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RESEARCH ARTICLE

Influence of Sturgeon Protein Fractions on Active Peptide Generation via Enzymatic Hydrolysis

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Abstract: To identify the sturgeon protein component responsible for generating bioactive peptides, including antioxidant peptides and alcohol dehydrogenase (ADH) promoting peptides, sturgeon protein and its isolated protein components (sarcoplasmic, myofibrillar, and stromal proteins) were studied. Among the four isolated protein components, stromal protein demonstrated the highest susceptibility to enzymatic hydrolysis (P<0.05). Besides, pepsin and alkaline protease are the most effective enzymes for the hydrolysis of sturgeon protein to produce 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity peptides. For pepsin, the hydrolysate of sarcoplasmic protein has strong DPPH radical scavenging activity, while myofibrillar protein and stromal protein have better performance for alkaline protease. Moreover, sturgeon protein can produce an ADH-promoting peptide only under the action of trypsin. Myofibrillar protein can produce ADH-promoting peptides, while sarcoplasmic protein and stromal protein mainly produce ADH-inhibiting peptides. Therefore, the three protein components play a positive role in the production of antioxidant peptides. However, about the ADH-promoting peptides, only the myofibrillar protein plays a positive role, while the sarcoplasmic and stromal proteins play negative roles.

Keywords: Sturgeon Protein, Protein Fractions, Hydrolysis, Antioxidants, Adh-Promoting Peptides

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Introduction

Sturgeon is an economically important fish in China, with an annual output of 17,000 tons, accounting for nearly 80% of the world's total output [1]. Roe is the most valuable among all sturgeon resources and is often used during the manufacturing of caviar. However, the remaining by-products are faced with a low utilization rate and low-value-added processing. Among them, sturgeon meat accounts for about 40% of the sturgeon stroma, and the main destinations are direct sale, processing into animal feed, simple hot processing into conditioning food or direct discarding, which has the disadvantages of low value-added and environmental pollution [2]. Therefore, an exploration of the deep processing methods of sturgeon meat is crucial to the development of the sturgeon industry, and the preparation of bioactive peptides is an important direction of high-value processing.

So far, antioxidant peptides are the most widely studied functional active peptides, and many studies have reported that antioxidant peptides can be obtained from enzymolysis of fish protein, including mackerel meat and tilapia protein [3, 4]. In

addition, Noman et al. have successfully prepared collagen peptides with antioxidant properties from the processing of sturgeon by-products, such as cartilage and skin [5]. In addition to antioxidant peptides, alcohol dehydrogenase (ADH) promoting peptides have attracted increasing attention in recent years. ADH-promoting peptides are a type of active peptide that can promote ethanol metabolism in the liver, which is of great significance for the prevention and treatment of chronic diseases caused by alcohol, such as alcoholic liver disease. Fish proteins, such as the collagen of salmon and the protein in the swim bladder of crucian carp, are effective sources of ADH-promoting peptides [6]. Moreover, functional peptides that relieve alcoholic liver disease are also found in shellfish such as Musculus senhousei [7]. Undoubtedly, it would be of great significance for the enrichment of active peptides and the development of high-value-added products to determine the influence of relevant parameters on the production of active peptides.

Numerous relevant studies have focused on the effects of enzymatic parameters, such as enzyme type, time and temperature, on the production of active peptides during proteolytic hydrolysis [8-10]. However, little attention has been paid to the effects of protein components. In recent years, with the deepening of the study of protein, it has been found that protein is actually a mixture of different protein components, including sarcoplasmic, myofibrillar and stromal proteins. There is no doubt that the different protein components have structural specificity and, in response to the action of different enzymes, produce active peptides with distinct functions. It has been proven that carp myofibrillar protein is more likely to produce antioxidant peptides than sarcoplasmic protein [11]. In addition, Wang et al. found that stromal protease hydrolysates from grass carp had higher OH- scavenging activity than other protein preparations [12]. Therefore, identification of the protein component that plays a leading role in the enzymatic hydrolysis of sturgeon meat in the preparation of active peptides would be of great significance for the efficient preparation of active functional peptides of sturgeon. However, existing studies in this topic are rare.

In this study, we intend to explore the production rules of active peptides in sturgeon meat, including antioxidant peptides and ADH-promoting peptides, from the perspective of protein components. In this study, sarcoplasmic, myofibrillar and stromal proteins from sturgeon meat were prepared and treated with the appropriate enzymes to explore the production rules for antioxidant peptides and ADH-promoting peptides in different fractions of sturgeon protein. This paper provides a reference for research and processing in the sturgeon industry and fills the knowledge gap in the research direction of the pharmacological value of active peptides in sturgeon meat.

