

Preparation and Functional Analysis of a New Cosmetics Raw Materials with Sika Deer Skin Peptide and Angelica Dahurica

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Abstract Based on the traditional medicine cosmetic formula, sika deer skin peptides (SDSP) and the effective ingredients extracted from *Angelica dahurica* were added for preparation of a new cosmetic raw material. In this study, the composite protease group was selected as the tool enzyme by single factor orthogonal test and response surface method for hydrolyzing the total protein of deer skin. The optimal enzymatic hydrolysis process of deer skin protein was as follows: temperature at 51°C, pH 7.0, 6000 U/g of enzyme addition and reaction time in 5h and SDSP was obtained. The molecular weight of SDSP was 851 Da to 11 092 Da by exclusion chromatography analysis. The analysis of amino acid composition showed that SDSP were rich in Gly, Ala, Pro, Hyp and terpeneine. Under the same extraction conditions, ethanol extract content from *Angelica dahurica* showed a highest level, 8 kinds of furocoumarines compounds were identified in it by HPLC-ESI-MS analysis. The results also demonstrated that mixture of *Angelica dahurica* extraction and SDSP with the rate of 2:1 indicated the best moisturizing effect. Free radical scavenging ability of facial base liquids reached 81% in 2.0 mg/mL. The inhibition rates of tyrosinase reached 61.12% at 8 mg/mL. This new cosmetics raw materials provided a basic process for producing cosmetic raw material with remarkable whitening effect and excellent moisturizing performance.

Keywords: *Cervus nippon*, commercial facial materials, *Angelica dahurica*, HPLC-ESI-MS

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1. Introduction

Sika deer (*Cervus nippon*) [1] was an economic animal with high medicinal value and health care function which inhabited in northeast Jilin province of China for a long history of breeding industry. However, few report discussed medicinal value of sika deer skin [2], the mainly used of sika deer skin was focused on high-grade leather raw materials. In recent years, the extraction and activity of animals skin peptides [3] had been widely studied, which had the functions of promoting cell proliferation, immune regulation, anti-oxidation, anti-fatigue and showed widely application prospect in food, cosmetics, medicine and other industries.

Over the past thirty years, people's skin quality declined with the reason of life and work stress, the demand for whitening and moisturizing [4,5] commercial facial materials [6] was increasing. The traditional skin lighteners [7] such as hydroquinol induced genic mutation of cells and even caused sensitization [8] by skin contact.

For improving skin conditions, traditional Chinese medicine facial material [9] was a kind of beauty cosmetic products which integrated cleaning and maintenance with the advantages of remarkable efficacy [10], mild effect, small side effects and low toxicity [11]. *Angelica dahurica* [12] which was a common Chinese herbal medicine had the ability to eliminate excessive pigment accumulation in tissues and promote the metabolism of skin cells, the previous research suggested that the main components of *Angelica dahurica* were coumarins [13], which were the main active substance for whitening function, inhibiting photosensitive toxicity [14,15] and tyrosinase activity [16].

The following article provided a production process of cosmetic raw material by adding *Angelica dahurica* extraction [17] and sika deer skin peptides (SDSP) based on traditional commercial facial materials ingredients [18,19]. The composition of amino acids and relative molecular mass are analyzed of SDSP at the same time. The extraction process of *Angelica dahurica* was optimized by HPLC-ESI-MS and the moisturizing ability in vitro [20], antioxidant ability [21] and whitening ability [22] of cosmetic raw material [23] were tested.

2. Materials and Method

2.1. Material

The sika deer skin used in this was obtained from Menshi Deer Industry of Jilin Province in China and stored at -20°C. *Angelica dahurica* was provided by The Great Jilin Medicine Store. The reagents xanthan, Methyl Paraben, betaine, D-Trehalose anhydrous, glycerol, glyceride, PhenoXyaethanolum, alcohol, DPPH (1,1-diphenyl-2-trimethylphenylhydrazine), L-tyrosine, tyrosinase were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Papain (400 U/mg), neutral protease (200 U/mg), alkaline protease (60 U/mg) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Other chemical reagents were provided by own laboratory.

2.2. Treatment of Sika Deer Skin

1kg frozen Sika deer skin was thawed in water and removed the skin fat from the skin, than cut into 10 mm×10 mm small pieces. The skin pieces were treated with five times degreasing solution (40°C, 8% Na₂CO₃ Solution) twice, each time washed 10 min, finally washed with clean water about 5min before drying. The skin pieces powder for subsequent experiments was crushed by a micro sample grinder after drying the treatment skin pieces.

2.3. Optimization for the Reaction Condition of Preparing SDSP

To prepared the Sika deer skin peptides (SDSP), the papain, neutral protease, alkaline protease and composite protease (including papain, neutral protease, alkaline protease) 4 kinds of protease were added to reaction system mixed with 100 mg skin pieces powder above and water, respectively. Four aqueous solution of 5% deer skin

polypeptide was prepared and the initial pH value (pH of phosphate buffer) was adjusted according to Table 1. After enzymatic hydrolysis process, the temperature was raised to 95°C about 10 minutes in order to end the enzymatic hydrolysis reaction, centrifugation time was 10 min at 5000 r/min, than the SDSP was obtained from the supernate of enzymatic hydrolysate.

The orthogonal test factors including enzymolysis temperature, reaction pH, enzyme addition and the reaction time to the reaction system were investigated to determine the appropriate enzymic hydrolytic conditions based on hydrolytic degree (DH) rate (Table 2). To further optimized the SDSP hydrolysis process, response surface analysis method was used to carry out a three factors-levels response surface design with influencing factors as enzymatic hydrolysis temperature, enzyme addition, reaction pH and degree of DH was indicated the response value in experiment (Table 3). The results analysed by Design-Expert 12.0 software for discussing the interaction of each factors to the DH.

The content of amino-nitrogen was determined by method of formaldehyde titration, total nitrogen was confirmed by Kjeldahl method. The formula of DH showed as $DH = (N_1/N_0) \times 100\%$ (N_1 content of amino nitrogen in supernatant, N_0 total nitrogen of sample).

2.4. Extraction of *Angelica dahurica*

The *Angelica dahurica* powder was crushed into powder by micro plant sample grinder, through 0.42 mm sieve. 10 g of *Angelica dahurica* powder was accurately weighed and dissolved in 200mL water, ethanol, *n*-butanol and petroleum ether respectively, after ultrasound extracted for 8 h with the ultrasonic condition as temperature at 35°C, ultrasonic frequency of 80Hz. The extraction was filtered and stored at room temperature for further experiments.

