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Study on extraction of antioxidant polypeptides from byproducts of *Procambarus clarkii*

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Abstract
This article primarily focuses on studying the peptide extraction method and antioxidant properties of crayfish by-products using alkaline protease. Taking the degree of protein hydrolysis as a measurement indicator, and considering enzyme dosage, solid-liquid ratio, and hydrolysis time as optimization factors, we utilized single-factor and orthogonal experiments to refine the extraction process. Additionally, we assessed its antioxidant activity using the DPPH method. The findings revealed that the optimal process conditions were as follows: an enzyme dosage of 2% alkaline protease, a solid-liquid ratio of 1:20, and an enzymatic hydrolysis time of 2 hours. The measured protein hydrolysis degree was 21.08%, and its DPPH free radical scavenging rate reached 70.55%. When compared to vitamin C, it demonstrated superior antioxidant capabilities in scavenging free radicals.

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Keywords: Crayfish by-products, polypeptide, oxidize resistance

Introduction

In recent years, with the improvement of living standards, *Procambarus clarkii*, also known as crayfish, is an aquatic animal in the order Crustacea, Decapoda, and family Crayfidae. Crayfish is a type of river fresh fish with a high protein content. Its meat is soft and easy to digest, making it suitable as a nutritional food for those who need to take care of themselves after illness or are physically weak. In addition, the meat quality of crayfish is also loved by many people. In terms of nutrition, the meat of crayfish contains abundant magnesium, zinc, iodine, selenium, and other important minerals and trace elements needed by the human body, which have different health benefits. Therefore, regularly consuming crayfish can help people supplement nutrients, enhance their immune system, and improve their health; Shrimp meat is also rich in magnesium, zinc, iodine, selenium, and other nutrients. The nutritional components of crayfish have various cardiovascular protective effects. Zinc can reduce cholesterol levels in the blood, effectively preventing and improving cardiovascular diseases such as arteriosclerosis. In addition, it can promote coronary artery dilation, which has certain benefits for preventing hypertension and myocardial infarction. Therefore, regularly consuming crayfish is of great significance for protecting heart health. For promoting wound healing after surgery, shrimp can also be used as medicine to reduce phlegm and cough. Also known as red swamp crayfish and freshwater crayfish. It has a delicious taste, rich in high-quality protein and minerals, and high nutritional value, and is increasingly popular among people [1-2].

With the passage of time, the demand for crayfish in the market is increasing, and the demand for fresh crayfish is huge. The increasing demand for crayfish in people's daily lives will also lead to a significant increase in by-products such as shrimp heads and shells produced during the processing and consumption of crayfish. The processing methods for by-products of *Procambarus clarkii* are currently in a relatively crude stage, with the commonly used method being fish meal feed processing. However, the added value is low, and the utilization of its potential value is very insufficient, often resulting in waste of its by-products. Moreover, it can lead to the disposal of shrimp by-products, waste of resources, and even environmental pollution. New methods for treating shrimp by-products are urgently needed for the utilization and development of the by-products of *Procambarus clarkii*. Therefore, the sustainable and effective reuse of by-products from *Procambarus clarkii* has become a hot research topic in the food, medical, cosmetics and other industries today.

The by-products have been appropriately treated, such as Sui Wei, Wang Yan, Duan Shan, etc., using enzymatic hydrolysis to extract proteins, providing technical support for the comprehensive recycling and full utilization of by-products from the freshwater shrimp.

After processing and utilizing crayfish, a large amount of by-products are produced, including shrimp heads, shells, feet, and low value shrimp parts. These by-products typically account for about 85% of the raw materials. These by-products contain a large amount of protein, fat, minerals, and various active ingredients such as astaxanthin, astaxanthin, and chitin. Among them, protein has the highest content, accounting for about 36% -40% of by-products [5-6]. The protein in the by-products of crayfish contains various functional peptides, which are highly safe, have no toxic side effects on the human body, and are easily absorbed by the human body. These peptides are abundant in content and have strong antioxidant and antibacterial abilities. Therefore, these peptides can be used to prepare functional foods, achieving protection and promotion of human health. The prepared food can have health benefits such as enhancing immunity, improving intestinal health, and protecting cardiovascular health, which is of great significance to people's health.

