

Analysis of Ant Composition and Exploration of Protease Digestion Conditions

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Abstract: This experiment took black ants in Changbai Mountain as the experimental object, simply explored some basic components contained in them, and mainly studied the best conditions of ants by comparing the degree of the enzymatic digestion of various proteases. The experimental results show that the water content of black ants in Changbai Mountain is 41.53%, ash content 4.17%, crude protein content 39.3%, crude fat content 12%, amino acid content 43.91%, containing 26 kinds of fatty acids. The optimal single enzyme solution of protease was set as 2 hours, temperature 55°C, three alkalases pH 8.2, pepsin pH 5.2, substrate 0.5 g, a total amount of 0.03 g, and the two enzymes were 0.015 g. Double trypsin and pepsin have the highest degree; neutral proteinase, trypsin and papain have the highest degree; higher than double enzyme, and higher than all double and triple enzyme.

Keywords: Changbai Mountain Black Ant; Chemical Composition; Enzymatic Lysis Conditions; Amino Acid; Fatty Acid

1.Introduction

Ants is hymenoptera ant insects on earth, insect body has a high medicinal value, ant is rich in protein and a variety of amino acids, are necessary for the human body is called miniature animal nutrition treasure and natural medicine processing plants, can fitness and cure, very suitable as a new medicinal resources development and utilization, provide medical products, safeguard people's health, etc. In recent years, with the in-depth research on its medicinal chemistry, pharmacology and clinic, people pay more and more attention to the medicinal value of ants and the development and utilization of their resources, which is of great significance to further expand the medicinal resources and maintain human health[1]. Ants

are rich in protein, above 42% and as high as 67%. Proteins contain 27 amino acids. The fat content of ants is about 12% fatty acids, including oleic acid 62.44%, palmitic acid 21.14%, palmitoleic acid 11.03%, stearic acid 2.29%, linoleic acid 1.39%, linolenic acid 1.21% and mydamic acid 0.53%. The dosage form of medicinal ant preparation has been decoction, oral liquid, powder, powder, granule, capsules, pills, wine agent and so on. In terms of the development and utilization of ant food resources, its products are also limited to ant health wine, pure ant powder, ant powder capsule, ant oral liquid, ant health tea and other quasi-drugs with certain therapeutic effects. There is no precedent in the market of applying the active ingredients of ants in dairy products, baking products, beverage products, convenience food and so on[2]. According to studies, ants have anti-inflammatory and analgesic effects[3]. The research found that ants can be described as the whole body is a treasure, the body contains a variety of nutrients needed by the human body, including the protein content as high as 55.96%, containing 18 kinds of amino acids, including 8 kinds of amino acids necessary for the human body, and a variety of other vitamins, minerals, especially zinc and other rich content[4-6].

Amino acids are a kind of nutrients with special significance. They are essential nutrients for body growth and development, regulating metabolic function, enhancing disease resistance and strengthening aging, among which there are 8 essential amino acids. Amino acids also have a great use in medicine. Surgical infusion is added with various amino acids to increase nutrition and enhance the resistance of patients[7]. For hydrolysis of protein enzymes has many kinds of ants, ants on the market products also have a lot of, if the hydrolysis of ant protein enzymes can have better selection, then can improve the efficiency of ant protein hydrolysis, and if you can through more enzyme hydrolysis ant protein,

increase the ant protein hydrolysis, can save the amount of enzymes, get higher hydrolysis of ant protein products[8].

In recent years, some developed countries in the United States, Germany and other developed countries have gradually increased the research on the medicinal value of ants. Through experiments, it is proved that ants contain steroids, triterpenoids, similar adrenoids and a variety of alkaloids, and have developed drugs for the treatment of rheumatoid and other diseases[9]. China's Ministry of Health has officially designated ants as a new resource of health care products in 1993. At present, the development and utilization of ant medicinal resources has been from a single treatment of rheumatoid arthritis, rheumatoid arthritis, etc., to the treatment of hepatitis b, diabetes, asthma and other immune dysfunction disease and deficiency disease, and analgesia, anti-inflammatory, kidney aphrodisiac, stress, enhance resistance, and recently is used in cancer treatment.