Table 1. The reaction conditions of the different proteas

enzymes	pH	Enzymatic hydrolysis time/h	temperature/°C	volume of enzyme/(U/g)
Papain protease	6.5	5	45	5000
Neutral protease	7.0	5	45	5000
alkaline protease	8.0	5	45	5000
composite protease	7.0	5	45	5000

Table 2. The factor levels of the orthogonal test

level	temperature (A)/°C	ph(B)	volume of enzyme(C)U/g	Enzymatic hydrolysis time (D)/h
1	50	6	5000	4
2	55	7	6000	5
3	60	8	7000	6

Table 3. The factor levels of the Box-Behnken test

Experimental factors	Factor condition	level of factor		
		-1	0	1
X ₁	temperature/°C	45	50	55
X ₂	pH	6	7	8
X ₃	volume of enzyme/(U/g)	5000	6000	7000

2.5. Analysis of Molecular Weight of SDSP by Exclusion Chromatography

Precisely weighed by the compound enzyme optimal conditions enzyme-decomposed deer collagen peptide powder 100 mg, placed in a 10 mL capacity bottle, water dissolved and fixed to the scale, shake the concentration of 10 mg/mL collagen peptide water solution, as a test solution. TSk super SW2000 column (300 mm×4.6 mm, 4 μ m), flow phase: 0.1 mol/L phosphate buffer solution (phosphate buffer saline,) PBS (pH-7.0) plus 0.1 mol/L sodium sulfate solution; detection wavelength: 214 nm; flow rate: 1 mL/min; sample size: 5 μ L. Molecular weight criteria were used: myoglobin (16,950 Da), peptidase (6,512 Da), insulin B (3,496 Da), growth hormone release inhibitors (1,521 Da), phenylalanine (165 Da). According to the molecular weight of standard products, the molecular weight of deer collagen peptides prepared by enzymes under optimal conditions by compound enzymes is calculated by external standard method Amino.

2.6. Amino Acid Components of SDSP

Accurately weight 0.02 g SDSP freeze-dried samples in hydrolysis tubes, then added 10 mL 6 mol/L HCl and 3 or 4 drops of phenol solution was added to hydrolysis tubes. Put the hydrolytic tube in the refrigerant, freeze 3 min to 5 min, hydrolysis 22 h in a thermostatic blow drying chamber at 110°C, after removed the cooling, the SDSP of hydrolyte was preserved through the filter. The hydrolyzed sample of SDSP was fixed to 20 mL, and the content of hydroxyproline was determined by the automatic amino acid analyzer and external standard method.

2.7. Identification of the SDSP Sequence

The SDSP sequence was analyzed used Q Exactive mass spectrometer. The liquid chromatography column (0.15 × 150 mm², RP-C18, Column Technology Inc.) was equilibrated with a 95 % A solution. The mobile phase A and mobile phase B were respectively 0.1 % formic acid aqueous solution and 0.1 % formic acid acetonitrile aqueous solution (84 % for acetonitrile). The raw data obtained using mass spectrometry were processed with the software MaxQuant.

2.8. Determination the Content of Active Components in *Angelica dahurica* Extraction

Accurately weighed 10mg, 20mg, 50mg, 100mg of psoralen standard respectively, put them into a volumetric flask (100mL), added ethanol to dissolve and fix the volume, then prepared psoralen standard solution (0.1, 0.2, 0.5, 1.0 mg/mL), the wavelength of UV spectrophotometer was set at 490nm to measure the absorbance of standard solution. The standard curve of psoralen was drawn with absorbance as ordinate and concentration as abscissa, the regression equation and correlation coefficient of the standard curve are obtained by drawing software. Then, the water, ethanol, n-butanol and petroleum ether extraction above were also detected by UV spectrophotometer at 490 nm, data analysis displayed the active components content of different extraction process from *Angelica dahurica* used with psoralen standard solution.

2.9. Detection of Active Components in *Angelica dahurica* Extraction by HPLC-ESI-MS

For HPLC-ESI-MS, 1mL the extraction of ethanol of *Angelica dahurica* were performed as above. The power of the sample extraction was obtained used nitrogen blowing instrument and set the temperature of the water bath equipment to 60°C. Then, the extraction power of water, ethanol, n-butanol and petroleum of *Angelica dahurica* were dissolved in 1ml methanol solution respectively.

The samples were separated on the Atlantis dC₁₈ column (3 μ m, 4.6mm×150mm; Waters, Milford, MA, USA). HPLC conditions was followed as: the mobile phase consists of a linear gradient of 0.05% (V/V) formic acid and deionized water (A) and methanol (B), 0-3min, 10-20% B (v/v); 3-5min, 20-25% B; 5-7min, 25-30% B; 7-8min, 30-35% B; 8-15min, 35-40% B; 15-20min, 40-80% B. The flow rate of the mobile phase was 0.3 mL/min, and the operation was carried out at room temperature. DAD was monitored in the range of 190-800nm. The analysis time was 60min.

ESI-MS conditions was followed as electrospray positive ion source, capillary temperature 270°C; Spray voltage 4.0kV, lens voltage -250V; Capillary voltage -41V; Quality detection range m/z: 200~1500; Injection pump flow rate 5 L/min; The sheath gas was nitrogen, and the flow rate was 40 mL/min.

2.9.1. Determination of the Moisturizing of facial Cosmetic Raw Material in Vitro

For analysis the moisturizing of *Angelica dahurica* extraction and sika deer skin peptides, human skin was imitated with medical breathable tape for detecting the moisturizing of facial cosmetic raw material liquid. The mass ratio of 500 μ L composite protease extract of sika deer skin protein and *Angelica dahurica* ethanol extract with rate of 1:1, 2:1, 5:1 material essence sample was coated directly and evenly on it, the quality was accurately weighed and recorded as H₀, 500 μ L of SDSP and *Angelica dahurica* extraction with mass ratio of 1:1, 2:1, 5:1 was directly and evenly coated on medical breathable tape, accurately recorded the weigh as H₀, after 0-24 h placed in a dryer environment with 25°C and 80% humidity, the weigh of medical breathable tape was recorded as H_n, the moisture retention rate (ϕ) was suggested that $\phi = (H_0/H_n) \times 100\%$.

2.9.2. Configuration of Facial Materials Liquid

Table 4 listed the formulation of 7 groups of facial materials liquid (The composition were mass fraction). The phase A which consisted of the following substances xanthan, Methyl Paraben, betaine, D-Trehalose anhydrous and glycerol dissolved in 60 mL water and heated to 70°C for 20 min, then the temperature dropped slowly to 40°C. Added the glyceride, PhenoXyaethanolum, SDSP, extraction of *Angelica dahurica* in turn and dissolved in 40 mL water to obtain phase B. Phase B was slowly added to phase A, after stirring the solution constantly for 20min, 7 kinds of facial materials liquid were obtained. The mass concentration of SDSP was 40.0 mg/mL and extraction of *Angelica dahurica* was 40.0 mg/mL.

