Advances in Biological Sciences Research (ABSR), volume 6
2017 2nd International Conference on Biological Sciences and Technology (BST 2017)

The Antioxidant Activity of Royal Jelly Water Soluble Proteins Hydrolysate from Xinjiang Black Bee

Jian-hui JIANG[1,2, a], Jian-bo ZHAO[1,2], Hui-ping DING[1,2], Wen-bo XIN[1,2] and Long CHEN[3,*]

[1]Engineering Laboratory of Chemical Resources Utilization in South Xinjiang of Xinjiang Production and Construction Corps, College of Life Sciences, Tarim University, Alar, Xinjiang 843300, China

[2]College of Life Sciences, Tarim University, Alar 843300, China

[3]College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China

[a]xjjjh78@163.com

*Corresponding author

Keywords: Response surface, Polysaccharide, Extract.

Abstract. In this paper, pepsin, trypsin and chymotrypsin were used to hydrolyze royal jelly water soluble proteins from Xinjiang Black Bee, and peptides with the molecular weight of more than 10kD, 3-10kD as well as less than 3 kD were obtained by using ultrafiltration. Through the determination of total antioxidant capability, 1, 1-Diphenyl-2-picrylhydrazyl radical scavening ability and inhibitory ability against oxidation of linoleic acid, the antioxidant activities of water soluble proteins in royal jelly and their hydrolysates were surveyed. The results indicated that all hydrolysates have antioxidant properties and hydrolysates hydrolyzed by pepsin and trypsin both showed the highest antioxidant activity. Besides, hydrolysates hydrolyzed by trypsin showed highest antioxidant activity than those by pepsin. We also found that peptides with the MW less than 3 kD showed the strongest antioxidant activity, then 3-10 kD peptides, and finally more than 10 kD peptides showed the lowest antioxidant activity.

Introduction

Xinjiang Black Bee belonging to the same strain with Apis mellifera, also known as Ili black bee, is one of the four major bee species in the world. The Black Bee mainly distributed in Xinjiang Ili, Tacheng, Altay, Xinyuan, Turks, Nileke, Zhaosu, Gongliu, Yining, Burqin and other places. The main food for Black Bee is pollution-free flowers growing at an altitude of 1800 to 2500 meters [1].

Royal jelly, also known as bee milk, is young worker bees' secretions of mandibular gland and hypopharyngeal gland, which is white or milky yellow viscous material, with a special smell, and used for feeding queen bee and larvae [2]. Fresh royal jelly contains a lot of active ingredients. What's more, the results showed that fresh royal jelly has the biological function of natural protein such as antioxidant, antibacterial, regulating immunity, lowering blood sugar, prevention cancer and anti-cancer, relieving fatigue, lowering blood pressure, protecting nerve cells, promoting cell proliferation [3-10]. This is widely used in the field of pure natural food production.

Studies shows that fresh royal jelly is complex mixture, which contains 60-70% of water, 10-16% of sugar, protein 12-15%, lipid 3-6%, vitamins, free amino acids, minerals and other 2-3%.

The variety and quantity of proteins, sugars and other major components obtained from royal jelly is influenced by many factors, such as types of bees, honey sources, production season, climate differences, growth and development of bees and so on [11-13].

Royal jelly has a wide variety of proteins and extremely rich in content, so the anti-oxidation effect is not the same.

In this experiment, the royal jelly water-soluble protein of the Black Bee from Xinjiang was used as the raw materials, and the enzyme products which were hydrolyzed by pepsin, trypsin and chymotrypsin were subjected to ultrafiltration with different specifications of the ultrafiltration tube. The enzyme products according to peptides molecular weight were divided into more than 10 kD, 3-10 kD and less than 3 kD, respectively. To study the antioxidant activity of the royal jelly hydrolyzates of Xinjiang Black Bee, we then measured the ability of removal of DPPH• and inhibition of linoleic acid oxidation and total antioxidant capacity.

Materials and Methods

Material and Instruments

Royal jelly from Xinjiang Black Bee (Yili Hong County apiculture limited liability company); Diphenyl-2-bitter radical radicals (DPPH· free radicals),bovine serum albumin, trypsin, pepsin, chymotrypsin, Coomassie brilliant blue G-250 Sigma Company; linoleic acid, Total Antioxidant Capability Assay Kit (Beijing Suolai Bao Technology limited Company); Phosphate buffer, ferricyanide, ferric trichloride, ammonium thiocyanate, anhydrous ferric chloride (Shandong Xiya limited Company); petroleum ether, 85% phosphoric acid, sodium chloride, formaldehyde, 95% ethanol, sodium hydroxide (Tianjin Zhiyuan Chemical Reagent Limited Company); distilled water.

