Nutrients and antioxidant properties of enzymatically hydrolyzed anchovy (Engraulis japonicus) paste

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Abstract

Anchovy, a low-value small marine fish, is mainly processed into fish meal, whose high-value functional components are not fully exploited. In this study, the enzymatically hydrolyzed anchovy (Engraulis japonicus) paste (EHAP) was prepared by hydrolyzing anchovy using Alcalase2.4L, and the nutrients and antioxidant properties of EHAP were assessed to increase its added value. Protein, total amino acids and oligopeptide of the nutrients in EHAP reached 73.9 ± 0.7%, 56.1 ± 0.8 g/100 g and 50.4 ± 1.6%, respectively. The molecular weight distribution of EHAP displayed a high dipetides and tripeptides proportion of 60.1 ± 0.4%. Furthermore, EHAP showed the highest antioxidant activities of ferrous ion chelating, lipid peroxidation inhibition and DPPH scavenging activity, reached about 34%, 40% and 49%, respectively. Collectively, the EHAP belonged to a product with strong antioxidant properties, and was rich in nutrients, which could serve as a valuable bioactive marine resource applied in the aquatic feed and functional food industry.

KEYWORDS
Anchovy; enzymolysis; nutrition; antioxidation

Palabras clave
Anchoa; Enzimólisis; Nutrición; Antioxidación

Nutrientes y propiedades antioxidantes de la pasta de anchoa (Engraulis japonicus) hidrolizada de forma enzimática

Resumen

La anchoa, un pequeño pez marino de escaso valor, se procesa principalmente como harina de pescado, cuyos componentes funcionales de alto valor no son aprovechados plenamente. En este estudio se preparó la pasta de anchoa (Engraulis japonicus) hidrolizando la anchoa enzimáticamente (EHAP), empleando para ello Alcalase2.4L. Posteriormente se evaluaron los nutrientes y las propiedades antioxidantes de la EHAP, para determinar cómo se incrementó su valor añadido. Las proteínas, los aminoácidos totales y los oligopeptídos de los nutrientes de la EHAP alcanzaron el 73.9 ± 0.7%, 56.1 ± 0.8 g/100 g y el 50.4 ± 1.6%, respectivamente. La distribución del peso molecular de la EHAP mostró una elevada proporción de dipetípidos y tripeptídos equivalente a 60.1 ± 0.4%. Además, en la EHAP se identificaron las mayores actividades antioxidantes de quelación de iones ferrosos, inhibición de la peroxidación de lipídios y actividad de eliminación del DPPH, mismas que alcanzaron valores de alrededor del 34%, 40% y 49%, respectivamente. En conjunto, la EHAP es un producto con fuertes propiedades antioxidantes y rico en nutrientes, por lo que su aplicación en las industrias de piensos acuícolas y alimentos funcionales podría servir como valioso recurso bioactivo marino.

1. Introduction

As a class of low-value marine small fish, anchovy (Engraulis japonicus) is widely inhabited in the coastal areas of China (Wang et al., 2021). The freshness of anchovy cannot be maintained without freezing because it will be gradually degraded after capture (Achmad et al., 2021). Therefore, most anchovies are processed into fish meal by fish meal processing enterprises through rough processing and used in the aquaculture industry (Li & Wu, 2020). Whereas anchovy is rich in protein, lipid and other high-value nutrients, it has high development and utilization values.

Enzymatic hydrolysis of macromolecular proteins from marine fishes can generate micromolecular active substances with antioxidant functions (Devita et al., 2021; Nikoo et al., 2021). Mongkonkamthorn et al. (2021) hydrolyzed tuna dark meat using alcalase, neutrase, flavourzyme, and found the antioxidant hydrolysate with free radical scavenging activity of 2,2’-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Chotphruethipong et al. (2021) prepared the enzymatic hydrolysate from sea bass skin using papain combined with alkaline protease, which inhibited the free radical activity of ABTS and DPPH. Jia et al. (2010) obtained the antioxidant peptides from pollack skin with DPPH free radical scavenging activity. These above mentioned enzymatic hydrolysates derived from marine fishes showed high nutritional values and antioxidant activities.