Table 4. The formula of the facial material liquid

ingredient		groups						
		1	2	3	4	5	6	7
Group A	Deionized water	Add to 100.00	Add to 100.00	Add to 100.00	Add to 100.00	Add to 100.00	Add to 100.00	Add to 100.00
	xanthan gum	0.20	0.20	0.20	0.20	0.20	0.20	0.20
	methyl nipogin	0.20	0.20	0.20	0.20	0.20	0.20	0.20
	glycerin	12.00	12.00	12.00	12.00	12.00	12.00	12.00
	phenoxyethanol	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Group B	Angelica dahurica extract	0.00	1.00	2.00	3.00	4.00	5.00	6.00
	sika deerskin extract	6.00	5.00	4.00	3.00	2.00	1.00	0

Table 5. Conditions for determination of tyrosinase inhibitory activity

Groups	Sample (mL)	Tyrosinase (mL)	PBS (mL)	L-tyrosine (mL)
A	0	1.5	2.0	3
B	0	1.5	4.5	0
C	1.0	1.5	1.5	3
D	1.0	1.5	4.0	0

2.9.3. Determination of Antioxidant Capacity of Cosmetic Materials

112 mg of DPPH (1,1-diphenyl-2-trinitrophenylhydrazine) was dissolved in anhydrous ethanol and diluted to 100 mL and 2 mL of the solution was measured as A_0 at 519 nm. The absorbance of the 7 groups of facial materials liquid (1 mL) mixed with DPPH solution (3 mL) was measured A_b , the mixture of DPPH solution (1 mL) and ethanol (3 mL) were determined as A_t by the same method at 519nm. The clearance rate of DPPH solution (S) was also calculated as the formula of $S=1-[(A_t-A_b)/A_0] \times 100\%$, VC was used as positive control.

2.9.4. Molecular Docking of SDSP on DPPH

The three-dimensional structures of the ligand molecule (DPPH) were obtained from Pub Chem. The structures of peptides were constructed used Chem Draw and Chem3D software. Auto Dock software was used to conduct the docking procedure. Among peptide and ligand docking, the optimal model was selected on the basis of the lowest binding energy and the major cluster. The docking results were further confirmed and visualized by used PyMOL software.

2.9.5. Determination of Inhibitory Activity of Tyrosinase

Sample solution of facial materials liquid, PBS buffer solution (pH=6.8), tyrosine solution and L-tyrosine solution were accurately measured with pipet-gun according to the Table 5. After mixing evenly, the solution heated at 37°C water bath for 10 min. Then, the tyrosinase solution rapidly added to the reaction for 5 min. The absorbance rapidly measured at 475 nm, A, B, C and D were measured respectively. The tyrosinase inhibition rate of facial materials liquid (I) was calculate by $I=[1-(C-D)/(A-B)] \times 100\%$, and through SPSS software analysis.

3. Results and Discussion

3.1. Optimum Extraction Process of SDSP

Hydrolysis Degree (DH) referred to the percentage of broken peptide chains in the total number of peptide bonds

in the substrate protein. DH was closely related to the molecular weight of peptide, peptide structure, bioactivity of peptide and exposure to some special amino acids (Amso Z et al.; Zhang C et al.; Balkhi B et al.) [24,25,26]. Based on Kjeldahl method, DH rate that the papain, neutral protease, alkaline protease and composite protease 4 groups of Sika deer skin peptides (SDSP) were discussed the influence factor to the enzymatic hydrolysis process from Figure 1. All the results demonstrated that the acomposite protease to the ability of composite protease group to hydrolyze sika deer skin polypeptide was better than other three enzymes groups from Figure 1 A, B, C, D. Figure 1 A, B, C, D also indicated that DH increased gradually with the increase of temperature, reaction pH, enzyme addition and the reaction time, DH reached the maximum with the condition of 50°C, pH 7.0, 6000 U/g of enzyme addition and 5h. DH decreased with the temperature was higher than 50°C, the reason to the result may explain that denaturation of protease caused by too high temperature reducing the rate of enzymatic hydrolysis reaction [27]. The substrate protein was relatively insufficient with the increase of enzyme addition dosage, resulting in the weakening of the reaction process and the decrease of DH. According to the research of Mohammadi et al. (2016) [28], the matrix transfer rate was the key factor to determine the hydrolysis efficiency. In a certain range of time, with the extension of enzymatic hydrolysis time, the content of soluble peptides increased rapidly, and reached the peak at 5 h. Therefore, the content of soluble peptides was time-dependent. However, the content of soluble peptides gradually decreased, which may be due to the further transformation of soluble peptides into amino acids with the further extension of time (Guo et al., 2009) [29]. Therefore, the optimal enzymolysis time was 5 h. The optimum extraction process of SDSP condition selected composite protease group with temperature at 50°C, pH 7.0, 6000 U/g of enzyme addition and reaction time in 5h.

The results of orthogonal test (Table 6) suggested that the optimal combination of all factors was $A_1B_2C_2D_2$. Therefore, DH of composite protease group reached 24.42% in condition with the hydrolysis temperature 50°C, pH 7, enzyme dosage 6000 U/g and recation time 5h. The

range analysis showed that the effect of four factors on DH followed: enzyme dosage > hydrolysis temperature >

pH > hydrolysis time. This results were in line with previous studies (Tahmouzi et al., 2016) [30].