Ultrafiltration tube; 14 K dialysis membrane; FD-1A-50 vacuum freeze dryer (Hangzhou Jutong electronic limited Company);HJ8 collector type constant temperature magnetic stirrer (Guangzhou Hu Ruiming Instrument limited Company);FA1004B electronic balance (Zhengzhou Bao Jing Electronic Technology limited Company); TD5Z high speed refrigerated centrifuge (Shanghai Zhao Di Biology limited Company); HH.S21-4 digital thermostatic water bath (Xingtai Runlian Machinery Equipment limited Company); SHA-B thermostat oscillator (Jintan City, Jiangsu Province, the Institute of Instrument); D-9143B-1 electric thermostatic blast oven (Instrument of Huier); TGL-16G high-speed desktop centrifuge (Beijing Pingjian Laboratory Equipment limited Company); KQ-100B ultrasonic cleaner (Shanghai Ultrasonic Instrument Limited Company); UV2400 spectrophotometer (Shunyu Hengping); pHS-3C precision pH adjuster (Shanghai Hongyi Instrument Factory Limited Company).

Methods

Preparation of Water Soluble Proteins (WSPs) of Royal Jelly. According to Osboren classification, we took fresh Xinjiang Black Bee royal jelly freezed to dry and got the powder. The dry powder was stirred in petroleum ether at 4℃ for 2 h, and filtered to get the solid. The process was repeated three times to obtain the dry powder. Take the appropriate amount of royal jelly which was degreased in distied water, and repeated this extraction three times, and then merge the extracts to get the total water-soluble proteins. The total water-soluble proteins were dialyzed with 14 K dialysis membrane, and were freezed to dry to obtain the main protein of royal jelly.

Prepare Hydrolyzate of Water Soluble Proteins and Different Molecular Weight Peptides in Royal Jelly. Weighing 2 g of Black Bee royal jelly water soluble proteins dissolved in 60 mL of ultra-pure water was divided into four copies, which were enzymed with pepsin, trypsin, chymotrypsin, pepsin and chymotrypsin in the optimum conditions [14-15]. At the end of enzyming, placing the four parts of the hydrolyzates above in a constant temperature water bath boiled for 15 minutes inactivating the enzymes. The inactivated hydrolyzates were obtained by centrifugation at 12,000 rpm for 10 min at 4℃, and the hydrolyzates in turn were named pepsin water soluble proteins (P-WSPs), trypsin water soluble proteins (T-WSPs), chymotrypsin water soluble proteins (C- WSPs), pepsin and chymotrypsin water soluble proteins (P-C-WSPs). The hydrolyzates were separated by ultrafiltration with 10 kD and 3 kD ultrafiltration tubes to obtain the hydrolyzates of less than 3 kD, 3-10 kD, more than 10 kD peptides.

Determination of Antioxidant Activity of Water Soluble Proteins. Refer to the method of Singh et al [16]. Remove 5 mL of 0.05 mg/mL sample solution in 25 mL scale tube, add 0.2 mmo/L DPPH·solution 2 mL, methanol volume, shake, at room temperature for 40 minutes. The absorbance A_1 was measured at a wavelength of 517 nm, and at the same time the absorbance A_0 at the initial concentration of DPPH was measured. The DPPH radical scavenging rate of the sample was calculated according to the following formula.

$$\text{DPPH radical scavenging rate } /\% = (1-A_1 / A_0) \times 100$$

Take the preparation of 0.1 mg/mL of the Black Bee royal jelly water-soluble protein components to be tested and the control group 1 mL, add 1 mL of linoleic acid anhydrous ethanol solution at a concentration of 2.50% (v / v), then add 2 mL of 0.05 mol/L phosphate buffer solution (pH = 7.0) and 1 mL of anhydrous ethanol (for the test sample) or deionized water (for control), respectively. Seal and place in the dark at 37 ℃ constantly. Blank group without antioxidant and the other steps the same as above [17].