Materials and Methods

Raw Materials

Hybrid sturgeon (Acipenserbaerii ♂ × Acipenser schrenckii ♀) was sourced from Haiquan aquatic products Co., Ltd. (Sishui, Shandong, China). The hybrid sturgeon meat was stripped from the other parts, such as head, fin and fat. Then, the muscle was macerated in a blender (MB950, Wiggens GmbH, German) for 2 min and frozen in a polyethylene bag (Suzhou Unfailing Material Technology Co. Ltd, China) at −18°C until use. All animal-derived tissues study was approved by the Ethics Committee of China Jiliang University (certificate number: 2022YW34).

The chemicals, DPPH, NaOH, HCl, Na2HPO4, KH2PO4, NaCl, Trichloroacetic Acid (TCA), ethanol, Na4P2O7·10 H2O were analytical grade, purchased from Aladdin Co. Ltd (Shanghai, China). ALD (7.5 KU/mg), pepsin (USP, 1:30K), alacaline protease (200 U/mg), neutral protease (200U/mg), trypsin (1:250), acid protease (50 U/mg) and papain (2 KU/mg) were biological grade, purchased from Shanghai Titan Technology Co. Ltd, China.

Different Types of Proteins

Crude Protein

The crude protein was prepared through the process of alkali dissolution and isoelectric precipitation, with pH values of 11 and 5.5 for alkali dissolution and isoelectric precipitation respectively [13]. The pH of the mixed solution was adjusted using 1 mol/L NaOH or HCl. Finally, the crude protein precipitate was obtained by centrifugation at 5000 g (H2500R, Hunan Xiangyi, China). The resulting precipitate was freeze-dried (Scientz 30F/A, Ninbo Scientz Biotechnolgy Co. Ltd, China) for 48 h.

Separation of Protein Fractions

Research has demonstrated that sturgeon protein is predominantly comprised of myofibrillar, sarcoplasmic and stromal proteins [14]. Extraction of myofibrillar, sarcoplasmic and stromal proteins were carried out based on methods of Mauriello et

al. and Wu et al. [15, 16]. The lipid level in meat or isolated protein was also determined by Soxhlet gravimetric method. The total protein content and non-protein nitrogen in supernatant solution of 50% TCA sediment was determined by Kjeldahl Method [17].

Crude protein was blended with the buffer A as described by Wu et al. [16]. The suspension was subjected to high-speed homogenization (10,000 rpm, 1 min), followed by continuous agitation (JB43-TP, Inesa, Shanghai, China) at 4°C for 1 hour. Subsequent centrifugation (10,000 rpm, 20 min) separated the soluble and insoluble fractions. The clarified supernatant was acidified with trichloroacetic acid to a final concentration of 10% (v/v), precipitating sarcoplasmic proteins, while the remaining soluble nitrogenous compounds were classified as non-protein nitrogen. The residual pellet from the initial extraction was further processed with the buffer B as described by Wu et al. under identical homogenization conditions [16]. After thorough mixing (1.5 h) and centrifugation (10,000 rpm, 10 min), the supernatant contained myofibrillar proteins, whereas the sedimented fraction represented stromal proteins. Finally, all three isolated protein fractions were lyophilized for 24 h to ensure stabilization.

Hydrolysis

The freeze-dried powder of crude, myofibrillar, sarcoplasmic and stromal proteins was dissolved to a concentration of 1% (w/w, distilled water) and was hydrolyzed by six kinds of enzymes at 12 U/mg-protein level, including alkaline protease, trypsin, papain, pepsin, acid protease and neutral protease, at 40℃ in a water bath (DK-8D, Shanghai Zhisun Equipment Co. Ltd, China). During hydrolysis, 2 mL solution was sampled at 1, 2, 4, 6 and 8 h for protein content and bioactive test. The solution was kept at 100℃ for 10 min to deactivate the protease and then was centrifuged at 8000 g. The supernatant was preserved at −18℃ until for test.

Hydrolysis Degree

The resulting enzymatic solution (enzymolysis for 1, 2, 4, 6 and 8 h) was mixed with a quarter volume of TCA (50%) and centrifuged at 8000 g for 10 min. Finally, the supernatant was extracted, and the protein or peptide content was determined by the Lowry method [18]. The hydrolysis degree (%) of the protein was calculated by dividing the TCA dissolved peptide content after digestion by the total protein content in mixture before digestion. The total protein content was determined by Kjeldahl Method [17]. The hydrolysis degree (%) of proteins at various times was fitted to the special enzymatic hydrolysis kinetic model of proteins, and the digestion curve was drawn according to the following formula [19].