The enzymatic hydrolysates derived from marine fishes or their by-products can be added to the aquatic feed as phagostimulant and antioxidant to improve the palatability of aquatic feed, increase the feed intake, and strengthen the immune system (Hard et al., 2006; Khosravi et al., 2015). Besides, the marine fishes enzymatic hydrolysates have various physiological activities such as antioxidant and bacteriostasis.
which can enhance the human body function, and can be used as functional food supplement in the field of functional food (Nakajima et al., 2009; Ohba et al., 2003). While the nutritional value of anchovy is not fundamentally different from that of the above main commercial marine fish species, its high-value functional components are not fully exploited.

Therefore in the present study, we hydrolyzed the low-value anchovy to prepare the enzymatically hydrolyzed anchovy paste (EHAP) using commercial proteases, and assessed the nutrients and antioxidant properties of EHAP. On the one hand our researches provided a new finding for the high-value application of EHAP in the aquatic feed and functional food industry. On the other hand our findings provided a new potential idea about developing alternative products of anchovy fish meal for fish meal processing enterprises.

2. Material and methods

2.1. Materials

Anchovy (Engraulis japonicus) materials were provided by Zhejiang Eiﬁne Marine Biological Products Co., Ltd. (Taizhou, China). Commercial proteases including Alcalase2.4 L, Neutrase0.8 L, Protamex1.6 and Flavourzyme500 MG were presented by Novozymes (China) Biotech (Tianjin, China). Standards of fatty acids were supplied by Sigma Chemical (St. Louis, USA). Amino acids mixture standard was purchased from Wako Pure Chemical (Osaka, Japan). Molecular weight distribution standards involving cytochrome C (12500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide (glycine-glycine-tyrosine-arginine, 451 Da) and tripeptide (glycine-glycine-glycine, 189 Da) were obtained from Sigma Chemical (St. Louis, USA). Other reagents were of analytical grade.

2.2. EHAP preparation

The whole anchovy were ground and mixed with distilled water at the weight ratio of 1 : 1, followed by homogenization. Then the enzymatic hydrolysis was conducted according to the method of Mongkonkamthorn et al. (2021) with some modifications as follows. The four equal parts of homogenate were taken to maintain at 55°C using water bath. Subsequently, Alcalase2.4 L (2.4 AU/g), Neutrase0.8 L (0.8 AU/g), Protamex1.6 (1.6 AU/g) and Flavourzyme500 MG (500 LAPU/g) were simultaneously added into the quartering homogenates at the weight ratio of 0.1:100, respectively. String was performed for 6 h, and the enzymatic hydrolysates were collected every 2 h. Finally, the EHAP was prepared by concentrating the supernatant of hydrolysate to the concentration of 40–50% using a vacuum rotary evaporator (N-1300D-WB, EYELA, Tokyo, Japan) at 70°C and ~0.07 MPa.

2.3. Basic chemical compositions and amino nitrogen assay

Basic chemical compositions including protein, lipid, ash and moisture were measured in accordance with the corresponding instructions from the Association of Official Analytical Chemists (Gaithersburg, 2003). Amino nitrogen content was determined using the Formol method as previously established by Young-Je et al. (2001).

2.4. Amino acid compositions, oligopeptide and fatty acid compositions assay

Amino acid compositions containing free and total amino acids were tested by a previously reported method (Wang et al., 2021) utilizing an automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). Oligopeptide content was calculated according to the method of Cai et al. (2014) by the formula: Co = Cc − Cf, where Co, Cc and Cf represent the content of oligopeptide, trichloroacetic acid-soluble proteins and free amino acids, respectively. Fatty acid compositions were determined in accordance with the approach of Xie et al. (2018). In brief, Fatty acids samples were prepared by extraction with ether/petroleum ether (1:1) and transesterification using 2 mol/L potassium hydroxide in methanol before analysis. Subsequently, the samples were analyzed employing a gas chromatographic system (Agilent, Santa Clara, USA) equipped with a TRACE TR-FAME capillary column (0.25 µm, 60 m × 0.25 mm, Thermo Fisher, USA).

2.5. Molecular weight distribution assay

Molecular weight distribution was detected in accordance with the approach of Tang et al. (2015), and some modifications were performed as follows. In short, the hydrolysate was dissolved in the mobile phase of acetoniitrile/water at the volume ratio of 45:55, followed by ultrasonic consecution and membrane filtration (0.22 µm pore size). Then the processed samples were separated utilizing a gel permeation chromatography (TSK gel G2000 SWXL, 300 mm × 7.8 mm, Tokyo, Japan) at the flow rate of 0.5 mL/min, and the separations were monitored at UV220 nm and 30 °C.