2. Materials and Methods

2.1 Materials and Equipment

The material uses black ants (scientific name of black prickly ant) as the raw material, which is purchased in Bozhou Kangmei Traditional Chinese herbal medicine market. Reagent: (1) petroleum ether (30-60°C), copper sulfate, potassium sulfate and other reagents are pure analytical reagents and purchased from Sinopharm chemical reagent co., LTD.(2) Double uret reagent A liquid, double urea reagent liquid B, neutral protease, trypsin, papain, pepsin, purchased from Maclin Biological Co., LTD.(3) 17 amino acid hydrolysis standard solutions, 60% acetonitrile, 6-aminoquinolyl-N-hydroxysuccinyl carbentamate acetate buffer solution pH5.2, provided by Water. Main equipment: (1) low temperature high-speed refrigerated centrifuge H1850R, Shanghai Lu Xiangyi instrument; (2) SKD200 Kai fixed nitrogen meter, Shanghai Peo Instrument Co., Ltd.; (3) Sox extraction instrument GY-QZCDY, Shanghai Guiyong Electronics Co., LTD.; (4) Waters ACQUITY UPLC (H-Class), with fluorescent detector and diode array detector, Acc Q · TagTM Ultra amino acid analysis column, NEVAP nitrogen blower, MilliQ ultra-pure water device, are all instruments of Millipore company.

2.2 Determination Method

Moisture content determination: direct drying method. In the analysis of balance weighing a certain amount of ants, into the petri dish, net weight, record weight, into the blast dryer, 80°C dry for two hours, weighing, record weight, again into the blast drying box, weighing every half an hour to the ant weight, record the ants finally weighing weight, the difference is the water content, conversion percentage content. Repeated tests were averaged in triplicate.

Measurement of ash content: direct burning method. After weighing a certain amount of ants, heat the sample to be smokeless, and then place it in a maver furnace, burning at $550 \pm 25^\circ\text{C}$ for 4h. Cool to about 200°C , remove, put into the dryer to cool for 30min, repeatedly burn to constant weight, calculate the ash content.

Crude protein determination: K-nitrogen method. Prepare 30% sodium hydroxide solution, 2% boric acid solution, 0.01M standard hydrochloric acid, methyl red bromocresol green mixed indicator, ant dry to weighing weight, grind grinding, and screen through 60 mesh. Add 0.4g of potassium sulfate copper sulfate (10:1) to the bottom of the digestion tube, add 7 mL of concentrated sulfuric acid, digest on the digester, digest 420°C for 90min until the liquid is blue, green and transparent. Cool to room temperature, add 40 mL of distilled water, distillation on a nitrogen, gas is absorbed with boric acid, distillation is titrated with 0.01M standard hydrochloric acid solution, record consumption standard hydrochloric acid volume, the test was repeated three times, and do blank test. The crude protein content was calculated by converting the nitrogen content (N) to the protein coefficient.

Crude fat content determination: the Soxhl extraction method. Dry to 60 pieces of ant powder 1g, put into the Soxter extraction glass instrument, add 125 mL petroleum ether (30-60°C), 60°C in the Soxter extraction instrument for 6 h, weigh the weight of the extracted fat after drying, and convert the percentage content.

Amino acid composition and content detection: gas chromatography method. The derivative agent is used in the dryer, and the amino acid standard is configured into standard vertebral fluid. After the preparation, the amino acid standard solution is derivatized, the sample solution is derived, and the column gradient is

unwashed.

Fatty acid composition and content detection: gas chromatography-tandem mass spectrometry internal standard method. The samples were methyl-esterified, and the content of 13 fatty acid methyl esters was measured by gas chromatography-tandem mass spectrometry. Then, whether the food was abnormal according to the content of fatty acid methyl esters, and the abnormal samples were further checked and confirmed.

2.3 Ant proteolytic Analysis

Ant zymatic hydrolysis step: washed ant drying grinding sieve defat degrease ant enzymatic hydrolysis inactivated crude filtrate centrifugation

Make standard curve: accurately weigh 50mg of serum serum protein in the beaker, add 5 mL of distilled water to the beaker and stir to dissolve. 10 mg/mL bovine serum protein solution. Take 6 test tubes and wash them and dry, The 0mL, 0.2mL, 0.4mL, 0.6mL, 0.8mL, 1.0mL bovine serum protein solution was added with a pipette, Add the distilled water to 1 mL, That is, the concentrations obtained were 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL and 10 mg/mL, 4 mL of urereagent was added to each tube, When placed at room temperature for 30min, The absorbance of the visible photometer at 540nm, Control with the absorbance of 0mg / ml bovine serum protein solution at 540nm, With the protein content mg/mL as the abscissa, With the absorbance value as the ordinate, Make the standard concentration-absorbance curve, And find out the linear equation.

Determination of peptide content of enzyme solution: 5 mL of the filtered enzyme solution was taken, 5 mL of 10% trichloroacetic acid was added for a period of time, and then centrifuged at 6000 r/min at 20°C for 10min, 3 mL of the supernatant was centrifuged into the test tube, and borate alkaline was added

In 3 mL of buffer, 4 mL of biurea reagent was shaken and mixed for a period of time. When the color was stable, the absorbance was measured at 540nm, and the control bovine serum protein standard was made to obtain the peptide content of enzyme solution.

