donors and form alkoxy radicals. So antioxidants can slow down the oxidation process by chelating the transition metals [23]. Figure 3a showed the iron chelating abilities of BPH, PPH and MPH. The activity of chelating iron increased with concentration of protein. In this study, the iron chelating activity of PPH was close to MPH at the concentrations from 0 to 5

mg/mL. MPH and PPH showed more powerful iron chelating activity than BPH. Pownall, Udenigwe [24] reported that pea protein hydrolysates exhibited a high iron chelating activity. Chelating metal ions might be one of the antioxidant mechanisms for peptides and maybe protect cells from oxidative damage [25].

Peng, Kong [26] found that whey protein hydrolysates produced by alkaline protease showed a good copper chelating activity. In Figure 3b, the activity curve of chelating copper was different from that of chelating iron to some extent. The copper chelating activity of MPH increased sharply from 0 to 5 mg/mL, while BPH showed a sharp increase from 0 to 1 mg/mL and PPH showed a sharp increase with concentration up to 0.6 mg/mL. At 5 mg/mL, the copper chelating activity was 27.09, 24.40 and 66.84% for BPH, PPH and MPH, respectively. The results indicated that MPH showed more potent copper chelating activity than BPH and PPH.



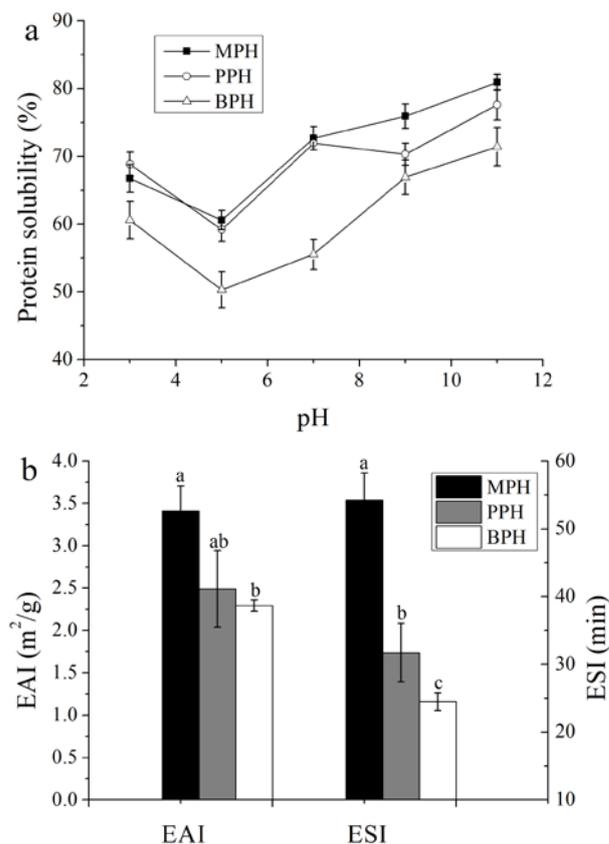
**Figure 3.** Iron chelating activity (a) and copper chelating activity (b) of BPH, PPH and MPH prepared by alkaline protease

The iron chelating activity could be due to the difference of amino acid composition. As shown in Table 2, the amino acid composition of BPH, PPH and MPH was significantly different. Storcksdieck, Bonsmann [27] reported that the amino acids of Asp, His and Gly would bind iron ions easily. The results in Table 2 showed the levels of Asp, His and Gly in BPH, PPH and MPH fluctuated dramatically. It is difficult to explain the different iron chelating abilities of BPH, PPH and MPH according to their levels of Asp, His and Gly. However, the activity of chelating metal for peptides also might be affected by many other factors, such as their molecular weight and structure.

### 3.6. Functional Properties

#### 3.6.1. Solubility

Solubility is closely related to hydrophilic amino acids, and it plays a crucial role in protein hydrolysates' functional property [28]. The solubility of BPH, PPH and MPH prepared for 270 min in the pH range from 3 to 11 were illustrated in Figure 4a. The solubility of all samples over the wide range of pH values exceeded 50%. As shown in Figure 4a, the solubilities of BPH, PPH and MPH were relatively high and their solubilities were over 50% at all the pH values except for pH 5. Only the solubility of BPH was below 50% at pH 5. This result indicated that the isoelectric point of BPH, PPH and MPH was about pH 5. What's more, the solubility of BPH was lower at all pH values than that of MPH and PPH, which may be caused by its lower DH value (Figure 1). In fact, MPH showed higher DH (Figure 1) and contained less large-molecular-mass peptides (Table 1), which had high solubility at all pH values than that of BPH and PPH ( $P < 0.05$ ). These results were consistent with previous report [29] that protein hydrolysates from zebra blenny's muscle exhibited the high solubility was attributed to its smaller molecular weight and high DH value. Hence, the differences of solubility in these samples might be related to their size of molecular weight, the charge groups, hydrophobic interaction, hydrophilic interaction, and so on.