This article mainly studies the peptide extraction method and antioxidant activity under the action of alkaline protease, with protein hydrolysis degree as the measurement index. In the early stage, single factor experiments were mainly used to analyze the effects of enzyme dosage, hydrolysis time, and solid-liquid ratio on protein hydrolysis degree; The optimal process conditions will be determined through orthogonal analysis in the later stage. The antioxidant activity is determined by measuring the enzymatic hydrolysis products under different enzymatic hydrolysis conditions using the DPPH method.

Experimental materials and equipment

Test Materials

Lobster: produced in Shayang Town, Shayang County, Jingmen City; Concentrated sulfuric acid: Wuhan Hongshan Zhongnan Chemical Reagent Co., Ltd. (analytical grade); Copper sulfate: Wuhan Hongshan Zhongnan Chemical Reagent Co., Ltd. (analytical grade); Potassium sulfate: Wuhan Hongshan Zhongnan Chemical Reagent Co., Ltd. (analytical grade); Sodium hydroxide: Tianjin Fuchen Chemical Reagent Factory (analytical grade); Boric acid: Delborium (analytical grade); Methyl Red and Bromocresol Green: Tianjin Chemical Reagent Factory No.1; Isoleucine: Yichang Sanxia Pronoting Biopharmaceutical Co., Ltd; Indene ketone: Hubei Rishengchang New Materials Technology Co., Ltd; Tin chloride: Hangzhou Jiuxi Chemical Co., Ltd. (analytical grade); Potassium dihydrogen phosphate: Hubei Chengfeng Chemical Co., Ltd. (analytical grade); Sodium dihydrogen phosphate: Wuhan Jiyesheng Chemical Co., Ltd. (analytical grade); Alkaline protease: Henan Yangshao Biochemical Engineering Co., Ltd; 1,1-diphenyl-2-trinitrohydrazine (DPPH): Shanghai Tengyue Biotechnology Co., Ltd; Hydrochloric acid: Xiangrui Chemical Co., Ltd. (analytical grade); 95% anhydrous ethanol: Wuhan Hongshan Zhongnan Chemical Reagent Co., Ltd; Vitamin C: Wuhan Xingzhongcheng Technology Co., Ltd.

Instruments and equipment

Table 1: Instruments and equipment

Instrument	Model	Manufacture
Electronic Balance	FA2104	Shanghai Puchun Measuring Instrument Co., Ltd
Spectrophotometer	UV6800	Shanghai Jingke Electric
Automatic Kjeldahl nitrogen analyzer	KT8400	Shanghai Guangdi Instrument Equipment Co., Ltd
Digestive Furnace	KDN-04C	Taicang Experimental Equipment Factory
Crusher	XPF-250	Taicang Experimental Equipment Factory
Freeze dryer	SR-A18N-80	Shanghai Furui Instrument Technology Co., Ltd
Temperature controlled water bath	DK-98-1	Tianjin Test Instrument Co., Ltd
Desktop centrifuge	TDL-5	Shanghai Anting Scientific Instrument Co., Ltd

Experimental methods

Raw material pretreatment

Remove the shell and meat from the lobster, then clean the shrimp head and shell, and dry them in a freeze dryer for 48 hours. After drying is complete, use a grinder to crush them.

Technological Process

Freeze dried powder of crayfish by-products→Add water to homogenize→Enzyme inactivation→Isothermal enzymatic hydrolysis→cooling→Centrifugal→Filter→Take the supernatant→Freeze drying→Crayfish peptide freeze-dried powder [7-8]

Main operating steps

After homogenizing the freeze-dried powder of crayfish by-products with water, the enzyme was killed at high temperature, and an appropriate amount of alkaline protease was added to adjust the appropriate pH at a certain temperature for enzymatic hydrolysis. After obtaining the enzymatic solution, it was filtered and centrifuged to obtain the supernatant for storage.