2.3.1 Single-enzyme digestion by a univariate test for optimal conditions.

This experiment with four kinds of protease (netropsin A, trypsin B, papain C, pepsin D)

compare enzyme hydrolysis, in each enzyme optimal conditions, after the determination of four enzyme solution peptide content, compare the size of four enzyme solution peptide content, select the four enzyme peptide content of single enzyme digestion single factor experiment. The three factors explored in this experiment include temperature, time, and pH. One factor test is one factor change, and the other conditions are fixed. By determining the value of the peptide content, explore the influence of the changed factors on the experiment. (1) Effect of temperature on enzyme digestion: pH was fixed at 8.2, substrate 0.5g, enzyme dosage 0.03g, enzyme digestion time was 2 h, temperature 40°C, 45°C, 50°C, 55°C and 60°C were selected for comparative experiments. (2) Effect of time on enzyme unwinding: pH was fixed at 8.2, substrate 0.5g, enzyme dosage 0.03g, temperature 55°C, 1 h, 2 h, 3 h, 4 h, 5 h for comparative experiments. (3) Effect of pH on enzyme digestion: the time was fixed at 2 h, substrate 0.5g, enzyme dosage 0.03g, temperature 55°C, pH 6.2, 7.2, 8.2, 9.2 and 10.2, respectively.

2.3.2 Orthogonal experiments with enzyme digestion conditions. Select the enzyme hydrolysis temperature a (a1, a2, a3), digestion time b (b1, b2, b3), pHc (c1, c2, c3) for orthogonal test. (The factors selected for orthogonal tests are one-factor tests, and the optimal value of each single factor and the two values of the optimal value are measured). The combination of single factors is a1b1c1, a1b2c2, a1b3c3, a2b1c2, a2b2c3, a2b3c1, a3b1c3, a3b2c1 and a3b3c2. The optimal conditions for single-enzyme digestion were obtained from the orthogonal test results.

2.4 Multienzyme Digestion Assay

2.4.1 duplex digestion. Four types of selected enzymes were netropsin, papain, trypsin and pepsin

Among them, neutropase, trypsin and papain are in the alkaline environment, Optimoptimal enzymatic conditions of pepsin is acidic environment, So when two enzymes with different digestion conditions, Resulting in both enzymes cannot be added simultaneously for enzymatic digestion, So this experiment takes an enzymatic solution first, The inactivation treatment after the enzymatic digestion under the same conditions, Adjust the pH value after inactivation, Add the same amount of another enzyme for the same conditions for the same time as before, They were tested for peptide content, Comparison of the enzyme digestion degrees was performed. In this experiment, four enzymes were numbered, namely netropsin A, trypsin B, papain C, and pepsin D, so the

combination of the two enzymes was AB, AC, AD, BC, BD, and CD. The conditions were set as 2 h, temperature 55°C, three alkaline enzymes pH8.2, pepsin pH5.2, 0.5g substrate, the total amount of enzyme used was 0.03 g, and the two enzymes were 0.015g respectively.

Enzymatic digestion process: (1) the two are alkaline enzymes, analytical balance weighing ant powder (60 order) 0.5 g add 20 mL distilled water to adjust pH 8.2 and add the two enzymes. (2) For enzymes with different properties of the two enzymes, the analytical balance weighed 0.5g of ant powder (60 orders) with 20 mL of distilled water regulating pH 8.2 with alkaline enzyme thermostatic enzyme activating pH 5.2 and pepsin for the same time.

2.4.2 Hydrolysis of the three enzymes

The enzymatic digestion of the three enzymes is the same as that of the two enzymes. The same enzymes are added at the same time, and the different proteases inactivate pH are added after regulation, so the combination of the three enzymes is ABC, ACD, BCD and ABD. The conditions were set at 2h, temperature 55°C, three alkaline enzymes pH 8.2, pepsin pH 5.2, 0.5 g substrate, the total enzyme dosage was 0.03 g, and the three enzymes were 0.01 g.

2.4.3 The enzyme digestion of the four enzymes

Four enzymes, three alkaline proteases were added at pH to 8.2, 55°C for 2h, the end of the enzyme activation, regulating pH 5.2 to add pepsin, 55°C for 2h, after the end of the enzyme activation, filtration. The enzyme solution was tested for the polypeptide content.