2.6. Antioxidant activities assay

Antioxidant activities were analysed by determining the ferrous ion chelating activity, lipid peroxidation inhibition activity and the four free radicals (ABTS, DPPH, superoxide and hydroxyl scavenging activities. Ferrous ion chelating activity and lipid peroxidation inhibition activity were respectively tested by the previously reported methods of Zhu et al. (2013) and Liu et al. (2013). Hydroxyl, superoxide DPPH and ABTS free radical scavenging activities were measured according to the approaches of Wang et al. (2015), Gu et al. (2012), Song et al. (2015) and Tang et al. (2010), respectively.

2.7. Statistical analysis

Triplicate samples were prepared, and the tested data were shown as mean ± SD. Statistical analysis was performed employing SPSS Statistics 13.0, and the significant differences (p < 0.05) were marked with different lowercase letters at the top right of the data.

3. Results

3.1. Enzymolysis effect of the four commercial proteases on anchovy

The amino nitrogen content of EHAP was selected to compare the enzymolysis effect of the four commercial proteases on anchovy. As shown in Figure 1, the highest amino nitrogen content was obtained with Alcalase2.4 L and
3.2. The basic chemical compositions of EHAP

The basic chemical compositions of EHAP, containing protein, lipid and ash, were shown in Table 1. The protein and fat content of EHAP gradually increased, while the ash content gradually decreased, with the prolongation of enzymolysis time. The protein and lipid content of EHAP reached the maximum 73.9 ± 0.7% and 18.4 ± 0.1%, respectively at 6 h (Table 1). Besides, the ash content of EHAP reached the minimum 8.34 ± 0.27% at 6 h (Table 1).

3.3. The oligopeptide of EHAP

Figure 2 showed the changing trend of oligopeptide content in EHAP. The oligopeptide content of EHAP gradually increased with the prolongation of enzymolysis time, which reached the maximum 50.4 ± 1.6% at 6 h (Figure 2).

3.4. The amino acid compositions of EHAP

The amino acid compositions of EHAP, containing free and total amino acids, were shown in Tables 2 and 3, respectively. The anchovy raw material contained about 55 g/100 g total amino acids, consisting of 21.2 ± 0.1 g/100 g essential amino acids (Lys, Phe, Met, Thr, Ile, Leu, and Val) and 33.8 ± 0.6 g/100 g non-essential amino acids (Asp, Ser, Glu, Gly, Ala, Cys, Tyr, His, Arg, and Pro) (Table 2). The total amino acids, essential amino acids and non-essential amino acids content of EHAP increased from 2 h to 6 h (Table 2). The anchovy raw material contained about 5 g/100 g total free amino acids, including 1.49 ± 0.05 g/100 g sweet and fresh amino acids (Table 3). The total free amino acids content of EHAP increased from 7.39 ± 0.09 g/100 g to 13.9 ± 0.2 g/100 g during the enzymolysis time of 2 h to 6 h (Table 3). Besides, the sweet and fresh amino acids (Thr, Asp, Glu, Ser, Gly, Ala and Pro) of EHAP showed a rising trend, reached the highest content of 5.00 ± 0.07 g/100 g at 6 h (Table 3).

3.5. The fatty acid compositions of EHAP

The fatty acid compositions of EHAP were shown in Table 4. The anchovy raw material contained 61.1 ± 0.2% unsaturated fatty acid (C14:1, C16:1, C18:1n9t, C18:1n9c, C18:2n6c, C18:3n6, C20:1, C18:3n3, C20:2, C20:3n6, C22:1n9, C20:4n6, C22:2, C20:5n3, C24:1, C22:6n3), including 23.5 ± 0.3% omega-3polyunsaturated fatty acid (C20:5n3, C22:6n3) (Table 4). The saturated fatty acid composition (C40, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0) of EHAP increased from 38.3 ± 0.3% to 41.5 ± 0.2%, with the prolongation of enzymolysis time from 2 h to 6 h (Table 4). The unsaturated fatty acid composition and omega-3polyunsaturated fatty acid composition of EHAP both displayed a slow decreasing trend, from 60.8 ± 0.1% to 58.4 ± 0.3% and 23.4 ± 0.2% to 22.2 ± 0.1%, respectively (Table 4).