Measurement indicators and methods

Total protein

Kjeldahl method (adding concentrated sulfuric acid, copper sulfate, and potassium sulfate to the sample for digestion and heating can destroy proteins, oxidize carbon and hydrogen to produce carbon dioxide and water, and convert organic nitrogen into ammonia and combine with sulfuric acid to form ammonium sulfate. Subsequently, the ammonia in the sample is evaporated by heating, and boric acid is used to absorb the remaining ammonia during the experiment. Next, standard hydrochloric acid solution is used for titration, and the protein content is calculated based on the required consumption of standard acid.)

Specific steps: Take 2g of freeze-dried crayfish by-product powder and place it in a dry and clean digestion bottle. Add 5g of finely ground concentrated sulfuric acid, 20ml of copper sulfate, and 10g of potassium sulfate in sequence, and

then put it into a digestion furnace for digestion until the solution becomes clear and green. Take out the digestion bottle and put it into an automatic Kjeldahl nitrogen analyzer. Add alkali distillation, and use boric acid with methyl red bromocresol green indicator to absorb ammonia gas. Then, use a hydrochloric acid standard solution with a concentration of 0.01000mol/L for titration. When the solution turns slightly red, it is the endpoint of the titration.

Amino acids

Indene ketone method (In alkaline solution, amino acids react with indene ketone to produce a blue purple compound. The color intensity of this compound is proportional to the concentration of amino acids and can be determined by absorbance method. Specifically, the maximum absorption wavelength of this compound is 570nm, so this wavelength can be used to determine the amino acid content in the sample.) [10].

Specific steps: Put 0.2000g of dry isoleucine into a beaker, add an appropriate amount of distilled water for dissolution, then transfer the quantitative solution to a 100ml volumetric flask, add enough distilled water, and bring the solution to the mark at the bottle mouth. Prepare a 200ug/ml amino acid standard solution, and then transfer different milliliters of amino acid standard solution, 0, 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, and 3.0ml, into a 25ml colorimetric tube. Add water to make up the volume to 4ml, and add water to the isoleucine solution in the volumetric flask to make up the total volume to 4ml. Then, add 1ml of indanone and 1ml of phosphate buffer solution, mix well, and heat in a water bath for 15 minutes. Subsequently, quickly cool to room temperature and add enough water to the mark, shake well. After waiting for 15 minutes, measure its absorbance using a spectrophotometer at a wavelength of 570nm and draw a standard curve based on experimental data. The absorbance measurement of the sample is consistent with that of the amino acid standard solution, and the micrograms of amino acids can be calculated by looking up the table to determine the content of amino acids.

Determination of protein hydrolysis degree

Kjeldahl nitrogen determination is used to determine the total nitrogen content in the raw material, and the indene ketone method is used to determine the amino acid content of the hydrolysate. The protein hydrolysis degree (DH) is calculated using formula (2-1): [11].

$$DH = \frac{The\ content\ of\ amino\ acids\ in\ the\ hydrolysis\ solution}{Total\ nitrogen\ content\ in\ raw\ materials} \times 100 \quad (2\text{-}1)$$

In vitro free radical scavenging assay

In vitro free radical scavenging assay using DPPH system (DPPH is a purple free radical that contains an unpaired electron in its molecule and has strong absorption ability. When DPPH reacts with antioxidants, the DPPH free radical decreases and appears pale yellow or colorless, and the absorbance changes to calculate the antioxidant capacity of the sample) [12]. Prepare crayfish by-product peptides at different concentrations and test their free radical scavenging ability using the DPPH system method.

Specific steps: Take sample solutions of different concentrations and add equal volumes of DPPH solution to form sample group A1; Add equal volumes of ethanol solution to sample solutions of different concentrations to form control group A2; Take DPPH solution and add an equal volume of ethanol solution to form blank group A0. After mixing the above experimental groups, react at room temperature in the dark for 30 minutes, and measure the absorbance at 517 nm. Calculate the DPPH radical scavenging rate according to formula (2-2):

DPPH radical scavenging rate (%)=1- $\frac{A_1-A_2}{A_0} \times 100\%$ (2-2)

Data analysis

All data were measured in parallel three times, and the experimental results were expressed in the form of mean plus standard deviation. During the drawing process, Excel 2019 software was used.