Take 5 mL of the above mixture, add 5.00 mL of 75% ethanol solution and 0.5 mL of 30% NH_4SCN, then add 0.5 mL 0.02 mol/L $FeCl_2$ dissolved in 3.5% hydrochloric acid, measure its absorbance at 500 nm after accurate reaction for 3 min. Futhermore, it was measured every 24 hours.

Prepare 0.1 mg/mL Black Bee royal jelly water- soluble protein samples of each component follow the instructions according to the T-AOC kit, measure absorbance at 520 nm. The test was repeated 3 times and averaged. In this experiment, the total antioxidant capacity was reflected according to the difference of the sample tube and the control tube at 520 nm (ΔA520), besides, the more the difference, the greater the total antioxidant capacity [18].

Results and Analysis

DPPH • Free Radical Scavenging by Samples

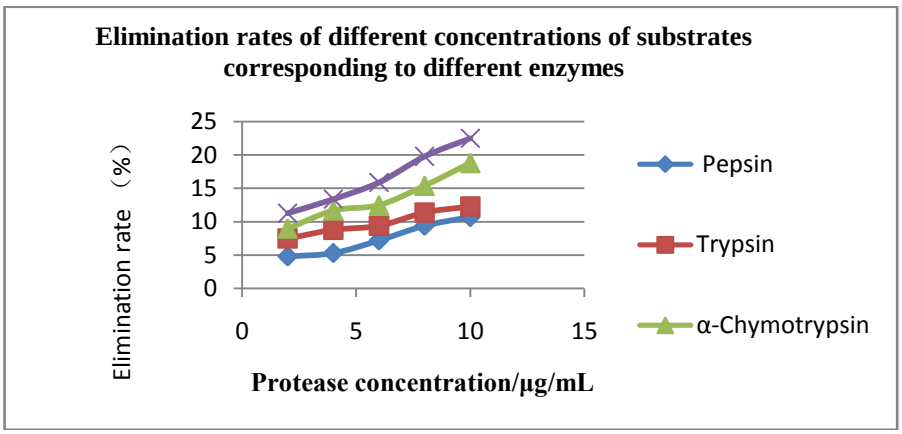

Figure 1. The substrate elimination rate of the different enzymes correspond to different concentrations (%)

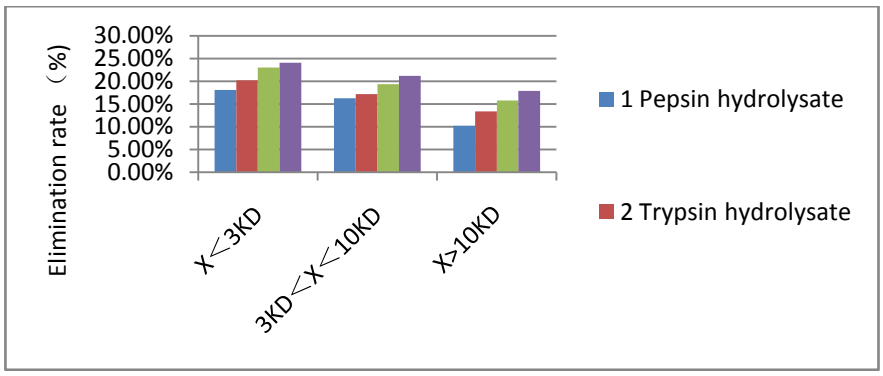

Figure 2. The components of royal jelly correspond to the elimination rates of different enzymatic hydrolysis (%)

Figure1: All components of the Black Bee royal jelly in Xinjiang have some antioxidant effect, and the antioxidant capacity of different enzymes is different. The ability of each hydrolyzate to DPPH· cleavage increases with the increase of protein concentration. Among them, the most effcient hydrolyzate is enzymed by pepsin and chymotrypsin, followed by the chymotrypsin, and then trypsin, and the weakest DPPH scavenging activity is pepsin hydrolyzate. The order of removal ability of DPPH ·was: pepsin and chymotrypsin hydrolyzate>chymotrypsin enzyme> trypsin hydrolyzate> pepsin hydrolyzate.