3.6. Molecular weight distribution of EHAP

Figure 3 depicted the molecular weight distribution of EHAP. Peptides in the anchovy raw material and EHAP were mainly composed of oligopeptides (180 1000 Da) and polypeptides (1000 10000 Da), both displayed a high proportion of about 96% with molecular weight ranging from 180 10000 Da (Figure 3). The proportion of polypeptides in EHAP reduced gradually with the prolongation of enzymolysis time from 2

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Table 1. Basic chemical compositions of the EHAP.

| Basic chemical compositions (dry basis %) | Protein | Lipid | Ash  |
|-----------------------------------------|---------|-------|------|
| Anchovy raw material                    | 72.1 ± 0.8<sup>b</sup> | 13.3 ± 0.4<sup>a</sup> | 12.4 ± 0.3<sup>a</sup> |
| 2 h                                     | 66.0 ± 0.2<sup>d</sup> | 14.6 ± 0.4<sup>a</sup> | 11.6 ± 0.4<sup>a</sup> |
| 4 h                                     | 72.3 ± 0.4<sup>b</sup> | 18.3 ± 0.3<sup>b</sup> | 9.03 ± 0.10<sup>c</sup> |
| 6 h                                     | 73.9 ± 0.7<sup>a</sup> | 18.4 ± 0.1<sup>a</sup> | 8.34 ± 0.27<sup>d</sup> |

Different lowercase letters marked in the same column representing significant differences (P < 0.05).
Las distintas letras minúsculas presentes en la misma columna representan diferencias significativas (P < 0.05).
h to 6 h. The proportion range from tetrapeptides to decapeptides (500–1000 Da) also dropped. Meanwhile, the proportion of free amino acids (<180 Da) also with dipeptides and tripeptides (180–500 Da) increased gradually. It was worth noting that the proportion of dipeptides and tripeptides in EHAP reached up to 60.1 ± 0.4% at 6 h (Figure 3).

3.7. Antioxidant activities of EHAP

Figure 4 summarized the antioxidant activities of EHAP. The anchovy raw material had relatively low antioxidant activities of the ferrous ion chelating activity, lipid peroxidation inhibition activity and the four free radicals scavenging activities (Figure 4). On the whole, the EHAP revealed a gradually increasing trend of antioxidant activities with the prolongation of enzymolysis time from 2 h to 6 h (Figure 4). The EHAP had relatively high antioxidant activities of ferrous ion chelating activity, lipid peroxidation inhibition activity and DPPH scavenging activity (Figure 4a,b,c). In addition, the highest ferrous ion chelating activity and lipid peroxidation inhibition activity of EHAP reached 33.9 ± 1.2% and 40.0 ± 0.7%, respectively, at 6 h (Figure 4a,b). The hydroxyl, superoxide, also with DPPH and ABTS free radical scavenging activities of EHAP, reached 13.8 ± 0.2%, 19.1 ± 0.6%, 48.2 ± 1.8% and 15.3 ± 0.3%, respectively, at 6 h (Figure 4c–f).

4. Discussion

Over the past few decades, a considerable number of bioactive substances have been discovered in enzymatic hydrolysates of marine fish proteins (Zhao et al., 2021; Zhu et al., 2020), showing a promising functional resource for aquatic feed and food industry. Therefore, anchovy might be a potential research object of enzymatic hydrolysis products. The basic components such as protein and lipid are crucial for the high-value development and utilization of low-value marine anchovy. The protein content of anchovy was similar to other marine commercial fishes, such as tuna (Khodabux et al., 2007) and hairtail (Tavares et al., 2018), reached about 73% (Table 1). The high proportion of protein components in anchovy provides an adequate substrate for enzymatic reaction and enlarges the interface between the protease and protein substrate. In addition, the ratio of essential to non-essential amino acids in anchovy was close to 0.65 (Table 2), exhibiting a well-balanced amino acid composition in accordance with the recommendation of United Nations Food and Agriculture Organization and the World Health Organization. It was worth noting that the lipid component of anchovy reached about 14% (Table 1), which was comparable to that of tuna. Even more surprising, the lipid of anchovy contained up to about 61% unsaturated fatty acids (Table 4). As a result, anchovy might be a typical development research model on account of its high protein level, well-balanced amino acid composition and abundant unsaturated fatty acids.