Single factor and orthogonal experiments

Single factor experiment

Effect of enzyme dosage on protein hydrolysis degree

Weigh crayfish peptide freeze-dried powder in water as a medium, at a temperature of 55 ℃, pH of 6, solid-liquid ratio of 1:20, enzyme dosage of 1%, 2%, 3%, 4%, 5%, and enzymatic hydrolysis for 2 hours to measure the degree of protein hydrolysis.

Effect of feed to liquid ratio on protein hydrolysis degree

Weigh crayfish peptide freeze-dried powder in water as a medium, at a temperature of 55 ℃, pH of 6, enzyme dosage of 2%, raw material to water ratios of 1:1, 1:5, 1:10, 1:15, 1:20, and enzymatic hydrolysis for 2 hours to determine the degree of protein hydrolysis.

Effect of Enzymatic Hydrolysis Time on Protein Hydrolysis Degree

Weigh crayfish peptide freeze-dried powder in water as a medium, at a temperature of 55 ℃, pH of 6, a solid-liquid ratio of 1:20, an enzyme dosage of 2%, and enzymatic hydrolysis times of 1h, 2h, 3h, 4h, 5h.

Orthogonal experiment

Based on the single factor results, with enzyme dosage, hydrolysis time, and solid-liquid ratio as optimization factors, three levels were set for each factor. The L_9 (3^3) orthogonal experimental table (Table 2) was selected, and the hydrolysis degree was used as the measurement index to study the effect of the optimal protease dosage (A), hydrolysis time (B), and solid-liquid ratio (C) on the protein hydrolysis degree of crayfish by-products.

Table 2: Orthogonal experimental design for protein hydrolysis of crayfish by-products

Level	A enzyme dosage (%)	B solid-liquid ratio (g/ml)	C enzymatic hydrolysis time (h)
1	2	1:10	2
2	3	1:15	3
3	4	1:20	4

Result and Analysis

The influence of enzymatic hydrolysis conditions on protein hydrolysis degree The effect of enzyme dosage on the hydrolysis degree of sample proteins.

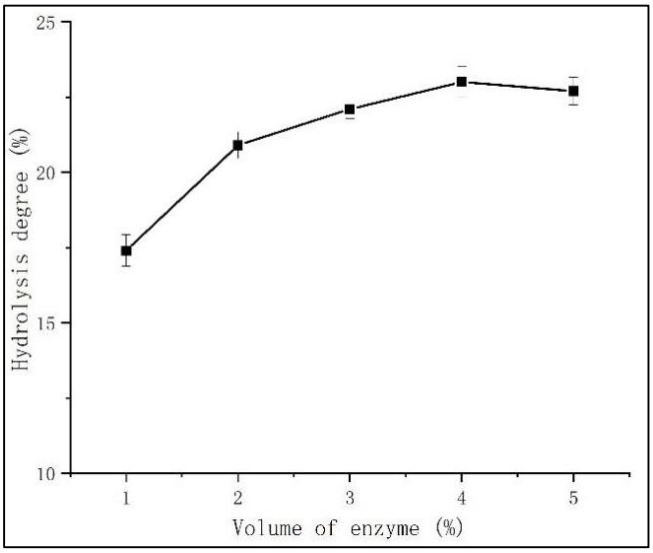

Fig 1: The effect of different enzyme dosages on protein hydrolysis degree

According to Figure 1, the protein hydrolysis degree of the sample increases with the increase of enzyme dosage. When the enzyme dosage exceeds 2%, the increase in protein hydrolysis degree of the sample is not significant. When the amount of enzyme added is small, and when the amount of enzyme added increases, the enzyme will bind to more substrates, thereby increasing the efficiency of enzymatic hydrolysis; As the amount of enzyme added increases, the lack of substrate binding to the enzyme does not significantly increase the degree of protein hydrolysis, and substrate enzyme binding may also inhibit protein hydrolysis in turn, resulting in a decrease in reaction rate or protein hydrolysis rate [13-14].