Hydrolysis degree (%) = Hydrolysis degree max × exp (− (half-life time × In2)/time)

Where, the enzymatic hydrolysis rate max is the degree of digestion at the final digestion time, and the half-life is the time when the enzymatic hydrolysis rate reaches half of the enzymatic hydrolysis rate max.

DPPH Scavenging Activity

The 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity was determined according to Adjimani and Asare with some modifications [17]. A 2-ml 0.1 mmol/L of DPPH ethanol solution was mixed with 1 mL of hydrolysate mixture, the mixture was reacted in dark condition for 5 min and then centrifuged at 1500 g for 10 min. The absorbance of the resulting supernatant was determined at a wavelength of 517 nm by a spectrophotometer (UV1100, Shanghai Meixi Instrument Co. Ltd, China). In the control group, distilled water was used to replace the sample, and in the blank group, ethanol was used to replace DPPH solution. DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \left(1 - \frac{A1 - A2}{A3}\right) * 100\%$$

Where A1, A2 and A3 denote the absorbance of sample, control and blank.

ADH Promotion Rate

Mix 1 mL of protease hydrolysate, 1.5 mL of phosphate buffer solution (pH = 7), 0.5 mL of ethanol solution (11.52%, v/v), and 1 mL of coenzyme solution [20]. After 5 min of stirring, add 0.1 mL of ADH solution and immediately measure the change in absorbance at 340 nm until stabilization. The reaction rate is calculated as the change in absorbance at 340 nm from the initial time point to stabilization divided by the time interval. The ADH activation rate (%) is determined by subtracting the reaction rate of the blank (distilled water) from that of the sample, then dividing by the sample reaction rate.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics v20.0 (IBM, USA). One-way analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test were applied to assess the significance of differences among results (n = 3). A p-value < 0.05 was considered statistically significant.

Results and Discussion

Protein Component

The chemical composition in sturgeon meat and isolated protein, including water, lipid, protein, ash, non-protein nitrogen (primarily consisting of single amino acids and small peptides), were shown in Table 1. In fish muscles, myofibrillar (65–80% of total protein) composed mainly of actin, myosin, tropomyosin and troponin, or connective tissue 3 to 5%, and sarcoplasmatic (20–30% of total protein) are mainly proteins [14]. As anticipated, sturgeon meat is characterized by high protein and low fat, with contents of 17.77% and 5.32% (Table 1), respectively, which render it a favourable substrate for the production of active peptides. The protein content and fat content were 82.7% and 2.3% in Sturgeon isolated protein, respectively, aligning with the observations of 82.3% and 2.7% in fish (Oreochromus niloticus) myofibrillar isolated protein [21].

In order to reduce the interference of lipids and other impurities on the enzymatic hydrolysis process and obtain more scientific results, the crude protein was separated by pH-shift. As can be seen from Table 1, after pH-shift, the lipid content decreased significantly from 5.32% to 2.32% (P<0.05). [22] Zhou and Yang (2019) also found that about 1%–10% of the lipids remained in the pH-shift proteins from various fish resources. In addition, we found that although the pH-shift method can reduce the influence of lipids on enzymatic hydrolysis, it can also lead to a significant decrease in non-protein nitrogen and sarcoplasmic protein (P<0.05), and a significant increase in myofibrillar protein (P<0.05). This is mainly due to the use of a large volume of water in the process of pH-shift, which will lead to the loss of sarcoplasmic protein, consistent with the results of Wu et al. [16].

Table 1: The compositions (%) of raw materials and prepared protein powders

Compositions	Sturgeon meat	Sturgeon isolated protein
Protein	17.77±1.43[a]	82.7±1.42[b]
Non-protein nitrogen	4.36±0.11[a]	4.05±0.18[b]
Water-soluble protein	37.00±0.22[a]	26.55±0.52[b]
Salt-soluble protein	42.84±0.58[a]	52.32±1.02[b]
Insoluble protein	14.39±0.44[a]	15.88±0.29[b]
Moisture	72.65±1.51[a]	10.71±0.69[b]
Lipids	5.32±0.75[a]	2.32±0.18[b]
Ash	3.74±0.15[a]	3.22±0.27[a]

[a,b] Followed by different letters in the same line demonstrated significant difference (P< 0.05).