3. Results and Analysis

3.1 Basic Components of Black Ants in Changbai Mountain

3.1.1 Water content of ants

From Table 1, we conclude that the water content of black ants in Changbai Mountain is about 41.53%. According to the data, the water content in the general ants is about 30%, and the measured

value is higher than the conventional content. The possible reasons are that the object of this study is black ants in Changbai Mountain. The high air humidity in Changbai Mountain area leads to the high water content in ants. Second, because the selection of ants is not live ants, are dry ants, because the farmers have cleaned them, it will also lead to the high measured value, so it is a normal phenomenon.

Table 1. Content of Basic Components of Ants in Changbai Mountain

Ant	Shuifen	Ash Content	Protein	Fat
Content (%)	41.53±0.3	4.17±0.2	39.3±2	12±0.5

3.1.2 Ash content of ants

It can be seen from Table 1 that the ash content of black ants in Changbai Mountain is about 4.17%, which is not much different from the ash content of other ants, so the measured value is normal.

3.1.3 Crude protein content of ants

As can be seen from Table 1, the protein content of black ants in Changbai Mountain is as high as 39.3%. According to the study, the protein content of black ants is very high, accounting for about 42%~67% of their dry mass, much more than pork, chicken, eggs, fish, and almost the same as beef liver^[10], Belongs to high protein edible food, so if it can be effectively used, it is of great significance for food nutrition research.

3.1.4 Crude fat content of ants

According to Table 1, the fat content of the black ants in Changbai Mountain is about 12%, and the measured value is normal, which is of great help for the subsequent degreasing.

3.1.5 Ant Amino Acid Content

It can be seen from Table 2 that the black ants in Changbai Mountain contain 17 kinds of amino acids, which can meet the needs of the human body, and Yang Zhixin^[11] The study showed that the total amount of amino acids in black ants was 43.91%, and the essential amino acids accounted for 32.49% of the total amino acids, indicating that black ants are a high-quality protein source, with the highest content of glutamate, followed by alanine and glycine. If it is developed into a product for people to eat, it is of very high value.

Table 2. The Amino Acid Content of Ants in Changbai Mountain

Amino Acid	Content Mg/g	Amino Acid	Content Mg/g
Asp Asp	54.261	Val Val	46.628
α-Aminoglutaric Acid Glu	47.314	Methionine Met	6.225
Ser Ser	26.524	Cys Cys	0
Glycocol Gly	52.821	Ile Ile	27.839
Thr Thr	25.511	Leu Leu	35.126
Tryptophan Try	8.152	Phe Phe	15.306
Arginine Arg	28.312	His His	43.116
Proline Pro	48.466	Lysine Lys	21.235
Ala Ala	34.257	p-Hydroxyphenylalanine Tyr	34.762

3.1.6 Fatty acid content of ants in Changbai Mountain

According to Table 3, there are as many as 26 kinds of fatty acid compounds in Changbai Mountain ants, according to the quality of Shen Lirong^[12] With a supercritical CO₂ et al₂ (SFE-CO₂) When ant oil was extracted and the relative content of various components was determined by GC-MS, the unsaturated fatty acid content was 64.58%. Wei Guining et al^[13] The fatty acid components of black

ant ethanol extract was extracted with petroleum ether, and the results showed mainly unsaturated fatty acids, with the highest content of methyl hexadecarene (60.77%), methyl hexadecane (18.99%) and methyl hexadecarene (9.31%). And Siharnala O et al^[14] It was found that 23%~24%, monounsaturated fatty acids 68.1%~77.0%, and polyunsaturated fatty acids 3.7%. It can be seen that black ant fat oil is mainly monounsaturated fatty acids, with a content of about 70%.

Table 3 Fatty acid detection results of ants in Changbai Mountain

Chemical Compound	Content Mg/g	Chemical Compound	Content Mg/g
Methyl Butyrate	0.12	Xvii Methyl Carbonate	0.62
Methyl Caproate	0.29	Cis-10-Heptadecapenoterenylate	0.26
Methyl Caprylate	0.41	18 Methyl Carbonate	7.25
Methyl Sulphate	0.37	Inverse-9-Octadecacenoenylate	0.5244
Eleven Methyl Carbonate	1.46	Cis-9-Octadecacenoacrylate	0.8624
Methyl Decyl Carbonate	0.34	Reverse, Anti-9,12-Octadecadienolate	2.37
13 Methyl Carbonate	2.26	Cis, Cis-9,12-Octadecadienolate	1.64
Xiv Methyl Carbonate	0.07	20 Methyl Carbonate	0.44
Cis-9-Tedecapenoprenylate	0.11	Methyl Eicosarene	1.61
Xx Methyl Carbonate	1.46	Twenty-One Methyl Carbonate	0.14
Cis-10-Hexacacapinoalkenylate	0.29	Methyl Docodioenyl Acid	5.14
Xvi Methyl Carbonate	11.86	x3 Methyl Carbonate	0.11
Cis-9-Hexadosanoacrylate	1.24	x4 Methyl Carbonate	0.52