Effect of feed to liquid ratio on the hydrolysis degree of sample proteins

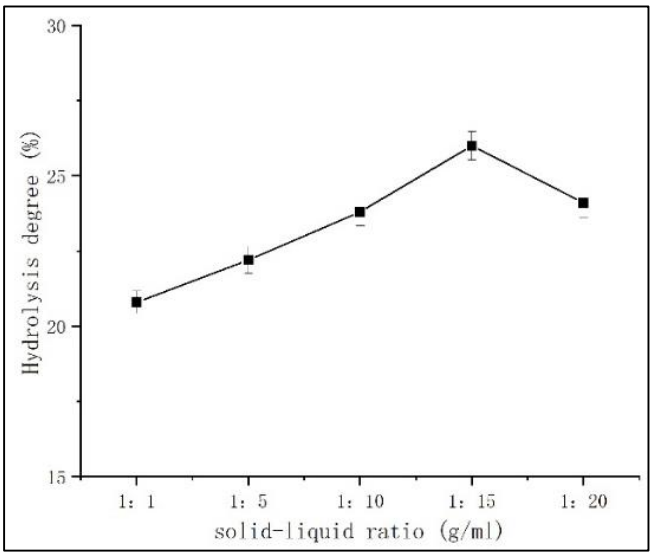

Fig 2: The influence of different material to liquid ratios on protein hydrolysis degree

From Figure 2, it can be seen that when the feed liquid ratio is low, the system becomes too viscous, resulting in clumping and sticking to the wall, incomplete enzymatic hydrolysis of the substrate, and low hydrolysis degree. As the ratio of feed to liquid increases, an appropriate amount of feed to liquid ratio is beneficial for the enzyme to act more efficiently on the substrate when it comes into full contact with the enzyme, thereby achieving better enzymatic hydrolysis results. However, if the ratio of material to liquid used is too high, it will lead to a decrease in substrate concentration or dilution of enzyme concentration, which will reduce the effectiveness of enzymatic hydrolysis, slow down the enzymatic hydrolysis rate, and cause the hydrolysis degree to flatten or decrease. Therefore, the effect of material to liquid ratio on hydrolysis degree can be clearly observed from the graph.

The Effect of Enzymatic Hydrolysis Time on the Hydrolysis Degree of Sample Proteins

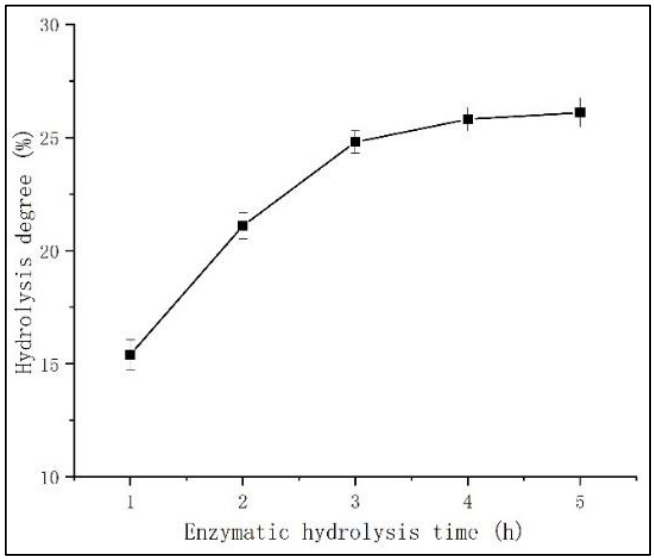

Fig 3: The Effect of Enzymatic Hydrolysis Time on the Hydrolysis Degree of Sample Proteins

From Figure 3, it can be seen that when the enzymatic hydrolysis time is less than 4 hours, the hydrolysis degree of the sample protein increases with the increase of enzymatic hydrolysis time. The hydrolysis degree increases significantly in the 1-2h stage, and reaches its maximum at 4 hours. There is not much difference between the hydrolysis degree at 3 hours and 4 hours, which may be due to the partial inactivation of the enzyme molecule itself and the enhanced inhibitory effect of the product caused by excessive heating time at 3 hours. The activity of the enzyme is inhibited and weakened, and the trend of increasing hydrolysis degree gradually slows down [15]. When the enzymatic hydrolysis time is short and the protein is not completely precipitated, the degree of protein hydrolysis is low. As the enzymatic hydrolysis time prolongs, the degree of hydrolysis first increases and then tends to plateau. It is also possible that after 4 hours of enzymatic hydrolysis, the activity of endogenous autolytic enzymes gradually decreases while the degree of hydrolysis remains basically unchanged [16].