**Figure 4.** Protein solubility (a), emulsifying activity index (EAI, b) and emulsifying stability index (ESI, b) of BPH, PPH and MPH prepared by alkaline protease

#### 3.6.2. Emulsifying Properties (EAI and ESI)

The freshly formed oil droplets can absorb peptide to their surface and form peptide membrane during

homogenization, and this peptide membrane prevents oil droplets from coalescing [29]. The EAI and ESI of BPH, PPH and MPH prepared with alkaline protease for 270 min at pH 7.0 were shown in Figure 4b. The EAI and ESI of BPH were relatively low, with DH value 13.79%, Partly because of its molecular weight distribution (Table 1). The EAI of MPH was much higher than those of PPH and BPH, and its ESI was also the highest ( $P < 0.05$ ). For the peptides obtained by enzymatic hydrolysis, the low-molecular-weight showed a better solubility than the high-molecular-weight, but the low-molecular-weight peptides might reduce their emulsifying property [30].

However, in our results, MPH showed higher DH and contained less large-molecular-mass peptides and showed more soluble than the others, but it showed higher emulsifying capacity than BPH and PPH ( $P < 0.05$ ). These results suggest that the relationship between emulsifying capacity and solubility is complicated, and further research is needed.

## 4. Conclusions

Hawk tea protein hydrolysates could be exploited and utilized as a good source of health products, and can be used as a natural food additive because of their antioxidant properties, or as an emulsifier. Here, we studied the protein hydrolysates of different mature degree Hawk teas. The protein hydrolysates of the older raw material, MPH, showed higher degree of hydrolysis by alkaline protease, and contained less large-molecular-mass peptides than that of the tender raw materials, BPH and PPH. Furthermore, MPH showed the best metal chelating activities and relatively good functional properties. In order to explore and utilize these resources better, further studies need to be done, including further purification and identify their sequence.

## Acknowledgements

This research is financially supported by the program of Young Fund Project of Sichuan Provincial Department of Education (11ZB054). Sincere thanks to Xu Li and Shian Shen for their supports in study process.