3.2 Analysis of Ant Enzyme Digestion Results

3.2.1 Standard curve drawing

Table 4. Bovine Serum Protein Absorbance

Potency Mg/MI	0	2	4	6	8	10
Absorbance At 540nm	0.099	0.184	0.268	0.354	0.392	0.360

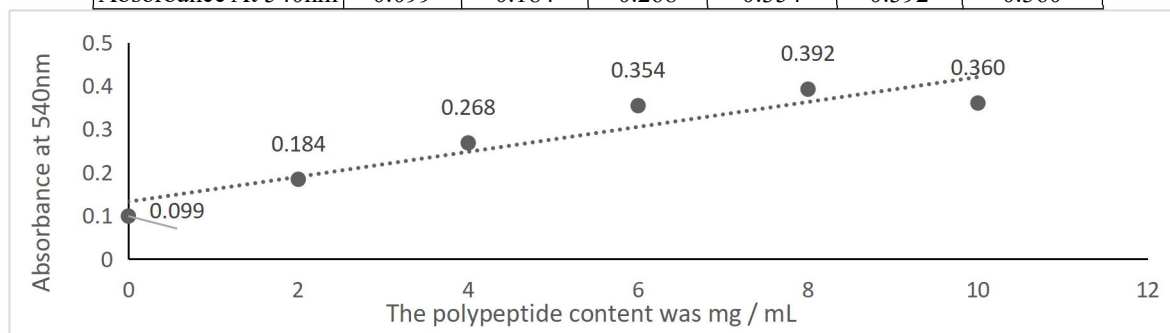


Figure 1. Standard Curve of Bovine Serum Protein

3.2.2 Comparison results of enzyme preparations
As can be seen from Table 5, after the hydropeptide content of the four enzymes, the highest and the lowest trypsin is trypsin, so neutral proteinase is selected as the hydrolase to explore the single enzyme hydrolysis factors, which is conducive to the exploration of the best conditions for enzymatic hydrolysis.

3.3.1 Effect of the enzyme digestion temperature on the enzyme digestion degree

It is known from Figure 2 that in the range of 40-60°C, the content of the enzyme solution polypeptide gradually increased, reaching the maximum value of 4.44 mg/mL at 55°C, and then began to decrease gradually after 55°C. So in the range of 40 – 60°C, the optimal digestion temperature is 55°C.

3.3 Analysis of enzymatic digestion

Table 5. Absorbance of the Different Enzymes

Enzyme Species	Neutral Proteinase	Papain	Trypsase	Pepsase
Absorbance At 540nm	0.749	0.735	0.670	0.721
The Polypeptide Content Of Mg/MI	21.41	20.9	18.67	20.44

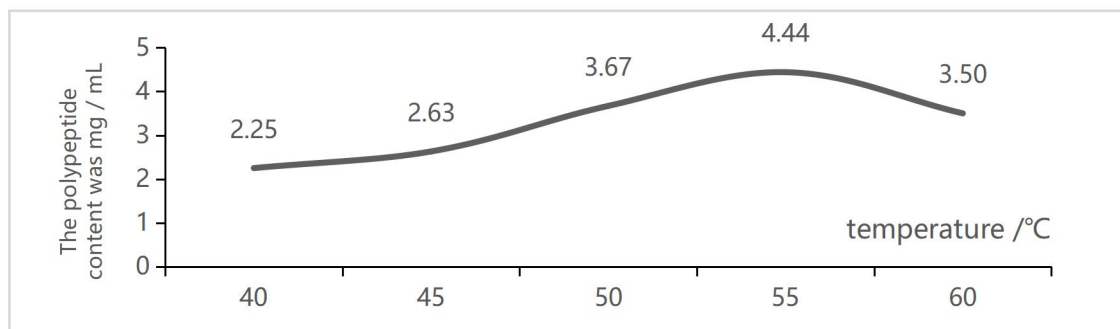


Figure 2. Effect of the Temperature on the Enzymatic Hydrolysis

3.3.2 Effect of the enzyme digestion time on the enzyme digestion degree

Figure 3 shows that with the same other conditions, the polypeptide content in the enzyme solution in the test tube increased gradually over

time, reaching the maximum value of 5.13 mg / mL at 4 h, and then gradually decreased later. At 1 to 3 hours, the increase was more obvious, and after 4 hours, the decrease was not too large. So in the range of 1 – 5 h, the optimal time for enzymatic digestion was 4 h.