Process optimization of orthogonal experiments

Based on the results of a single factor experiment, the protein hydrolysis degree was used as an indicator to optimize the protein extraction of crayfish by-products through L_9 (3^3) orthogonal experiment. The results and trend charts of the orthogonal experiment are shown in Table 3 and Figure 4, respectively.

Table 3: Results of orthogonal experiment on hydrolysis degree

Experiment number	A(Enzyme dosage)	B (Material liquid ratio)	C (Enzymatic hydrolysis time)	D (Protein hydrolysis degree /%)
1	1	1	1	19.22
2	1	2	2	18.47
3	1	3	3	20.15
4	2	1	2	17.25
5	2	2	3	16.75
6	2	3	1	17.59
7	3	1	3	15.23
8	3	2	1	16.45
9	3	3	2	14.22
K1	57.84	51.70	53.26	
K2	51.59	51.67	49.94	
K3	45.90	51.96	52.13	
k1	19.28	17.23	17.75	
k2	17.20	17.22	16.65	
k3	15.30	17.32	17.38	
R	3.98	0.10	1.11	

From the above table, it can be seen that through the results of orthogonal experiments and range analysis, the influence of various factors on the protein hydrolysis degree of the sample varies in magnitude, with the importance level being A (enzyme dosage)>C (enzyme hydrolysis time)>B (feed to liquid ratio). According to the results of orthogonal experiments on hydrolysis degree, the optimal combination for orthogonal experiments is A1C1B3, which means that the enzyme dosage is 2%, the enzyme hydrolysis time is 2 hours, and the feed to liquid ratio is 1:20 (g/mL). At this time, the protein hydrolysis degree measured under this experimental condition is 21.08%, reaching the maximum value of protein hydrolysis degree of crayfish by-products under all enzymatic hydrolysis conditions in this experiment.

Determination of antioxidant capacity of crayfish by-products in vitro

After dissolving with DPPH, the solution turns purple and generates single electron free radicals. When combined with free radical scavengers, the original color fades away. Quantitative analysis is performed by quickly measuring the absorbance. The degree of hydrolysis of samples that have been hydrolyzed under different enzymatic conditions is taken as the X-axis and measured using the DPPH method [17]. As shown in Figure 4. Compare the clearance rate of crayfish by-products with the clearance rate of vitamin C to reflect the antioxidant capacity of crayfish by-products, as shown in Figure 5.

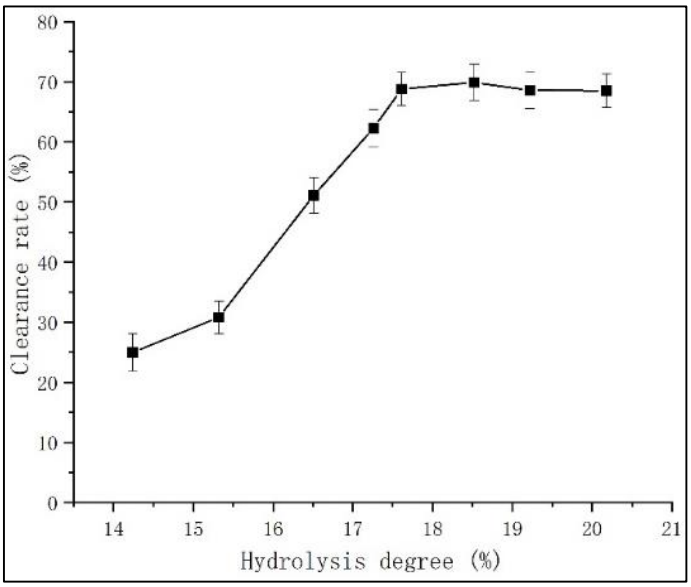

Fig 4: The scavenging effect of crayfish by-products on DPPH

From Figure 4, it can be seen that the ability to clear free radicals gradually increases with the increase of protein hydrolysis degree, the deeper the enzymatic hydrolysis degree, the higher the free radical clearance rate, and the stronger the antioxidant capacity. When the hydrolysis degree reaches 17-20%, the scavenging effect of crayfish by-products on DPPH no longer changes significantly, which may be due to the effect of increased reaction time on the antioxidant capacity of crayfish by-products or the influence of air. The antioxidant peptides in crayfish by-products can demonstrate their antioxidant capacity by scavenging free radicals and reducing them [18-19].