Hydrolysis Degree

Fig. 1 and 2 present the enzymolysis kinetic curves and associated kinetic characteristic parameters of various protein samples, including crude, sarcoplasmic, myofibrillar, and stromal proteins, when subjected to the influence of six enzymes. The enzymolysis rates of the four protein samples exhibited a consistent pattern over time, with a rapid increase observed between 1 and 2 h, followed by a gradual deceleration and eventual stabilization. This phenomenon can be attributed to the prolonged duration of enzymolysis, which leads to a decrease in the efficiency of peptide bond hydrolysis and consequently results in a corresponding decrease in the rate of enzymolysis. Moreover, an increase in the concentration of reaction products in similar reactions inhibits enzyme activity [23]. On the other hand, for the same protein substrate (crude protein, sarcoplasmic protein, myofibrillar protein, or stromal protein), the hydrolysis degree were significant differences (P<0.05) between the six enzymes from 30.14% to 2.51%, which were also consistent with Hernández-Figueroa et al. [24]. The main reason is that cleavage sites vary among enzymes. For instance, alkaline proteases predominantly cleave the polypeptide chain at hydrophobic amino acids, whereas trypsin cleaves at the C-terminal end of lysine or arginine residues.

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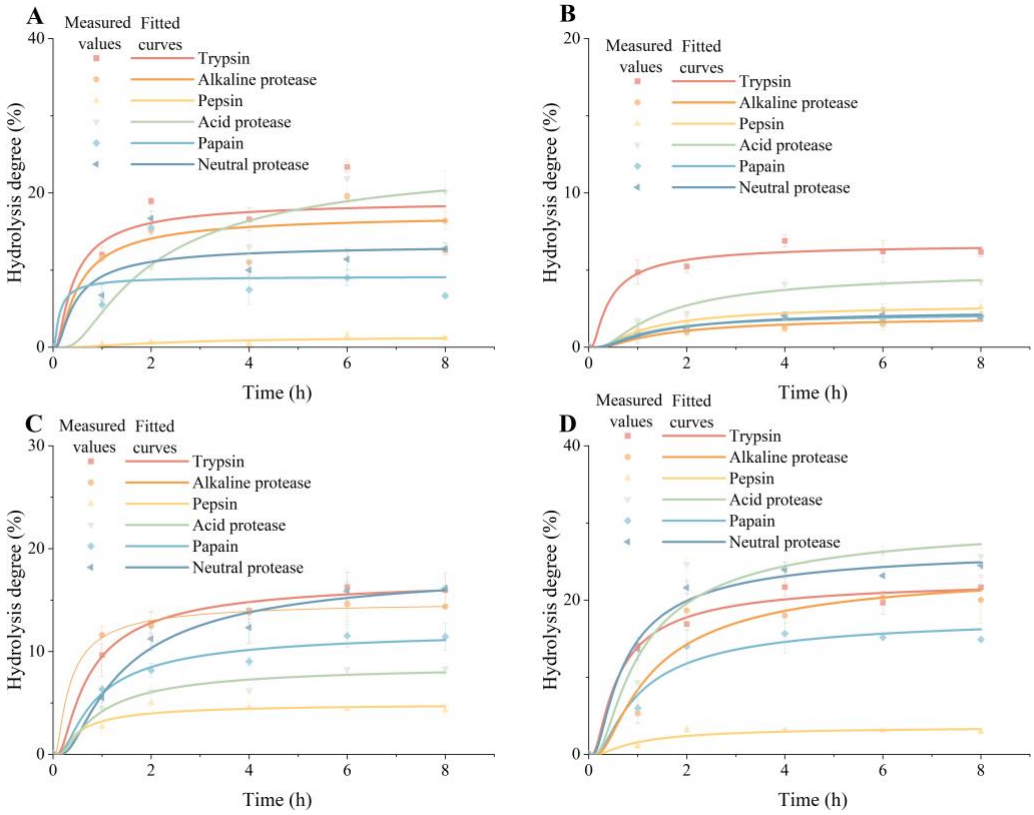

Fig. 1: (A), (B), (C), and (D) represent the enzymatic hydrolysis curves of crude, sarcoplasmic, myofibrillar, and stromal proteins, respectively

For crude proteins, following an 8 h enzymolysis period, acid protease exhibited the highest enzymolysis rate of 25.17%, with trypsin, alkaline protease and neutral protease following closely behind and pepsin demonstrating the lowest enzymolysis rate (2.51%). Pepsin exhibited the lowest enzymatic activity rate, as determined by Korkmaz and Tokur, who also revealed that alkaline protease outperformed both papain and neutral protease in the enzymatic hydrolysis of fish protein [25].