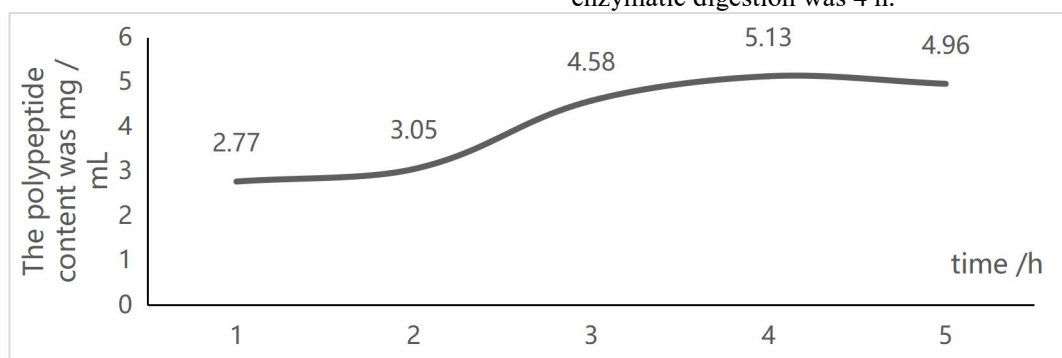


Figure 3. Effect of Time on Enzymatic Hydrolysis

3.3.3 Effect of pH digestion on enzyme digestion

As is known from Figure 4, when other conditions remain unchanged, in the range of pH 6.2 to 9.2, the enzyme polypeptide content increases as PH

increases, reaching a maximum at 8.2, and then starting to decline again. Around pH 8.2, the increase and decrease in the peptide content of the enzyme solution is very significant, so in the range of pH 6.2-9.2, at pH 8.2.

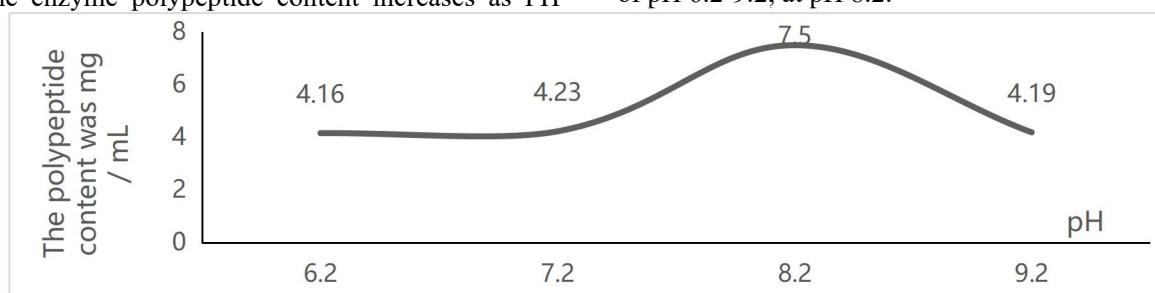


Figure 4. Effect of pH on Enzymatic Hydrolysis

3.3.4 Results of the one-factor orthogonal experiments

According to the basis of one factor experiment, orthogonal test design is used to arrange the test, the conditions of enzymatic hydrolysis are

optimized, and the experimental results are analyzed as shown in Table 6. Table 6 shows that the enzyme digestion reached the maximum at 50°C, pH 8.2 and 4h, so experiment 2 was the best condition for the orthogonal test.

Table 6. Orthogonal Design of Ant Proteins and Results

Test Number	a Temperature /°C	b Time /h	c p h	The Polypeptide Content Of Mg/MI
1	1 (50)	1 (3)	1 (7.2)	5.73
2	1	2 (4)	2 (8.2)	8.61
3	1	3 (5)	3 (9.2)	6.24
4	2 (55)	1	2	7.35

5	2	2	3	5.24
6	2	3	1	4.26
7	3 (60)	1	3	2.13
8	3	2	1	3.45
9	3	3	2	4.61

3.4 Analysis of the multienzyme digestion results

3.4.1 duplex digestion

Figure 5 shows that the combination of trypsin and trypsin is the highest, and the combination of trypsin and xyliglobynase is the lowest.