Table 2. Total amino acids of the EHAP.

| Amino acid | Anchovy raw material | 2 h       | 4 h       | 6 h       |
|------------|----------------------|-----------|-----------|-----------|
| Lys        | 4.56 ± 0.14          | 4.18 ± 0.09 | 4.45 ± 0.19 | 4.57 ± 0.16 |
| Phe        | 2.04 ± 0.07          | 1.68 ± 0.04 | 2.31 ± 0.09 | 2.42 ± 0.13 |
| Met        | 1.73 ± 0.07          | 1.28 ± 0.03 | 1.62 ± 0.13 | 1.81 ± 0.06 |
| Thr        | 2.37 ± 0.11          | 1.40 ± 0.12 | 2.20 ± 0.03 | 2.35 ± 0.08 |
| Ile        | 2.54 ± 0.51          | 1.63 ± 0.07 | 2.87 ± 0.08 | 2.94 ± 0.03 |
| Leu        | 4.69 ± 0.09          | 2.66 ± 0.08 | 4.55 ± 0.09 | 4.72 ± 0.06 |
| Val        | 3.25 ± 0.08          | 2.68 ± 0.09 | 3.36 ± 0.12 | 3.49 ± 0.05 |
| Asp        | 5.85 ± 0.06          | 5.26 ± 0.13 | 5.91 ± 0.11 | 6.05 ± 0.12 |
| Ser        | 1.67 ± 0.05          | 1.13 ± 0.03 | 1.63 ± 0.01 | 1.70 ± 0.02 |
| Gli        | 9.93 ± 0.11          | 9.16 ± 0.11 | 9.70 ± 0.18 | 9.89 ± 0.10 |
| Gly        | 3.99 ± 0.25          | 3.83 ± 0.11 | 4.09 ± 0.10 | 4.20 ± 0.04 |
| Ala        | 4.09 ± 0.25          | 3.44 ± 0.09 | 3.87 ± 0.07 | 3.92 ± 0.11 |
| Cys        | 0.12 ± 0.02          | 0.10 ± 0.01 | 0.11 ± 0.02 | 0.12 ± 0.02 |
| Tyr        | 1.31 ± 0.15          | 0.76 ± 0.02 | 1.38 ± 0.04 | 1.43 ± 0.08 |
| His        | 1.18 ± 0.02          | 0.64 ± 0.03 | 1.04 ± 0.05 | 1.06 ± 0.03 |
| Arg        | 3.32 ± 0.07          | 2.26 ± 0.06 | 3.22 ± 0.07 | 3.28 ± 0.10 |
| Pro        | 2.12 ± 0.01          | 1.27 ± 0.06 | 2.10 ± 0.09 | 2.13 ± 0.04 |
| Total essential | 21.2 ± 0.11       | 15.3 ± 0.1   | 21.4 ± 0.2   | 22.3 ± 0.2  |
| Total non-essential | 33.8 ± 0.6          | 27.9 ± 0.1   | 32.1 ± 0.2   | 32.8 ± 0.5   |
| Total      | 55.0 ± 0.7           | 43.4 ± 0.1   | 54.4 ± 0.7   | 56.1 ± 0.8  |

Different lowercase letters marked in the same row of the total amino acids; the total essential amino acids and the total non-essential amino acids in EHAP representing significant differences (P < 0.05).

Las distintas letras minúsculas presentes en la misma fila de los aminoácidos totales, los aminoácidos esenciales totales y los aminoácidos no esenciales totales de la EHAP representan diferencias significativas (P < 0.05).
A large number of studies have shown that enzymatic hydrolysis using proteases can effectively release small molecule active substances from marine fishes (Huang et al., 2012; Yang et al., 2010). Four typical commercial proteases from Novozymes, the world’s leading enzyme preparation company, were utilized to hydrolyze the anchovy for selecting the appropriate protease, firstly. Eventually Alcalase2.4L was chosen to hydrolyze the anchovy because of the maximum amino nitrogen produced in the hydrolysate (Figure 1), which was the same protease that used by Kim et al. (2016) in the research on enzymatic hydrolysis of anchovy fine powder. Then the EHAP was prepared successively by enzymolysis, deslagging and concentration. The nutrients and antioxidant activities of the EHAP were analyzed subsequently.