Figure2: The DPPH · free radicals scavenging ability of each ultrafiltration component of water soluble proteins of royal jelly decreased with the increase of molecular weight of royal jelly protein. Among the three peptide fragments isolated from water-soluble protein hydrolyzate, the efficiency is less than 3 kD, followed by 3-10 kD, the weakest is more than 10kD when take the DPPH ·radical scavenging ability into consideration. The order of DPPH ·free radical scavenging ability of the ultrafiltration components of the royal jelly peptides was as follows: less than 3kD>3-10kD>more than10 kD. Comprehensively, after digestion with chymotrypsin and trypsin, the peptide of the water-soluble protein of the Black Bee royal jelly with molecular weight less than 3 kD was better for DPPH ·radical scavenging ability.

Hydrolysis of Royal Jelly Water Soluble Proteins to Inhibit the Oxidation of Linoleic Acid

Table 1. Changes in absorbance at 500 [nm] of absolute ethanol solution of linoleic acid-NH_4SCN-$FeCl_2$ added with various WSPs hydrolysates with time

Day Sample	1	2	3	4	5
P-**WSPs**>10kD	0.074	0.1	0.123	0.151	0.143
P-**WSPs**3-10kD	0.055	0.081	0.104	0.152	0.131
P-**WSPs**<3kD	0.052	0.064	0.096	0.132	0.112
T-**WSPs**>10kD	0.064	0.099	0.122	0.145	0.137
T-**WSPs**3-10kD	0.043	0.078	0.104	0.137	0.125
T-**WSPs**<3kD	0.035	0.067	0.099	0.125	0.124
C-**WSPs**>10kD	0.055	0.076	0.089	0.112	0.108
C-**WSPs**3-10kD	0.023	0.067	0.056	0.098	0.103
C-**WSPs**<3kD	0.024	0.038	0.087	0.084	0.075
P-T-**WSPs**>10kD	0.023	0.061	0.102	0.095	0.088
P-T-**WSPs** 3-10kD	0.015	0.054	0.063	0.125	0.094
P-T-**WSPs**<3kD	0.013	0.036	0.059	0.093	0.087

It can be seen from Table 1, the same concentration of the Black Bee royal jelly water-soluble protein components of the enzymatic products of linoleic acid oxidation have a certain inhibitory effect, the royal jelly water-soluble protein enzymed by pepsin and trypsin on the inhibition of linoleic acid is the strongest, followed by the chymotrypsin, and then trypsin, the lowest was enzymed by pepsin. The order of antioxidation of linolenic acid was as follows: pepsin and chymotrypsin hydrolyzate>chymotrypsin hydrolyzate> trypsin hydrolyzate> pepsin hydrolyzate, which was the same as free radical scavenging of DPPH·. Judging from the experimental data, the inhibitory effects of different peptides on the oxidation of linoleic acid were different, and the smaller the molecular weight, the more obvious the inhibitory effect. Overall, the order of the ability of different peptides of Black Bee royal jelly water soluble proteins inhibit linoleic acid oxidation is: molecular weight less than 3 kD> 3-10 kD> more than 10 kD.

Determination of Total Antioxidant Capacity of Enzyming Ultrafiltration Products of Royal Jelly Water-solube Protein

We use the antioxidants which can make Fe^{3+} reduced to Fe^{2+} to determine the total antioxidant capacity, and Fe^{2+} can form stable complexes with phenanthrene. We will know the total antioxidant capacity by the method of colorimetric. Besides, the greater the difference in absorbance between the determine tube and the control tube, the stronger the antioxidant capacity. The total antioxidant capacity of the royal jelly extracted at the same concentration was investigated and the results are shown in figure three.

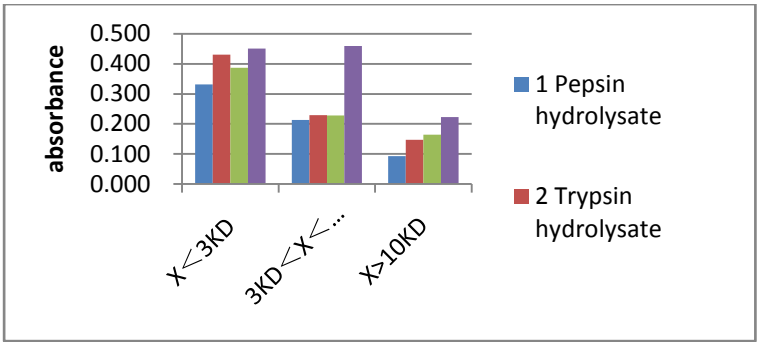

Figure 3. The total antioxidant capacity of different components in royal jelly

Figure 3: At the same concentration, the order of the total antioxidant capacity of the different components was: pepsin and chymotrypsin hydrolyzate>chymotrypsin hydrolyzate> trypsin hydrolyzate> pepsin hydrolyzate. The weakest antioxidant capacity is the peptide> 10 kD from the hydrolyzate of royal jelly water soluble proteins, the strongest is the peptide<3 kD.