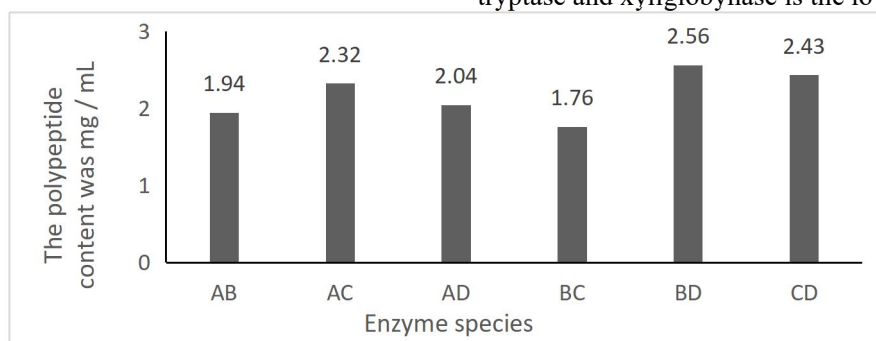


Figure 5. Polypeptide Content of Duplex Enzymes

3.4.2 Hydrolysis of the three enzymes. From figure 6, three enzyme digestion, including neupsin, trypsin and papain, and trypsin, papain and pepsin combination without much change, the

reason may be related to the optimal pH of enzyme, including netropsin, papain and trypsin are all alkaline protease, and pepsin is acid protease. The difference for this reason.

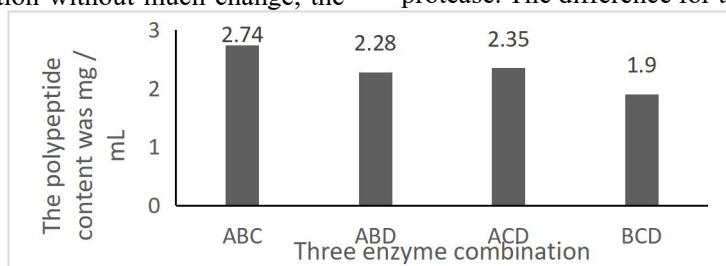


Figure 6. Polypeptide Content of Trienzymes

3.4.3 The enzyme digestion of the four enzymes According to the experimental results, the four enzymes at the same time were 3.12mg / ml.

3.4.4 Comprehensive comparison of different species of enzymes

From Table Table 7, for the comparison of the combination of multiple enzymes, the degree of the solution of the combination of three enzymes and the degree of the combination of three enzymes. Due to the different characteristics of each enzyme, the role of fragment is different, so the combination of double enzyme and three

enzymes, the enzyme solution peptide content difference, four enzyme combination, because for this experiment to explore the four enzymes are added, so the enzyme solution contains the peptide should be the highest in theory, and the results of the actual test, is verified the theory is correct. However, the polypeptide content of the combined enzyme solution of the three enzymes is lower than that of the combined enzyme solution because of the different enzymes, or because the enzyme is not completely inactivated in the experiment, which may result in the difference of experimental results.

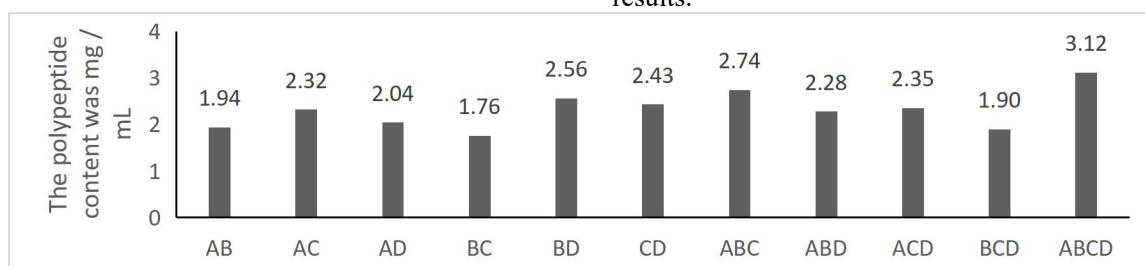


Figure 7. Polypeptide Content of Different Enzyme Combinations

For the comparison of the double enzyme results and the three enzyme results, according to the data in Table 7, the peptides in the combined pepsin and trypsin was 1.94 mg/mL, while the content of pepsin and papain was 2.28 mg/mL. In the combination, the first two did not change, and the content of the polypeptide increased after the addition of the third enzyme. Similarly, the peptide content of the combination of neutral protease and papain is 2.32 mg/mL, and the peptide content of trypsin or pepsin and pepsin will increase, indicating that increasing the type of enzyme can improve the enzymatic hydrolysis of ant protein. But the data in Figures 7 also suggest that not all enzyme combinations increase hydrolysis. If trypsin and pepsin contain 2.56 mg/mL, Whereas in the case of both the unchanged enzyme species, Increased as the enzymatic digestion of the three enzymes, When the third type is a neutral protease, The polypeptide content of the enzyme solution was 2.28 mg/mL, When the third species is a papain, The polypeptide content of the enzyme solution was 1.90 mg/mL, Are lower than the content of duplex hydropeptide, For this result, Possibly because it is the different amount of enzyme added, On duplex digestion, The amount of both enzymes is half each, The amount of each enzyme is one-third. Because of the different activities of the different enzymes. However, most of the results are that the three enzymes is higher than the two enzymes, and the digestion of the four enzymes is higher than all of the three enzymes. So it turns out that the addition of multiple enzymes does improve the digestion of ant protease.