## References

- [1] Young, I.S. and J.V. Woodside, "Antioxidants in health and disease," *Journal of Clinical Pathology*, 54(3), 176-186, 2001.
- [2] Deetae, P., P. Parichanon, P. Trakunleewathana, C. Chanseetis, and S. Lertsiri, "Antioxidant and anti-glycation properties of Thai herbal teas in comparison with conventional teas," *Food Chemistry*, 133(3), 953-959, 2012.
- [3] Maryline Abert Vian, Z.H., A.S. Fabiano-Tixier, M. Elmaataoui, O. Dangles, and F. Chemat, "A remarkable influence of microwave extraction: Enhancement of antioxidant activity of extracted onion varieties," *Food Chemistry*, 127(4), 1472-1480, 2011.
- [4] Najafian, L. and A.S. Babji, "A review of fish-derived antioxidant and antimicrobial peptides: their production, assessment, and applications," *Peptides*, 33(1), 178-185, 2011.
- [5] Vercruyse, L., J. Van Camp, and G. Smagghe, "ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: a review," *Journal of Agricultural and Food Chemistry*, 53(21), 8106-8115, 2005.
- [6] Pihlanto-Leppälä, A., "Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides," *Trends in Food Science & Technology*, 11(9), 347-356, 2000.
- [7] Gauthier, S.F., Y. Pouliot, and D. Saint-Sauveur, "Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins," *International Dairy Journal*, 16(11), 1315-1323, 2006.
- [8] Intarasirisawat, R., S. Benjakul, W. Visessanguan, and J.P. Wu, "Antioxidative and functional properties of protein hydrolysate from defatted skipjack (*Katsuwonus pelamis*) roe," *Food Chemistry*, 135(4), 3039-3048, 2012.
- [9] Sarmadi, B.H. and A. Ismail, "Antioxidative peptides from food proteins: A review," *Peptides*, 31(10), 1949-1956, 2010.
- [10] Zhao, Q., H. Xiong, C. Selomulya, X.D. Chen, H.L. Zhong, S.Q. Wang, W.J. Sun, and Q. Zhou, "Enzymatic hydrolysis of rice dreg protein: Effects of enzyme type on the functional properties and antioxidant activities of recovered proteins," *Food Chemistry*, 134(3), 1360-1367, 2012.
- [11] Jia, X.J., L.H. Dong, Y. Yang, S. Yuan, Z.W. Zhang, and M. Yuan, "Preliminary structural characterization and antioxidant activities of polysaccharides extracted from Hawk tea (*Litsea coreana* var. *lanuginosa*)," *Carbohydrate Polymers*, 95(1), 195-199, 2013.
- [12] Carrasco-Castilla, J., A.J. Hernández-Álvarez, C. Jiménez-Martínez, C. Jacinto-Hernández, M. Alaiz, J. Girón-Calle, J. Vioque, and G. Dávila-Ortiz, "Antioxidant and metal chelating activities of peptide fractions from phaseolin and bean protein hydrolysates," *Food Chemistry*, 135(3), 1789-1795, 2012.
- [13] Carrasco-Castilla, J., A.J. Hernández-Álvarez, C. Jiménez-Martínez, C. Jacinto-Hernández, M. Alaiz, J. Girón-Calle, J. Vioque, and G. Dávila-Ortiz, "Antioxidant and metal chelating activities of *Phaseolus vulgaris* L. var. Jamapa protein isolates, phaseolin and lectin hydrolysates," *Food Chemistry*, 131(4), 1157-1164, 2012.
- [14] Razali, N., R. Razab, S.M. Junit, and A.A. Aziz, "Radical scavenging and reducing properties of extracts of cashew shoots," *Food Chemistry*, 111(1), 38-44, 2008.
- [15] Zhu, K.X., C.X. Lian, X.N. Guo, W. Peng, and H.M. Zhou, "Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ," *Food Chemistry*, 126(3), 1122-1126, 2011.
- [16] He, R., A.T. Girgih, S.A. Malomo, X.R. Ju, and R.E. Aluko, "Antioxidant activities of enzymatic rapeseed protein hydrolysates and the membrane ultrafiltration fractions," *Journal of Functional Foods*, 5(1), 219-227, 2013.
- [17] Castellani, O., V. Martinet, E. David-Briand, C. Guerin-Dubiard, and M. Anton, "Egg yolk phosphovitin: preparation of metal-free purified protein by fast protein liquid chromatography using aqueous solvents," *Journal of Chromatography B*, 791(1), 273-284, 2003.
- [18] Adler-Nissen, J., "Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid," *Journal of Agricultural and Food Chemistry*, 27(6), 1256-1262, 1979.
- [19] Li, X., H. Xiong, K.W. Yang, D.W. Peng, H.L. Peng, and Q. Zhao, "Optimization of the biological processing of rice dregs into nutritional peptides with the aid of trypsin," *Journal of Food Science and Technology*, 49(5), 537-546, 2012.
- [20] Saiga, A., S. Tanabe, and T. Nishimura, "Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment," *Journal of Agricultural and Food Chemistry*, 51(12), 3661-3667, 2003.
- [21] Kim, E.K., S.J. Lee, B.T. Jeon, S.H. Moon, B. Kim, T.K. Park, J.S. Han, and P.J. Park, "Purification and characterisation of antioxidative peptides from enzymatic hydrolysates of venison protein," *Food Chemistry*, 114(4), 1365-1370, 2009.
- [22] Wu, H.C., H.M. Chen, and C.Y. Shiau, "Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scorpaenopsis auroguttatus*)," *Food Research International*, 36(9), 949-957, 2003.
- [23] Kumar, N.S.S., R.A. Nazeer, and R. Jaiganesh, "Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*)," *Amino Acids*, 42(5), 1641-1649, 2012.
- [24] Pownall, T.L., C.C. Udenigwe, and R.E. Aluko, "Amino acid composition and antioxidant properties of pea seed (*Pisum sativum* L.) enzymatic protein hydrolysate fractions," *Journal of Agricultural and Food Chemistry*, 58(8), 4712-4718, 2010.

- [25] Chen, N., H.M. Yang, Y. Sun, J. Niu, and S.Y. Liu, "Purification and identification of antioxidant peptides from walnut (*Juglans regia* L.) protein hydrolysates," *Peptides*, 38(2), 344-349, 2012.
- [26] Peng, X.Y., B.H. Kong, X.F. Xia, and Q. Liu, "Reducing and radical-scavenging activities of whey protein hydrolysates prepared with Alcalase," *International Dairy Journal*, 20(5), 360-365, 2010.
- [27] Storcksdieck, S., G. Bonsmann, and R.F. Hurrell, "Iron-binding properties, amino acid composition, and structure of muscle tissue peptides from in vitro digestion of different meat sources," *Journal of Food Science*, 72(1), S019-S029, 2007.
- [28] Trevino, S.R., J.M. Scholtz, and C.N. Pace, "Amino acid contribution to protein solubility: Asp, Glu, and Ser contribute more favorably than the other hydrophilic amino acids in RNase Sa," *Journal of Molecular Biology*, 366(2), 449-460, 2007.
- [29] Ktari, N., M. Jridi, I. Bkhairia, N. Sayari, R.B. Salah, and M. Nasri, "Functionalities and antioxidant properties of protein hydrolysates from muscle of zebra blenny (*Salaria basilisca*) obtained with different crude protease extracts," *Food Research International*, 49(2), 747-756, 2012.
- [30] Chobert, J.M., C. Bertrand-Harb, and M.G. Nicolas, "Solubility and emulsifying properties of caseins and whey proteins modified enzymically by trypsin," *Journal of Agricultural and Food Chemistry*, 36(5), 883-892, 1988.