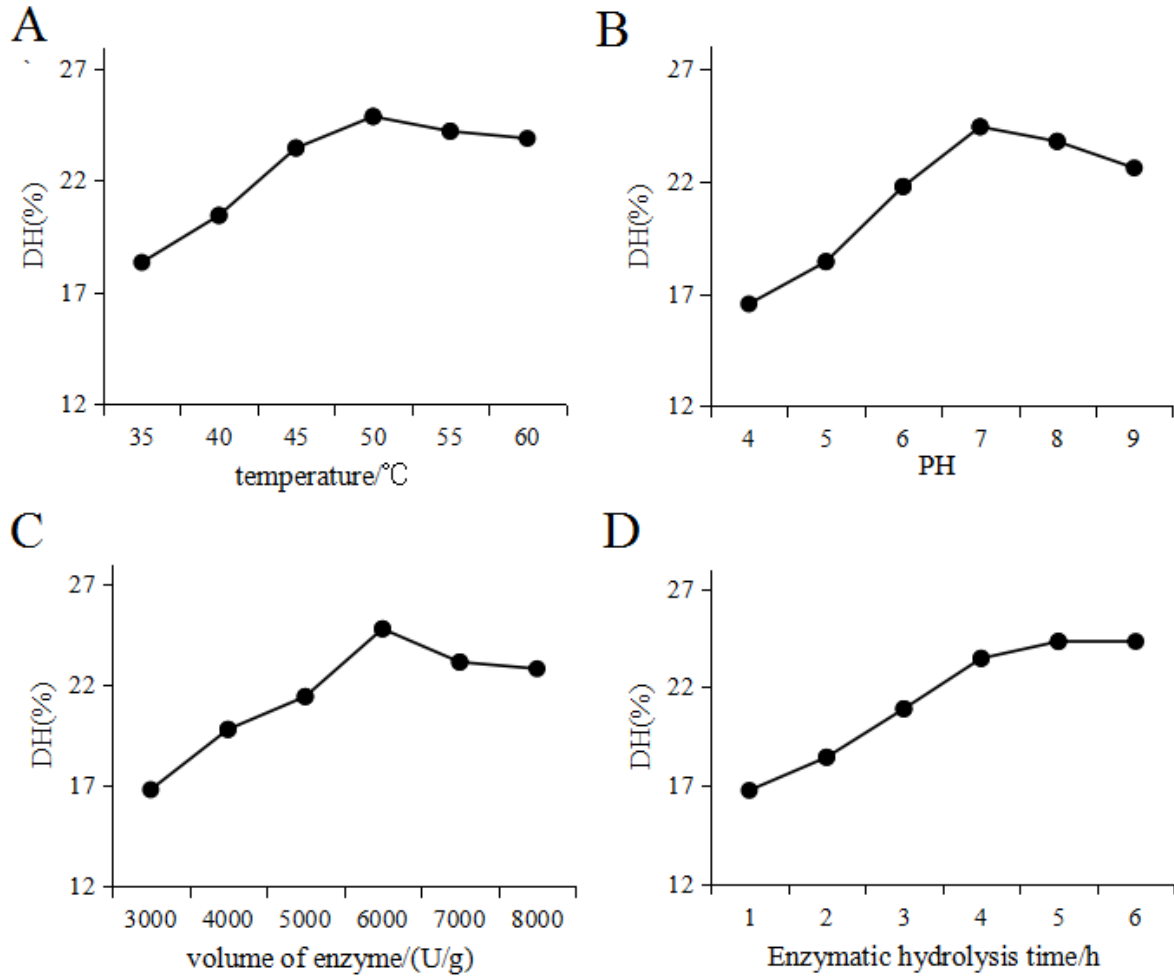


Figure 1. Effect of different enzymatic hydrolysis conditions on hydrolysis degree. A: Effect of temperature on hydrolysis degree. B: Effect of PH on hydrolysis degree. C: Effect of enzyme dosage on hydrolysis degree. D: Effect of enzymatic hydrolysis time on hydrolysis degree

Table 6. Orthogonal test results

Test numbers	A	B	C	D	DH/%
1	1	1	1	1	17.34
2	1	2	2	2	24.42
3	1	3	3	3	19.46
4	2	1	2	3	18.75
5	2	2	3	1	16.54
6	2	3	1	2	14.88
7	3	1	3	2	14.32
8	3	2	1	3	16.79
9	3	3	2	1	17.46
K ₁	61.22	50.41	49.01	51.34	
K ₂	50.17	57.75	60.63	53.62	
K ₃	48.57	51.80	50.32	55.00	
k ₁	20.41	16.80	16.34	17.11	
k ₂	16.72	19.25	20.21	17.87	
k ₃	16.19	17.27	16.78	18.33	
R	4.22	2.45	3.87	1.22	

Design-Expert 12.0 software was used to treat the response surface analysis data (Table 7 and Table 8). The aim of response surface methodology was find out the optimal enzymatic hydrolysis conditions for the maximum soluble peptide content. When the response surface test was designed to examine the interaction between the two factors, other variables should be kept constant. The slope change of response surface reflected the influence of each factor on the response value (Cho et al., 2005; Bezerra et al., 2008) [31,32]. The quadratic multiple regression equation model was established with hydrolysis temperature (x_1), pH value (x_2) and enzyme dosage (x_3) as independent variables and DH as dependent variable (response value y),

$$Y = 24.62 + 0.9512x_1 + 0.7825x_2 + 0.9137x_3 \\ + 0.1475x_1x_2 - 0.1300x_1x_3 \\ + 0.4575x_2x_3 - 5.10x_1^2 - 3.56x_2^2 - 4.00x_3^2.$$

From Figure 2, the quadratic model was used to fit the relationship between each factor and DH, the $P < 0.0001$

of the model showed that the experimental model was extremely significant, the linear effect and square effect of factors x_1 , x_2 and x_3 on DH were extremely significant, meanwhile DH was not affected by the interaction of x_1x_2 , x_1x_3 and x_2x_3 . According to the absolute value of the coefficient of the first regression equation, the main and secondary factors affecting DH were: the amount of enzyme addition $> \text{pH} > \text{enzymatic hydrolysis temperature}$. A similar result also verified that the ratio of enzyme to substrate was the key factor which affected the DH. According to the results of experimental design and quadratic multiple regression equation, the optimal combination of enzymolysis process was obtained as follows: enzymolysis solution temperature 50.47°C, enzymolysis time 4.93 h, enzyme addition 6119.43 U / g, initial pH 7.12. Considering the convenience of process operation, the parameters were adjusted as follows: enzymolysis solution temperature 51°C, enzymolysis time 5 h, enzyme addition 6000 U / g, initial pH 7.0.

Table 7. Experimental design and results for response surface analysis

Numbers	X_1 temperature/°C	X_2 pH	X_3 Volume of enzyme/(U/g)	DH/%
1	0	-1	-1	15.28
2	0	0	0	24.42
3	-1	0	1	15.43
4	0	1	-1	16.78
5	1	-1	0	16.33
6	-1	-1	0	14.87
7	0	0	0	25.38
8	1	0	-1	15.87
9	0	0	0	24.26
10	1	1	0	17.34
11	-1	0	-1	13.56
12	0	1	1	19.74
13	-1	1	0	15.29
14	0	-1	1	16.41
15	1	0	1	17.22
16	0	0	0	24.65
17	0	0	0	24.39

Table 8. Analysis of variance of response surface regression model

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	276.41	9	30.71	90.17	<0.0001	significant
X_1 -temperature	7.24	1	7.24	21.25	0.0025	
X_2 -PH	4.90	1	4.90	14.38	0.0068	
X_3 -volume of enzyme	6.68	1	6.68	19.61	0.0030	
X_1X_2	0.0870	1	0.0870	0.2555	0.6287	
X_1X_3	0.0676	1	0.0676	0.1985	0.6694	
X_2X_3	0.8372	1	0.8372	2.46	0.1609	
X_1^2	109.41	1	109.41	321.23	<0.0001	
X_2^2	53.51	1	53.51	157.12	<0.0001	
X_3^2	67.45	1	67.45	198.05	<0.0001	
Residual	2.38	7	0.3406			
Lack of Fit	1.58	3	0.5277	2.64	0.1862	not significant
Pure Error	0.8010	4	0.2002			
Cor Total	278.79	16				