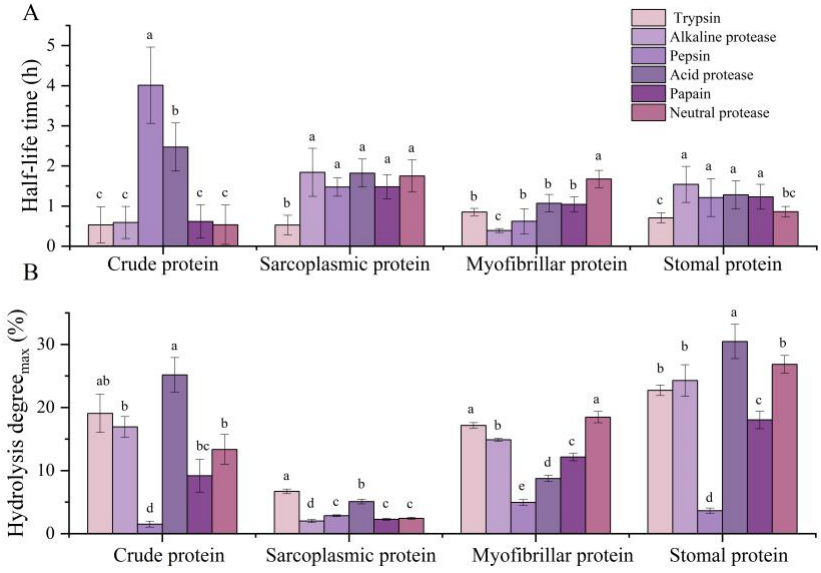

Fig. 2: Hydrolysis half-life (A) and maximum hydrolysis rate (B) of crude protein and three protein components by different proteases

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Furthermore, there were notable distinctions in the enzymolysis characteristics among the three protein components. Specifically, trypsin exhibited superior enzymolysis efficacy on sarcoplasmic protein (the hydrolysis degree of 7.3%), while neutral and acidic proteases demonstrated optimal enzymolysis effects on myofibrillar protein and stromal protein, the hydrolysis degree of 18.1% and 30.14%, respectively. Our study revealed that the hydrolysis of myofibrillar protein (half-life of 0.43) by alkaline protease resulted in a significantly shorter half-life compared to the other two components ($P<0.05$), sarcoplasmic protein (half-life of 1.85) and stromal protein (half-life of 1.54). Additionally, neutral protease was found to have the shortest half-life among the three components, which may reflect differences in the difficulty of enzymolysis of protein samples by corresponding enzymes [26] (Zou et al., 2018). In contrast to crude protein, the enzymolysis results of the three protein components demonstrated a significant reduction in the half-lives of pepsin and acid protease, with a slight increase observed in the half-lives of other enzymes. This result was not only due to the difference in the above protease cleavage sites leading to the difference in enzyme sensitivity but was also possibly due to the removal of lipids and other impurities that could compete for cleavage sites after separation during the process of separation of protein components. However, with the exception of pepsin, the remaining five enzymes exhibited superior enzymolysis effects on stromal proteins, with myofibrillar and sarcoplasmic proteins following suit. This phenomenon may be attributed to the lower content of branched-chain amino acids in myofibrillar protein compared to sarcoplasmic protein, resulting in less challenging hydrolysis [27].

With the exception of acid protease, the enzymatic hydrolysis efficacy of the remaining five enzymes on myofibrillar protein and stromal protein closely resembled that of crude protein, with alkaline protease yielding the most favorable outcomes. On the other hand, pepsin is also the most effective enzyme at forming antioxidant peptides for crude proteins, almost as effective as alkaline protease. However, of the three protein components, only sarcoplasmic protein produced the most effective antioxidant peptide under pepsin action (about 80%), while only approximately 60% were found in myofibrillar protein and matrix protein. Therefore, sarcoplasmic protein is the main component of crude protein from which antioxidant peptides can be produced under the action of pepsin, while under the action of alkaline protease, myofibrillar and matrix proteins are paramount.