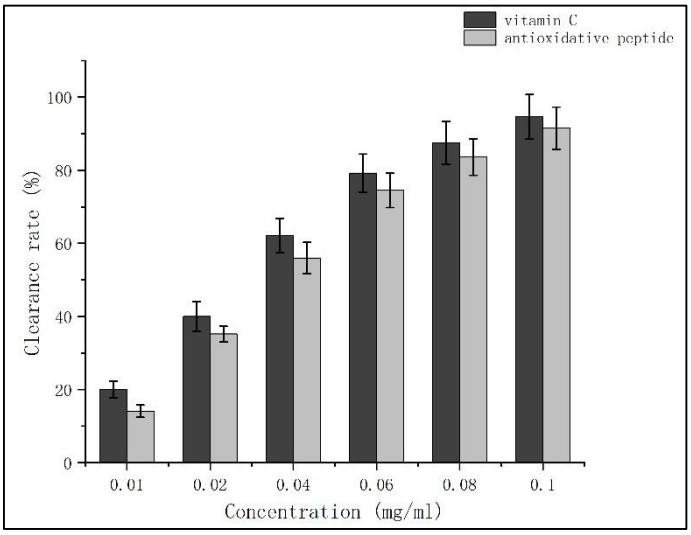

Fig 5: The clearance effect of vitamin C on DPPH

From Figure 5, it can be seen that compared with the control group of natural antioxidant vitamin C, the antioxidant capacity of antioxidant peptides in crayfish by-products is limited. Under the concentration of less than 0.1mg/mL, the scavenging rate of DPPH is lower than that of vitamin C. Under the hydrolysis conditions of the above experiment, its antioxidant capacity is significantly different from that of natural antioxidant vitamin C, but its ability to scavenge free radicals is relatively stable and regular.

Summary

Enzymatic hydrolysis was used to prepare shrimp protein peptides from the protein in the head and shell of *Procambarus clarkii* using alkaline protease. The optimal enzymatic hydrolysis process was a 2-hour hydrolysis time, a solid-liquid ratio of 1:20, and an enzyme dosage of 2%. Under these conditions, the degree of protein hydrolysis was 21.08%. The in vitro antioxidant activity of crayfish by-products was studied by DPPH method, and it was found that their ability to scavenge free radicals was relatively stable and regular. The clearance rate showed a significant upward trend with the increase of protein hydrolysis degree, and the maximum DPPH free radical clearance rate was 70.55%

Its antioxidant capacity is weaker than that of natural antioxidant vitamin C, and its maximum free radical scavenging rate is significantly different from that of natural antioxidant vitamin C. The shrimp protein peptide extracted from crayfish by-products has multiple benefits for the human body due to its antioxidant capacity and as a biological peptide. Through process optimization, it can also be more fully applied in the food industry, medical industry, and cosmetics industry. This study provides a theoretical basis and scientific guidance for the process optimization of alkaline protease for extracting peptides from crayfish by-products.