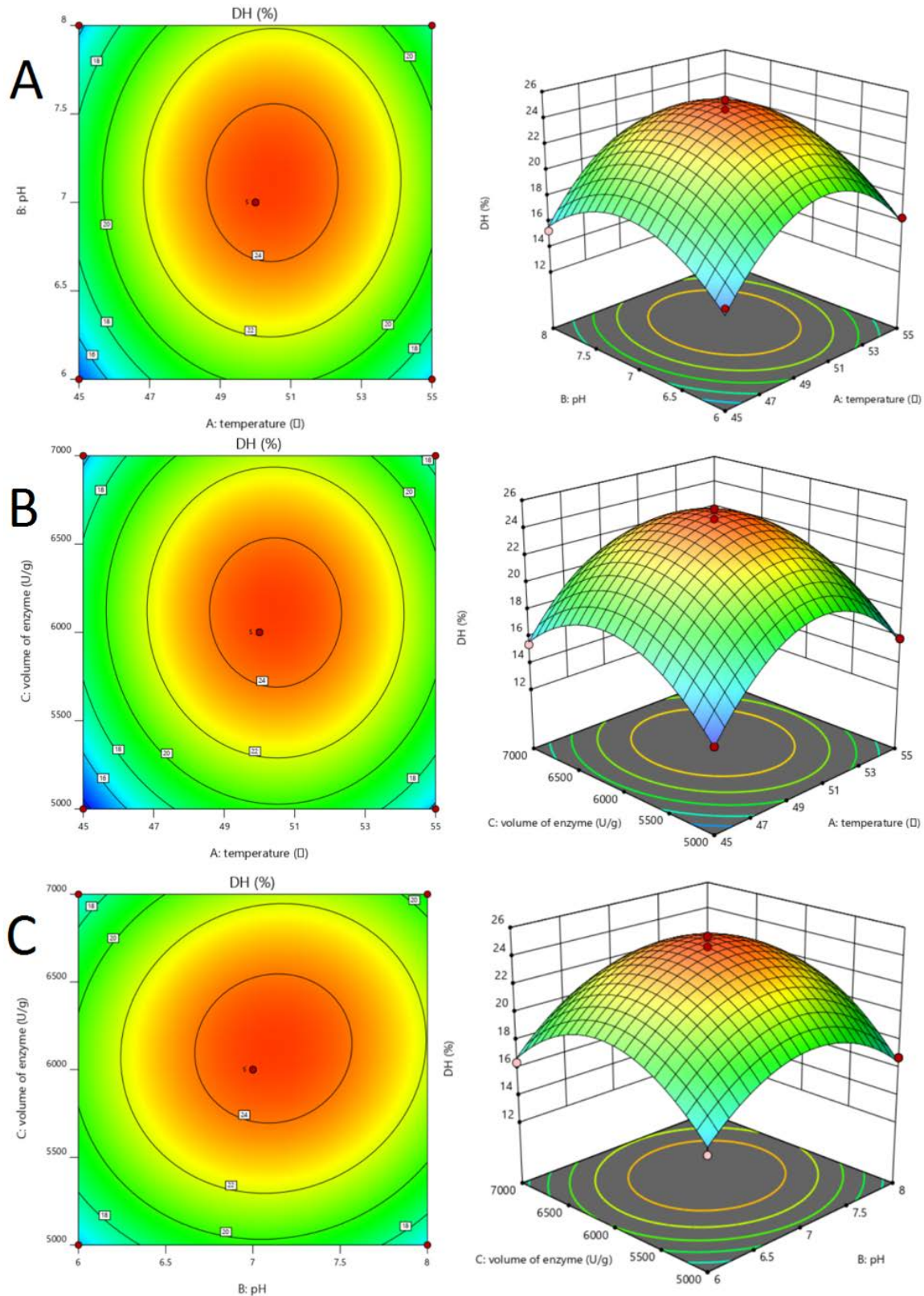


Figure 2. Influence of each factor interaction on the hydrolysis degree. A: Interactions of temperature and PH. B: Interactions of temperature and volume of enzyme. C: Interactions of PH and volume of enzyme

3.2. Amino Acid Components of SDSP

The function of protein or peptide depends mainly on its amino acid components. The amino acid components of SDSP was shown in Table 9. The content of Cys, Tyr, Ile, His and Met were very small, the content of Glu, Gly, Ala, Pro, Hyp indicated a higher level in SDSP. Studies

had shown that 19 amino acids inhibit superoxyion anions and hydroxyl free radicals, and found that amino acids such as Tyr, Asp, Lys, Glu, Met, Gly and Asn have strong ability to remove free radicals [33], and the content of these amino acids found in SDSP was more than 39.50%, so that deer collagen peptides have strong antioxidant activity.

Table 9. Amino acid species and content of complex protease of SDSP

Amino acid species	Content (%)
Glu	8.11
Ser	4.02
Gly	26.14
Tyr	0.34
Ile	1.46
Thr	2.13
Ala	8.76
Pro	11.45
His	1.34
Val	3.89
Met	1.22
Cys	0.02
Arg	6.78
Leu	3.33
Phe	1.24
Lys	3.69
Hyp	7.46

3.3. Peptides Sequence of SDSP

Currently, bioinformatics has been applied to conduct bioactive peptides research, and is regarded as an effective method to evaluate new peptides. In this study, LC-MS/MS was used to identify the peptide sequence of SDSP. As shown in Table 10, 14 peptides were identified in SDSP. It was worth noting low molecular weight peptides ($MW \leq 1000$) were the main component. Low molecular weight peptides facilitate binding [34]. Among these peptides, the peptides sequences of Gly-X-Y (X and Y are usually proline and hydroxyproline) are found frequently, which was considered as the characteristic sequences of SDSP. The relative amount of the peptides was calculated based on the intensity of the peak. QPTEGEAGAVQDK and RKAEGGEEGTVFF for a large proportion of total peptide chain. Further, 10 of these peptides contained one or more acidic amino acids. Furthermore, we found that the “GPAGP” peptide appeared many times in the polypeptide chain, and the “GPAGP” peptide seemed to have a certain relationship with SDSP binding. The same findings were also reflected in Pacific cod skin gelatin iron chelating peptides [35] and Alaska cod skin collagen-derived mineral chelating peptides [36]. When developing new safe foods, the toxicity of peptides should also be considered. A bioinformatics tool called ToxinPred can help predict the toxicity of peptide chelate. The results predicted that 14 peptides were non-toxic.