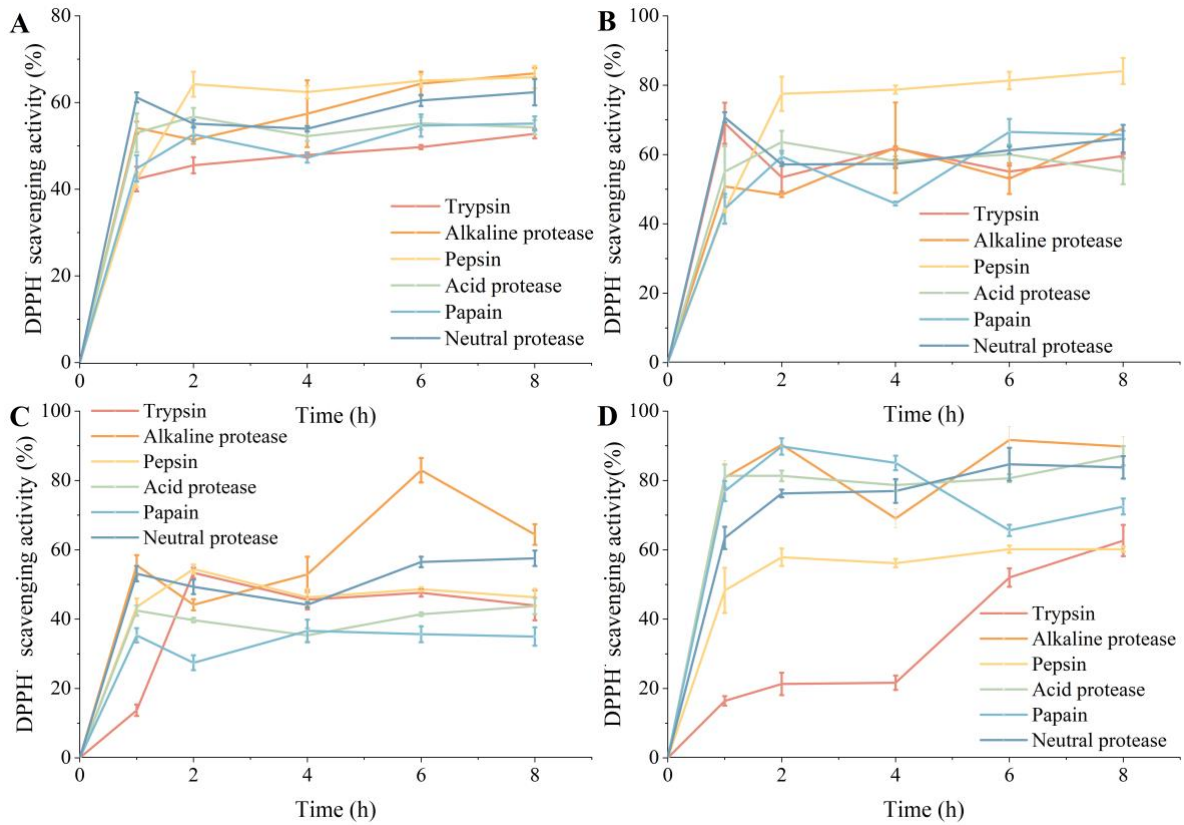

Fig. 3: (A), (B), (C), and (D) represent the DPPH scavenging activity of crude, sarcoplasmic, myofibrillar, and stromal proteins during the process of enzymatic hydrolysis, respectively

DPPH Scavenging Activity

Fig. 3 illustrates the variance in DPPH scavenging activity between crude protein and the hydrolysates of three protein components. Under the action of different enzymes, the changes in DPPH scavenging activity of the four protein samples all showed a similar increasing trend in the early stage and fluctuations in the later stage. Two factors contributed to the variability observed in the enzymatic hydrolysis curve. The first is that research has demonstrated an increase in the antioxidant activity (2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate, ABTS radical scavenging activity) of sturgeon head derived peptides as their molecular weight decreases [28]. The second is that antioxidant peptides produced during enzymolysis usually have phenylpropyl and tyrosine, and the number of these residues fluctuates with the change in protein structure during enzymolysis [29, 30].

For crude protein, after the enzymolysis solution was treated by six enzymes for 2 h, the DPPH assay showed strong antioxidant activity, which is in line with the study of Islam et al. [28]. Sturgeon muscle protease lysate exhibited a peak DPPH scavenging activity of approximately 63% following a 2-h period of pepsin enzymolysis or an 8-h period of alkaline protease. Among the other enzymes, neutral protease, acid protease, trypsin and papain all reached the maximum free-radical elimination rate, which was, in decreasing order, approximately 61%, 56%, 49% and 45%, at different times.

On the other hand, the variance in free-radical-scavenging activity observed in the enzymolysis solution of the three protein components is likely attributable to differences in amino acid composition. For example, Amakye et al. discovered that peptides containing amino acid residues such as histidine (His), leucine (Leu), tyrosine (Tyr), methionine (Met) or cysteine (Cys) tend to exhibit elevated levels of free-radical-scavenging activity [31]. The enzymatic hydrolysis of pepsin demonstrated the most effective scavenging effect on DPPH free radicals in sarcoplasmic protein, achieving a scavenging rate of approximately 80%, whereas other enzymes exhibited a lower rate of around 60%. Nevertheless, the myofibrillar and stromal proteins exhibited a maximum free-radical-scavenging rate of approximately 50% and 55%, respectively, following pepsin treatment, placing them fourth and fifth in a ranking of six enzymes. Hence, the primary factor contributing to the superior scavenging efficacy of the enzymatic hydrolysis solution of crude protein by pepsin is the significant involvement of sarcoplasmic protein. However, in combination with Fig. 1, sarcoplasmic protein has a low enzymatic hydrolysis rate. In addition to the high antioxidant activity of sarcoplasmic protease hydrolysis products, we speculate that the hydrolysate of sarcoplasmic protein strongly promotes the free-radical-scavenging activity of other enzymatic hydrolysis products. On the other hand, numerous researchers have conducted studies on the antioxidant peptides found in sarcoplasmic proteins derived from a range of raw materials, such as grass carp, beef brisket, beef liver and tilapia, among others, and we arrived at a similar conclusion [32-34].