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References

1. Xu Wensi, Li Baihua, Zhang Mengyuan, Yang Qifu, Yang Hongxing, Zhou Shunxiang. Research progress on processing and utilization of crayfish and its by-products [J]. Agricultural Products Processing. 2021;(01):60-63+68. DOI: 10.16693/j.cnki. 1671-9646 (X). 2021.101.016
2. Lu Jianfeng, Lai Nianyue, Cheng Yongxu. Comprehensive Utilization and Development Value of Freshwater Crayfish Resources [J]. Agricultural Product Processing (Journal), 2006;(10):47-52+63
3. Duan Shan, Ding Huixin, Xiong Yun. Enzymatic recovery of protein from shrimp heads and shells [J]. Agricultural Product Processing (Journal). 2008;(01):43-46
4. Sui Wei, Zhang Lianfu. Study on the technology of enzymatic shrimp processing scraps [J]. Chinese Condiments. 2005;(12):54-57+53
5. Wang Yan, Deng Fangming, Liu Yan, Liao Quan, Liu Wenqian. Enzymatic extraction of mid protein from the head and shell of *Procambarus clarkii* [J]. Food Science. 2013;34(12):1-5.
6. Zhao Xiangjie, Zhu Yu, Yang Rongling, Bi Yanhong, Zhu Qianlin. Research progress on comprehensive utilization of crayfish resources [J]. Agriculture and Technology. 2019;39(05):28-29. DOI: 10.19754/j.nyyjs.20190315010
7. Chen Tianzhong, Yao Xinhe, Wen Lixin. Research progress on comprehensive utilization of shrimp head and shell resources [J]. Hunan Feed. 2006;(04):35-36
8. Li Mingjie, Jiang Guoliang, He Jiaming. Optimization of preparation process and antioxidant determination of Antarctic krill peptide [J]. Food Industry Science and Technology. 2012;33(03):279-282+301. DOI: 0.13386/j.issn1002-0306-2012.03.079
9. Huang Manrong, Song Yanmei, Fan Guangcai *et al*. Optimization method for detecting protein content in dairy products using Kjeldahl nitrogen determination method [J]. China Dairy. 2023;254(02):87-90.
10. Li Yanting Research on the Methodology of Determining Amino Acid Content in Food [C]//(IFSN) Organizing Committee of the International Food Safety and Nutrition Health Summit Forum. Proceedings of the 4th International Food Safety and Nutrition Health Summit Forum and Seminar on High Quality Development of Plant based Foods [Publisher

unknown]; 2022:7. DOI: 10.26914/c.cnkihy.2022.056649
11. Zhao Mei, Wu Chengye. Response surface methodology optimization of enzymatic hydrolysis process for tilapia leftovers [J]. Food Research and Development. 2007;138(05): 48-53.
12. Pericarditis. Preparation of Calcium Protein from Eucommia ulmoides Leaves and Development of Functional Products [D]. Anhui University of Engineering; c2020.
13. Guo Gangjun, Zou Jianyun, Hu Xiaojing, Huang Yanli, Huang Kechang. Optimization of Enzymatic Hydrolysis Process for Peptide Preparation from Australian Nut Meal by Hydraulic Pressing [J]. Food Science. 2016;37(17):173-178
14. Zhao Li, Li Ting, Wang Qing, Hu Huogen, Chen Lili, Yuan Meilan. Preparation and antioxidant activity of protein peptides from *Procambarus clarkii* [J]. Chinese Condiments. 2017;42(06):22-28.
15. Mao Xiaoyu, Zhang Chunyu, Chen Xiaodan, Lin Simin, Huang Xiejun, Zeng Xingsheng. Research on the preparation of peanut peptides from peanut meal by enzymatic antipyretic pressing [J]. Modern Food Science and Technology, 2013;29(01):150-152+166. DOI: 10.13982/j.mfst.1673-9078.2013.01.017
16. Xia Zhen, Chen Bingbing, Huang Wen, *et al*. Preparation of selenium enriched oyster peptides and their antioxidant and angiotensin converting enzyme inhibitory activities [J]. Food and Fermentation Industry. 49(2):120-128 [XIAZ, CHEN B, HUANG W, *et al*. Preparation of selenium enriched oyster peptides and their antioxidant and angiotensin converting enzyme inhibitory activities [J] Food and Fermentation Industries, 2023,49(2):120-128.
17. Xie Ningning, Chen Xiao'e, Fang Xubo, Yu Yu. Research progress on antioxidant peptides in aquatic products [J]. Journal of Zhejiang Ocean University (Natural Science Edition). 2010;29(01):74-80.
18. Wang Ruixue, Sun Yang, Qian Fang. Antioxidant peptides and their research progress [J]. Food Science and Technology. 2011;36(05):83-86. DOI: 0.13684/j.cnki.spkj.2011.05.039
19. WU PP, MA GZ, LI NH, *et al*. Investigation of in vitro and in vivo antioxidant activities of flavonoids rich extract from the berries of Rhodomyrtus tomentosa (Ait.) Hassk [J]. Food Chemistry. 2015;173:194-20.