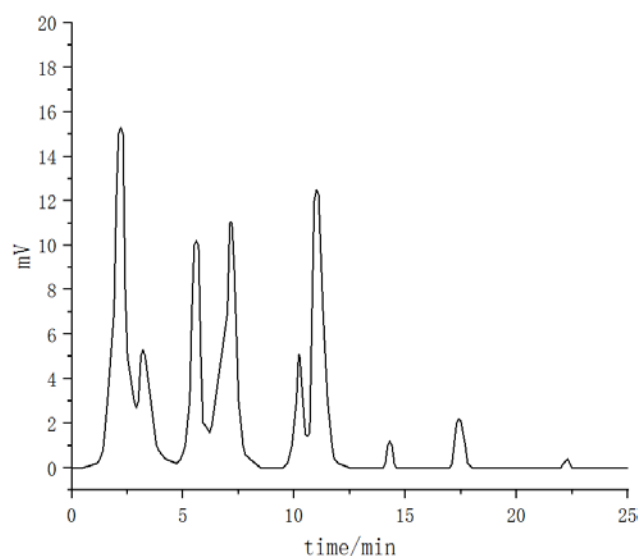
Table 10. The sequence description of SDSP

No	Sequence	Length	Mass (Da)
1	GPVGPTGPVG	10	837.45
2	GDRGETGPAGPAGPIGPV	18	802.90
3	GPAGPIGPV	9	764.43
4	ISVPGPM	7	350.69
5	VEDLLSLFR	13	1090.60
6	GIVLDSGDGVTH	17	1168.57
7	GPAGPQGPAGDRG	14	1135.54
8	TGPAGPQGPAGDRG	13	1236.58
9	AGPAGPAGPA	10	765.39
10	GPAGPAGPRG	10	418.72
11	SGDRGETGPAGPAGPIGPV	19	846.42
12	QPTEGEAGAVQDK	17	1328.62
13	GKSGDRGETGPAGPAGPIGPV	21	938.98
14	RKAEGGEEGTVFF	9	1425.69

3.4. Results of Exclusion Chromatography

According to the chromatography results of 5 standard substances of different molecular weight sizes, the relative molecular weight was ordinated ($\lg MW$) with its retention time of horizontal coordinate (T). The linear equation was calculated as $\lg MW = -0.2476T + 6.488$, $R^2 = 0.9973$.

The molecular blocking chromatography of SDSP, prepared by the compound enzyme under optimal enzyme solution conditions was shown in Figure 3, the retention time of composite protease group of SDSP was 10.521 min to 15.315 min and its corresponding molecular weight size was calculated by linear equations from 923 to 12192 Da.

**Figure 3.** Analysis of exclusion chromatography of SDSP

3.5. Detection the Concentration of *Angelica dahurica* Extraction

The average absorbance of the water, ethanol, n-butanol and petroleum ether four kinds of *Angelica dahurica* extraction were detected and the results showed in Table 11. According to the regression equation of the standard curve of psoralen, the concentration of the active compounds in each experimental extraction was obtained and the total concentration of the original extraction was identified by multiplied the dilution rate. The highest content was found in ethanol extract and the lowest in petroleum ether extract. The results suggested that the ethanol group of *Angelica dahurica* extraction should be select for following studies. The main demonstration of result was related to the different polarity of organic solvents [37].

Table 11. Absorbance of *Angelica dahurica* extracts and concentration

	Extracts				Concentration mg/mL
	Group A	Group B	Group C	Average	
Pure water	0.52	0.53	0.51	0.52	0.27
Ethyl alcohol	0.75	0.78	0.78	0.77	0.47
N-butyl alcohol	0.32	0.36	0.37	0.35	0.19
Petroleum ether	0.21	0.23	0.22	0.22	0.11

3.6. HPLC-ESI-MS Analysis of *Angelica dahurica* Extraction

HPLC-ESI-MS which was an ion source as a soft ionization method had widely used in similar compounds in plant research field. According to the previous reports and retention time, combined with molecular weight and MS/MS fragmentation rules in this study, 8 kinds of furocoumarines compounds were identified in ethanol extract form *Angelica dahurica* in positive ion mode (Figure 4), because these 8 compounds had the same skeleton and similar structure, MS/MS fragment ions was obtained by the same dissociation (CID) collision energy (Table 12). The main fragmentation patterns of furocoumarines compounds were hydrogen rearrangement

at C-5 and C-8 substituents, in the case of byakangelicol, the excimer ion peak m/z 317 $[M+H]^+$ was cleaved which occurred hydrogen rearrangement in the C-8 substituent resulting the lost of neutral molecule C_5H_8O and formed m/z 233 $[M+H]^+$ fragment ions (Figure 5). By comparing the lysis of several different furan coumarant compounds that replace the base, it descibed that the parent nucleus had similar neutral loss in which the position of the endoester ring was easily lost CO or CO_2 . Larger substrates were prone to neutral loss from hydrogen rescheduling, while smaller groups of methyl were prone to fracture to produce free radicals, however different substitution bases showed little effect on maternal fragmentation, which was slightly different from previously conclusion by Li et al [38].

Table 12. The chemical composition of *Angelica dahurica* by ESI

No.	tR(min)	Measured (m/z)	Formula	MS/MS (m/z)	Compound
1	5.16	271.72	$C_{16}H_{14}O_4$	203	Imperatorin
2	6.69	287.42	$C_{16}H_{14}O_5$	203	Isooxyeucedanin
3	7.08	317.24	$C_{17}H_{10}O_6$	233, 219	Byakangelicol
4	7.81	317.21	$C_{16}H_{15}O_7$	303, 219	Apaensin
5	11.74	247.59	$C_{13}H_{10}O_5$	219, 217	Isopimpinelline
6	11.94	287.58	$C_{16}H_{14}O_5$	203	Oxyeucedanin
7	14.03	247.36	$C_{13}H_{10}O_5$	215	Pimpinellin
8	16.05	305.61	$C_{16}H_{15}O_6$	203, 201	Prangeninhydrate