Conclusion

The results showed that the free radical scavenging ability overall strength was: pepsin and chymotrypsin hydrolyzate>chymotrypsin hydrolyzate>trypsin hydrolyzate> pepsin hydrolyzate. The hydrolyzate of the water-soluble protein of the Black Bee royal jelly that is separated by the membrane of the ultrafiltration tube becomes different peptides of hydrolysis, among them, the strongest antioxidant activity of the hydrolyzate is less than 3 kD peptide, followed by 3-10 kD peptide hydrolyzate, the lowest antioxidant activity is the hydrolysis products of that more than 10 kD peptide. The effect of hydrolysis of royal jelly water-soluble protein at the same concentration to inhibit the oxidation of linoleic acid and DPPH·radical scavenging is basically the same. We found that the total antioxidation of small molecule peptides was much better in the analysis of total antioxidation. So, we can infer that the smaller the peptides of the Black Bee water-soluble protein, the better the antioxidant capacity.

Summary

The results indicated that all hydrolysates of royal jelly have antioxidant properties and hydrolysates hydrolyzed by pepsin and trypsin both showed the highest antioxidant activity. Besides, hydrolysates hydrolyzed by trypsin showed higher antioxidant activity than those by pepsin. We found that peptides with the MW less than 3 kD showed the strongest antioxidant activity, then 3-10 kD peptides, and finally more than 10 kD peptides showed the least antioxidant activity.

Acknowledgement

Acknowledgments: This research was financially supported by the National Science Foundation of China (No. 21562035).

References

[1] P.K. Xia, J. Journal of bee. 1986, (04):17-18. In Chinese

[2] G.Z. Fang, Z. Guo, J.S. Fang, et al., J. China Daily Industry,1994(6): 2-4. In Chinese

[3] J. Majtan, E. Kovacova, K. Bilikova, et al., J. International Immunopharmacology, 2006, 6(2): 269-278

[4] S. Eshraghil, F. Seifollahi, J. Iranian Journal of PublicHealth, 2003, 32(1): 25-30.

[5] C. Bincoletto, S. Eberlin, C. Figueiredo, et al., J. International Immunopharmacology, 2005(5): 679-688

[6] M. Toshiro, Y. Akiko, D. Shima, et al., J. Journal of the Japanese Society for Food Science and Technology, 2006,53(4): 200-206

[7] M. Nomura, N. Maruo, Y. Zamami, et al., J. Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan, 2007, 127(11): 1877-1882

[8] S. Olivola, P. Gozalesv., J. Toxicol in Vitro, 2005, 19: 645-651

[9] L. Lu, Z.B. Lin., J. Herald of Medicine, 2004, 23(12): 887-890. In Chinese

[10] W.J. Ji, F.L. Hu, J. Journal of Bee, 2009(9): 8-11. In Chinese

[11] Y.L. Chen, J. Zhang., J. Food Research and Development, 2015,36(2):148-152. In Chinese

[12] C. Chen, S.Y. Chen. J. Food Chem, 1995, 54:195-200.

[13] Z.W. Zhang, B. Shan, W.J. Peng, et al., J. Food Research and Development, 2007, 28(10):32-36. In Chinese

[14] Y.W. Li., Study on preparation of royal jelly protein hydrolysates and its antioxidant activities [D]. Fujian Agriculture and Forestry University, 2012. In Chinese

[15] S. Cheng, S.H. Yin, J.L. Liu, et al., J. Journal of Yunnan Agricultural University (Natural Science),2016, (02):288-293. In Chinese

[16] N. Singh, P.S. Rajini. J., Food Chemistry, 2004, 85: 611-616.

[17] J.X. XU, Z.W. Zhang, Y.D. Liu, et al., J. Food Science, 2009, 30(05):72-75. In Chinese

[18] B. Chen, D.P. Xu., J. Food and Fermentation Indurtries, 2012, (07):124-127. In Chinese