Both of the protein and lipid in the EHAP displayed a gradual increase trend with the prolongation of enzymolysis time.
molecular proteins in anchovy, releasing the water-soluble micro-molecular proteins, as well as the lipids from the protein-lipid complex, thus increasing protein and lipid levels of the EHAP. The ash of EHAP consists mainly of water-soluble salts. The increased protein and lipid proportion resulted in a gradual decrease of ash in the EHAP (Table 1). The total amino acids of EHAP increased gradually with the prolongation of enzymolysis time, which might be due to that the enzymolysis increased the proportion of water-soluble proteins (Mezenova et al., 2021) of EHAP (Table 2). Based on total amino acid content, the essential amino acids of EHAP accounted for up to 40% at 6 h (Table 2), which was similar to the study conducted by Piotrowicz and Mellado (2015) with Argentine anchovy hydrolysate using Alcalase 2.4 L, which was 43.5% of the total amino acids. Free amino acids have great influence on the flavor and taste of EHAP. The total free amino acids of the EHAP displayed a gradual increasing trend, and the ratio of sweet and fresh amino acids accounted for about 38% of the total amino acids in EHAP of 6 h (Table 3). The high level of sweet and fresh amino acids formed the flavor basis (Yao et al., 2021) of EHAP.

The proportion of saturated fatty acids in EHAP showed a slow increasing trend (Table 4). In contrast to that the
proportion of unsaturated fatty acids displayed a slow decreasing trend (Table 4). It might be caused by that a small amount of unsaturated fatty acids were oxidized during enzymatic hydrolysis (Haldorsdottir et al., 2014). It was worth noting that the omega-3polyunsaturated fatty acid, a class of crucial functional fatty acids associated with decreasing risk for many chronic diseases (Yates et al., 2009), also had a small reduction. Therefore, the appropriate antioxidant measures, such as addition of the antioxidant vitamin C (Santos et al., 2022), might be taken to reduce the oxidation of unsaturated fatty acids during the enzymatic hydrolysis process of anchovy. Oligopeptide, a type of micro-molecular antioxidative compounds, is composed of 2 to 10 amino acid residues with the molecular weight ranging from 180 to 1000 Da (Qin et al., 2020). The oligopeptide was continuously generated by enzymatic hydrolysis of anchovy protein (Chatterjee et al., 2020) using Alcalase2.4 L, leading to a gradual increase trend of oligopeptide content in the EHAP (Figure 2). The maximum oligopeptide content of EHAP reached about 50% at 6 h, which was lower than that obtained by Wang et al. (2018) from enzymatically hydrolyzed anchovy fish meal. The explanation might be that, on the one hand, the lipid and salt in anchovy raw material were enriched into the EHAP, resulting in a high level of lipid and ash (Table 1). On the other hand, it might be related to the fact that Wang et al. (2018) used the defatted anchovy fish meal as raw material and desalted the oligopeptides with separation membrane. Thus it can be seen that the EHAP represented a high nutritional value for its rich nutrients such as protein (Table 1), amino acids (Tables 2, 3), unsaturated fatty acids (Table 4) and oligopeptide (Figure 2). Nevertheless, there are limitations to analyze only the nutrients of EHAP, the analysis of bioactive activities is equally important.

Molecular weight distribution displayed a high proportion (about 60%) of dipeptides and tripeptides with molecular weight ranging from 180–500 Da in the EHAP at 6 h (Figure 3). The increasing evidences have been reported to support the view that the dipeptides and tripeptides have strong antioxidant activities. For instance, glutathione, a tripeptide composed of glutamate, cysteine and glycine, has strong quenching effect on reactive oxygen species (Rai et al., 2021). Likewise, Du et al. (2019) discovered that the screened 36 dipeptides displayed varying degrees of individual antioxidant effects in vitro. In view of this, the high levels of dipeptides and tripeptides, and the low molecular weight distribution allowed us to assume that the EHAP might possess potential antioxidant activities. In our study, the EHAP at 6 h showed the highest antioxidant activities of ferrous ion chelating activity, lipid peroxidation inhibition activity and DPPH scavenging activity, reached about 34%, 40% and 49%, respectively (Figure 4a,b,e). These were comparable to that reported in other researches (Chen et al., 2017; Wang et al., 2018).

Based on all of the above-mentioned discoveries, the EHAP belonged to a marine fish processing product rich in nutrients, including protein, amino acids, unsaturated fatty acids and oligopeptide. Meanwhile, it exhibited the strong antioxidant properties, which could serve as a valuable bioactive marine resource applied in the aquatic feed and functional food industry.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### Data sharing statement

All data generated or analyzed during this study are available from the corresponding author HaiyanWu upon reasonable request.

### Author contributions

Authors Jiang Sun and HaiyanWu conceived and designed the experiments. Jiang Sun, Yongchang Su, Linghua Wang and Feng Lv performed the experiments. Jiang Sun and Yongchang Su analyzed the data. Finally, authors Jiang Sun and HaiyanWu prepared the manuscript. All authors have read and approved the final manuscript.

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