Furthermore, for the crude protein, the activity of the enzymolysis solution obtained by alkaline protease was weaker than that of pepsin in the early stage, but the antioxidant peptides were produced in large quantities after 2 h enzymatic hydrolysis and reached the maximum level of free-radical clearance of pepsin. Meanwhile, enzymatic hydrolysis of pepsin remained in a relatively stable state in the subsequent period. In addition, we found a similar pattern in the enzymatic hydrolysis of myofibrillar protein. Under the action of alkaline protease, its antioxidant activity still increased significantly after 4 h and reached 80% at 6 h. On the other hand, as shown in Figure 1, myofibrillar protein is also the main protein component of sturgeon protein. Therefore, this phenomenon is primarily responsible for the lagging effect of myofibrillar protein in the production of antioxidant peptides under the action of alkaline protease.

ADH Promoting Peptide

The variations in ADH promotion rates between crude protein and three protein components are illustrated in Fig. 4. Similar to antioxidant peptides, the peptides with ADH-promoting activity from various protein samples also exhibited notable distinctions ($P<0.05$). In particular, among the various enzymolysis solutions tested, only trypsin demonstrated a significant positive effect on crude proteins, with a promotion rate reaching approximately 40% at 4 h before gradually declining to a 30% inhibition effect at 8 h. However, the enzymolysis solution of alkaline protease and pepsin had almost no promoting effect on ADH, with only slight fluctuation over time. It can be noted that the results of the other three enzymes were similar, and all showed obvious inhibitory effects, with inhibition rates between 20% and 40%. These results suggest that the proteins did not generate functional peptides with ADH-promoting activity but instead produced active peptides with inhibitory effects when acted upon by the aforementioned enzymes. Similarly, Liu et al. found that digestion of low-processed or ultra-processed (excessive use of additives) proteins in red pork tenderloin caused liver inflammation in mice [35].

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Besides, we found that ADH-promoting activity was detected only in the enzymolysis solution of myofibrillar protein, and inhibitory activity was detected almost exclusively in sarcoplasmic and stromal protein. In the presence of alkaline protease, trypsin, pepsin and acid protease, the promotion of peptide production in myofibrillar protein by ADH exhibited a consistent trend of an initial increase followed by a subsequent slight decrease or stabilization. In addition, the maximum promoting effect was achieved at 4 h of pepsin hydrolysis, reaching about 47%. Conversely, the enzymatic solution containing papain and neutral protease reached its highest ADH-promoting rate after 1 h but exhibited a decrease or slight increase as the duration of hydrolysis increased. When papain enzymatically hydrolysed chickpea protein, it also generated a peptide with high ADH-promoting activity, which was consistent with our own results [36].