4. Conclusion

From the analysis of the experimental results, it can be seen that the water content of the black ants in Changbai Mountain is relatively high at about 40%. Because the water content used in this experiment is not live black ants in Changbai Mountain, but dry ants, farmers will clean them, resulting in high water content, which is also a normal phenomenon. The fat content of ants is about 12%. Because the defat conditions of black ants are not explored, the temperature, time and ant particle ularity can affect defat, resulting in incomplete defat, and there may be deviations in the determination of crude fat. The protein content of black ants in Changbai Mountain is about 42%, which contains relatively high protein content and is higher than many edible products. If they can be effectively used and made into protein products for human consumption, black ants will become a resource for key development. For the species and content of amino acids in the black ants of

Changbai Mountain, its body contains a variety of amino acids, and the content is not low, and there are 8 kinds of amino acids that can meet the daily needs of human beings, if used is also very important.

For the experiments of ant proteins, the four proteases were compared, where the degree of netropsin was the highest, and the lowest for trypsin. Then enzyme factors exploration experiment, this paper to explore the three single factors, temperature, time and pH, respectively to explore the optimal value of each single factor, and then through orthogonal test to explore the best combination of enzymatic conditions, because this paper to explore single factors is not much, so explore the best condition is relatively, there are a lot of single factors to explore, such as enzyme concentration and the ratio of enzyme and substrate. So the experiment has yet to be optimized.

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References

- [1] Sun Xiaoling, Ren Bingzhong. Ant resources and their research progress in Northeast China [J]. Songliao Academic Journal (Nature Science edition), 2001 (03): 20-23.
- [2] Zhao Dajun, Yu Xiaolei. Research on the processing techniques of ant functional food [J]. Food Industry Technology, 2008 (09): 279-282.
- [3] Cao Ping, Guo Qiaosheng. Study on the anti-inflammatory and analgesic effects of ant oral liquid [J]. Jiangsu Pharmacy and Clinical Research, 1996 (03): 9-11.
- [4] Yin Yong, Liu Jinbao, Liu Xiaoyu. Determination and analysis of amino acids and vitamins in Xinjiang brown forest ants [J]. Journal of Xinjiang Medical University, 2007 (03): 241-242,245.
- [5] Liu Jinbao, Yin Yong, Chen Zhaohui, et al. Determination of major vitamins and mineral elements in Xinjiang brown forest ants [J]. Journal of Xinjiang Medical University, 2007 (07): 695-696.
- [6] Liu Jinbao, Yin Yong, Wu Wei, et al. Determination and analysis of the main nutrient components of brown forest ants in Xinjiang [J]. Tissue Engineering Research

- and Clinical Recovery in China, 2007 (16): 3162-3163.
- [7] Du Fangling. Technological research and taste preparation of oral solution [J]. Shandong Agricultural Science, 2008 (09): 94-95.
- [8] Guo Cheng, Zhang Wenbo. The development and utilization of medicinal ants in China [J]. Journal of Chifeng University (Natural Science Edition), 2011,27 (07): 22-24.
- [9] Bo Shiru, Wu Yalin, Wang Quankai. Status and research progress of ant product development and utilization [J]. Jilin Agriculture, 2017 (17): 73.
- [10] Zhao Dacheng, Qin Xuemei, Shen Guohua, et al. Leucine in ant medicine and its rheumatic wine by thin layer scanning [J]. Pharmacy in China, 2004 (02): 47-48.
- [11] Yang Zhixin, Liu Hui, Xu Guijun, et al. Progress in the chemical composition, pharmacology and clinical application of black ants [J]. Pharmacy in China, 2020,31 (09): 1148-1152.
- [12] Shen Lirong, Ren Yucui. Progress in the development and utilization of edible and medicinal ants in China [J]. Knowledge of Insects, 1999 (04): 251-253.
- [13] Wei Guining, Su Qibiao, He Fei, et al. Screening and chemical composition analysis of the active site of reducing serum uric acid level in ethanol extract of Pant [J]. Chinese Journal of Pharmacology and Toxicology, 2013,27 (04): 67677.
- [14] Siharnala O, Bhulaidok S, Shen LR, et al. Lipid and fatty acid composition of dry-eating red ants and black ants [J]. Agricultural Science of China, 2010,9 (07): 1072-1077.