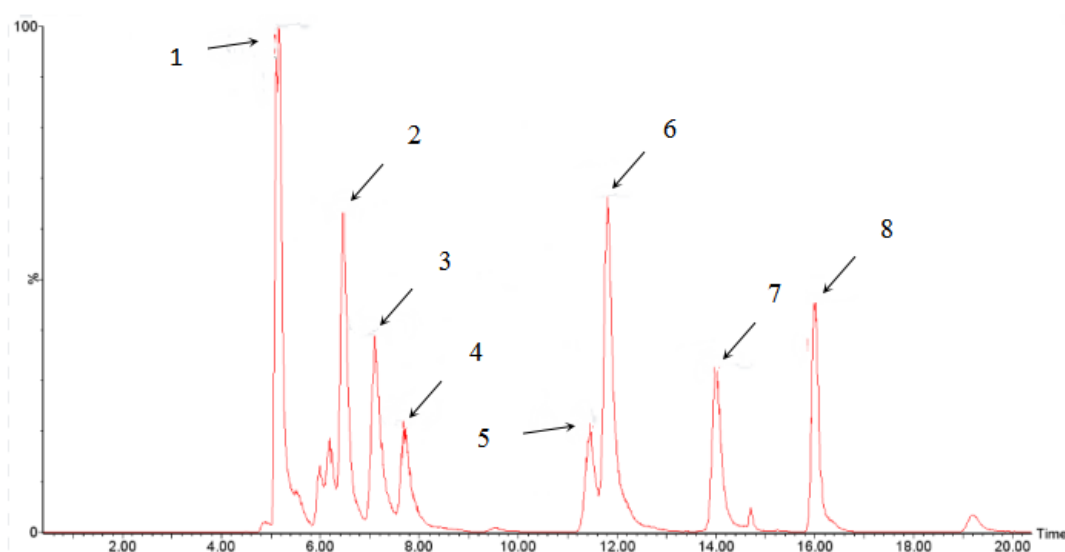


Figure 4. The ESI of *Angelica dahurica*

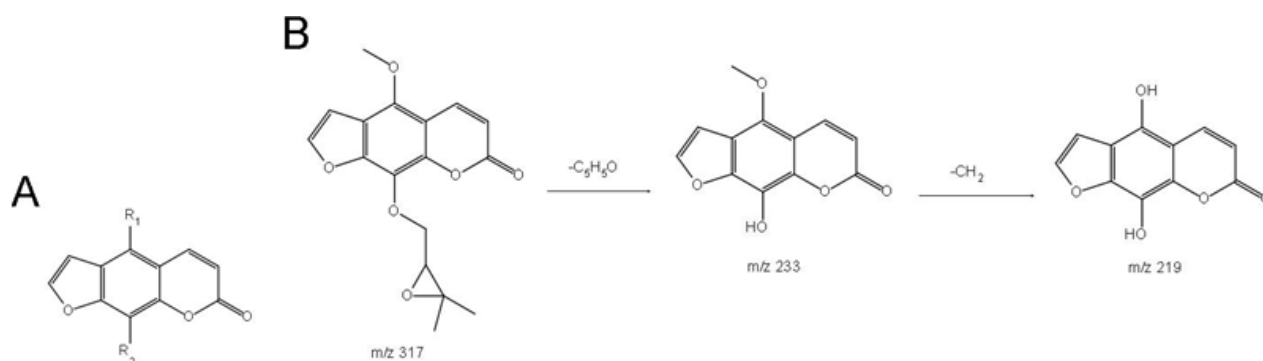


Figure 5. A: Patent nucleus structure of furocoumarin. B: Byakangelicol first level of mass spectra

3.7. Results of the Moisturizing of Facial Materials Liquid in Vitro

As shown in the Table 13, the moisture retention rate of the samples decreased gradually with the increase time and tended to be stable after 12h, which indicated that SDSP had a certain moisture retention property. The ability of moisture retention of the mixture of SDSP and *Angelica dahurica* concentrate in 2:1 was higher than the mixture rate with 5:1 and 1:1 groups. The mean of results was strongly demonstrated that SDSP had the function of anti drying and used in the development of moisturizing skin care products.

Table 13. Results of in vitro moisture retention test of the rate of SDSP and *Angelica dahurica* ethanol extraction

The rate of SDSP and <i>Angelica dahurica</i> ethanol extraction	Moisture retention rate				
	0h	6h	12h	18h	24h
1:1	95%	83%	72%	67%	62%
2:1	95%	88%	76%	73%	70%
5:1	95%	85%	73%	68%	64%

3.8. Analysis of Antioxidant Results

The DPPH free radical scavenging test was used to evaluate the free radical clearance capacity of 7 groups of facial materials liquid and VC. The absorbance value at the wavelength of 519 nm was linearly related to the scavenging intensity of free radicals. The scavenging ability of facial materials liquid on DPPH free radical was strong and gradually increased with the increase of concentration. Overall, the antioxidant activity level of facial materials liquid was lower than control group VC. When the sample dosage reached 2.0 mg/mL, the DPPH scavenging rates of groups 2, 3 and 4 (Figure 6) were 70%, 84% and 71% respectively, the free radical scavenging rate had little change when the concentration of facial materials liquid was more than 2.0 mg/mL. SDSP and *Angelica dahurica* extraction were the main antioxidant compounds in samples. (Ji et al., Ambreen et al.) [39,40]. have also reported a positive correlation between antioxidant capacity in polypeptide and furocoumarines compounds. Considering the cost of SDSP, the second, third and fourth groups facial materials liquid were used as the subsequent tyrosinase inhibitory activity experiment.