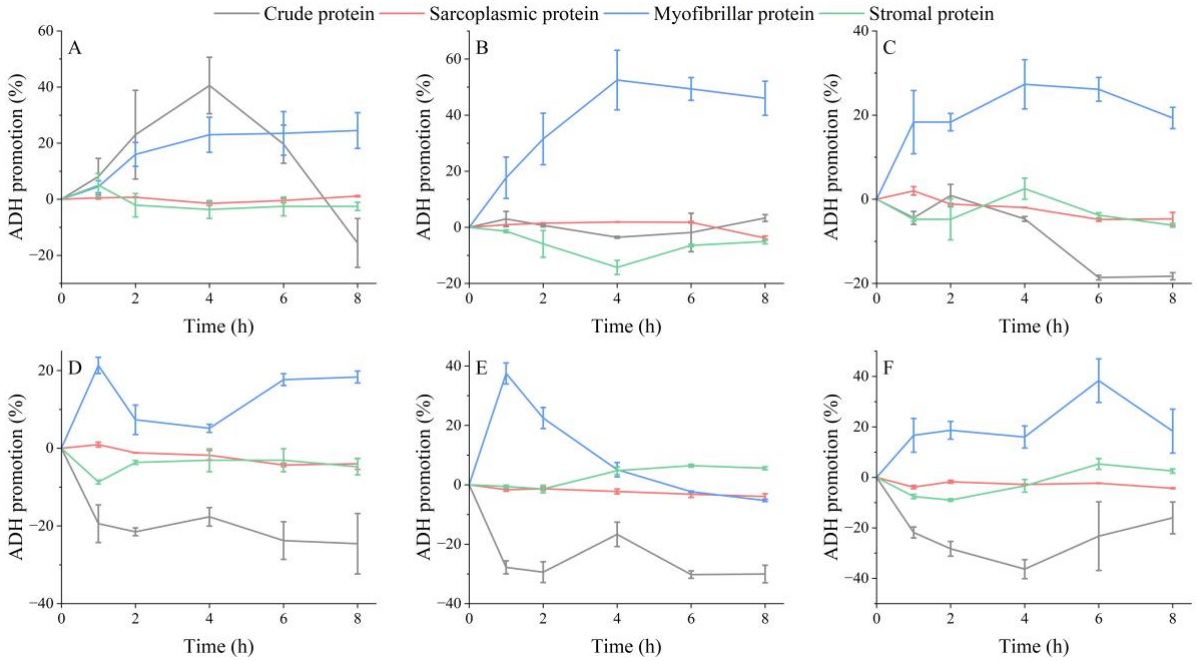

Fig. 4: The ADH promotion rates produced by trypsin (A), alkaline protease (B), pepsin (C), acid protease (D), papain (E) and neutral protease (F), respectively

However, we found that for both crude and myofibrillar protein, trypsin and pepsin, respectively, had the greatest ADH-promoting effect, which seems counterintuitive. This may be because sarcoplasmic proteins and stromal proteins produce more peptides with inhibitory effects under the action of pepsin, as shown in Fig. 4, although myofibrillar protein has the best ADH-promoting activity under the action of pepsin. The enzymolysis solution of pepsin and acid protease exhibited the most significant inhibitory effect on sarcoplasmic protein, and the inhibitory rate reached about 5% at 6 h. For stromal proteins, the enzymolysis solution after 4 h of treatment with alkaline protease had the highest inhibition rate, nearly 16%, while the inhibition rate of pepsin enzymolysis solution was second only to that of alkaline protease.

In general, as anticipated, protein components were critical to the generation of active peptides, with distinct protein components exhibiting notable variations in the synthesis of various functional active peptides. Three protein components can generate antioxidant peptides through the catalysis of six enzymes, with the most effective antioxidant peptides being stromal protein, sarcoplasmic protein and myofibrillar protein. Considering the low content of stromal protein and the similarity of antioxidative peptide production between sarcoplasmic protein and crude protein, it is suggested that sarcoplasmic protein plays a significant role in the generation of antioxidative peptide during proteolytic processes in sturgeon. For ADH-promoting peptides, however, the outcomes varied significantly. Of the three protein components examined, only the enzymolysis solution of myofibrillar protein demonstrated ADH-promoting activity, whereas the enzymolysis solutions of the remaining two proteins exhibited primarily inhibitory effects. These findings suggest that myofibrillar protein plays a pivotal role in the generation of ADH-promoting peptides during the proteolytic process of sturgeon. Moreover, the production of ADH-promoting peptide by sturgeon protein is influenced by the synergistic effect of the production of ADH-promoting peptide by myofibrillar protein and ADH-inhibiting peptide by sarcoplasmic protein and stromal protein. Therefore, we believe that the separation of the target protein components before enzymatic hydrolysis is an effective means to improve the yield and concentration of the target active peptide, which is of great significance for its preparation.

Conclusion

This study explored the enzymatic hydrolysis of sturgeon protein fractions (sarcoplasmic, myofibrillar, and stromal proteins) to identify their roles in producing bioactive peptides. The stromal protein was the most susceptible to hydrolysis, while pepsin and alkaline protease were the most effective enzymes for generating antioxidant peptides. The sarcoplasmic protein hydrolysates exhibited the strongest antioxidant activity (~80% DPPH scavenging activity) under pepsin treatment, whereas myofibrillar and stromal proteins performed better with alkaline protease. For ADH-promoting peptides, only myofibrillar protein showed positive activity under trypsin and pepsin, while sarcoplasmic and stromal proteins produced inhibitory effects. These findings highlight the importance of protein fractionation to optimize bioactive peptide production, offering practical strategies for high-value sturgeon meat processing. Future research should focus on peptide purification, identify the ADH-promoting peptides sequences and verify their functionality *in vivo* validation to enhance applications in functional foods and health products.

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Author's Contributions

Yejing Sun and Xiaolin Bai: Designed, performed the experiments, drafted the manuscript.
Yingmei Hou and Mengjie Lu: Collected data, plotted graphs, did some experiments and revised the manuscript.
Yizhou Fang and Guangrong Huang: Analyzed the experimental data and revised the manuscript.

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