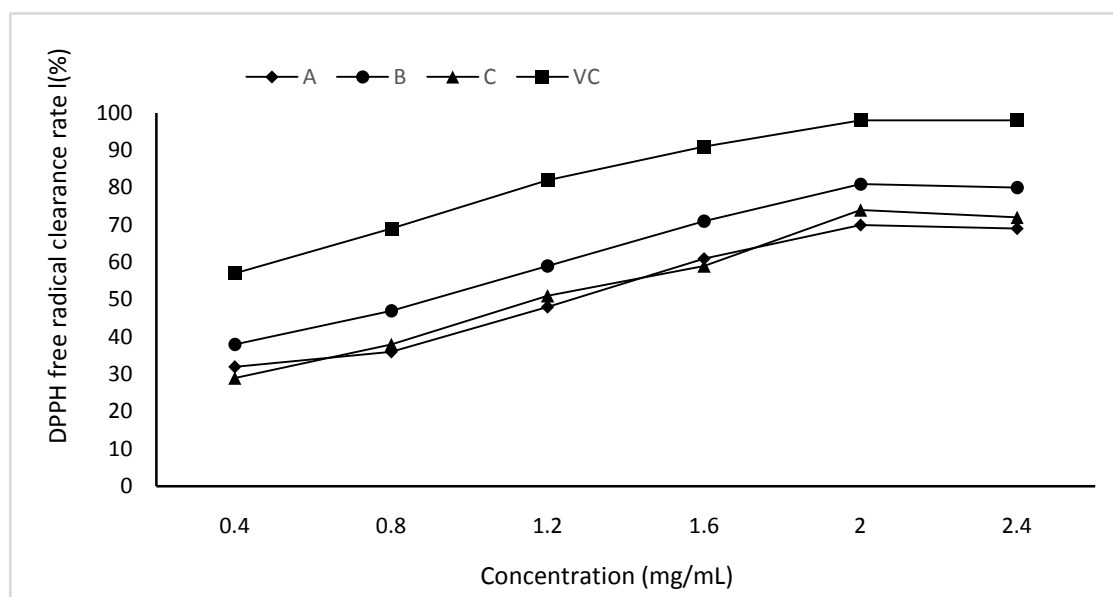


Figure 6. The DPPH free radicals clearance rate of the concentration

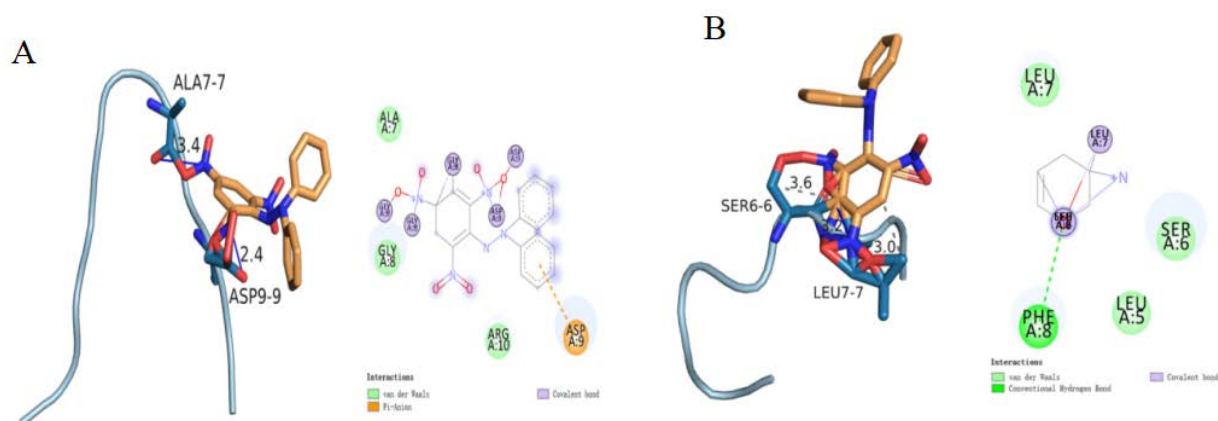


Figure 7. Molecular docking of antioxidant peptides derived from Sika deer skin polypeptide with DPPH radical

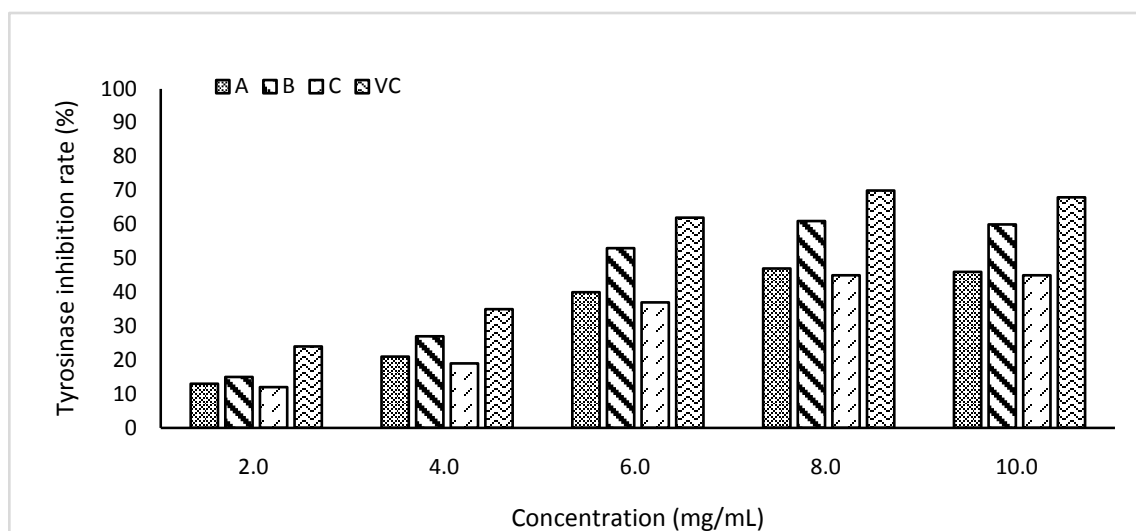


Figure 8. The tyrosinase inhibition rate of the material liquid concentration

3.9. Molecular Docking

Molecular docking is widely used to predict interactions between ligands and receptors. To better understand the antioxidative mechanism of SDSP, the schematic diagram of possible antioxidative mode was constructed by Autodock and presented in Figure 7. These two identified peptides presented strong hydrogen bonds and hydrophobic interactions with the target molecule (DPPH radical). Each peptide could form at least one hydrogen bond with DPPH. Both peptide chains combined with DPPH, with multiple binding sites. Xu [41] demonstrated by molecular docking of calcium ions with PIE that the acidic amino acid (glutamic acid) was the main chelation site. Also, DPPH was found to interact with hydrophobic amino acid residues such as leucine, alanine and proline via forming hydrophobic interaction. These results were in accordance with the previous studies.

3.9.1. Inhibitory Activity of Tyrosinase of Facial Materials Liquid

The inhibition rates of tyrosinase in 2, 3, 4 groups of facial materials liquid and VC were shown in Figure 8, facial materials liquid and VC solution reach the plateau stage at 6 mg/mL. The inhibition rates of tyrosinase of 2, 3, 4 groups of facial materials liquid and VC were 53.21% and 62.18% respectively. The IC₅₀ value was obtained by SPSS software analysis: A 0.68g/L, B 0.55g/L, C 0.70g/L. Facial materials liquid had weak inhibition on tyrosinase compared with VC, but it still displayed obvious inhibition. The inhibition intensity increased with the increase of sample concentration, the tyrosinase inhibition rate of VC was 70.03% and inhibition rate of 3 group of facial materials liquid reached 61.12% at 8 mg/mL which revealed tyrosinase inhibition activity, it proved that facial materials liquid had whitening effect in vitro.

4. Conclusion

For SDSP, the optimal enzymatic hydrolysis process was selected composite protease group above with the conditions of 51°C, pH 7.0, 6000 U/g and 5h. The

molecular weight of SDSP was 851 Da to 11 092 Da and SDSP were rich in glycine, alanine, proline, hydroxyproline and terpeneine. 8 kinds of furocoumarines compounds were identified from ethanol extraction from *Angelica dahurica* by HPLC-ESI-MS analysis. Through in vitro moisturizing experiment, the best moisturizing effect was obtained when the ratio of SDSP and *Angelica dahurica* ethanol extraction was 2:1. The free radical scavenging ability of facial material liquids increased with the increase of the concentration in the range of 0-2.4 mg/mL, which indicated a effect antioxidant activity of facial material, the result showed that free radical scavenging ability of group 3 of facial base liquids reached 81% in 2.0 mg/mL. By studying the inhibitory activity of tyrosinase, it was found that facial base liquids group 3 had a certain inhibitory effect to tyrosinase and its inhibition rate was 61.12% with 8 mg/mL.

Supplementary Materials

The following supporting information can be downloaded at: Supplementary Materials.

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Conflicts of Interest

The authors declare that they have no competing interests.

Data Availability Statement

Data will be made available on request.

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