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STUDY ON THE EXTRACTION OF PIGMENT FROM FRUIT OF ROSA DAVURICA PALL. WITH MICROWAVE COENZYME METHOD AND ITS STABILITY AND INOXIDIZABILITY

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ARTICLE DETAILS

ABSTRACT

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To explore the best extracting process, stability and antioxidation activity in vitro of pigment extracted from fruit of *Rosa davurica* Pall., orthogonal design method was applied to determine optimal results. Antioxidant ability of pigment can be determined by its scavenging activity and reducing power to DPPH, NaNO₂. The stability of pigment was tested using UV-Vis spectrophotometry. The best process to extract pigment was as follows, methyl alcohol (50 %) was used as extractant. The complex phosphoesterasum was used as enzymes type formed by cellulase and pectinase. Enzyme dosage was equal to 1.2% of quality of dry fruit of *Rosa davurica* Pall. (FRDP). Solid-liquid ratio was selected as 1:30 and microwave power was 600 W. The extraction was conducted in the solution with pH 6.0 for 17 minutes at 65 °C. The stability testing showed that pigment was not stable in the alkaline solution with Fe³⁺. Other factors had poor effect on stability of pigment. The antioxidant ability of pigment was confirmed by anti-oxidative experiment.

KEYWORDS

Fruit of *Rosa davurica* Pall, microwave coenzyme, pigment, antioxidant.

1. INTRODUCTION

Based on a study, FRDP belongs to *Rosa* of Rosaceae and is a kind of dried dahurian rose fruit whose active constituents include flavonoid, organic acid, coumarins and polyphenols [1-3]. Research showed it is expected to be fully exploited and utilized [4]. According to the previous study, pigments of plant possess a strong oxidative resistance, which can help prevent cancer, protect the skin and improve body immunity [5]. Being safe and reliable without other side-effect are feature of natural pigments. Besides, natural coloring gives it an important role in food additives [6,7]. Therefore, with the developing of food industry, natural pigments will be highly valued which will replace of artificial pigments [8,9].

The traditional method to extract pigment will consume a mass of solvent, which takes a long time but has a poor effect. According to a research, with the advantages of high efficiency, simplicity of operator, high yield and the easily purified product, microwave coenzyme will be widely used in extraction of natural pigment [10-12]. Enzyme will break the cell wall of FRDP, which can promote the extraction of effective constituent. Microwave coenzyme was used to extract pigment in the test. At the same time, the stability and oxidative resistance of pigment were study in the research which will lay a solid foundation on the further study.

2. MATERIALS AND METHODS

2.1 Materials and reagents

FRDP was picked from Jade Dragon Mountain in LongTan district, JiLin City and was authenticated by Dr.JianfeiXue of pharmacy department of JiLin Instituite of Chemical Technology as a kind of dried dahurian rose

fruit in Botanical hillbelongs to *Rosa* of Rosaceae. The rest of reagents are all analytical reagentsor food additives.

2.2 Instruments and equipments

Ultravioletand visible spectrophotomer(TU-1810), thermostat water bath(W5-100SP), electronicanalytical balance (FA2004N), SHZ-IIID(SHB-IIIA), electric heating air-blowing drier(DHG-9076A), supersonic cleaner(KQ118), microwave extractor(MAS-II).

2.3 Experimental methods

2.3.1 Extraction of pigment

According to the result of single factor experiment, the fixed extractant was based of 50% methyl alcohol. The complex phosphoesterasum which was formed by cellulase and pectinase was used as enzymes type. The experiment was based on 5 factors: Dosage of enzyme, solid-liquid ratio, microwave power, extraction duration and extraction temperature. Orthogonal experiment is designed on the base of five factors and four levels. The factors and levels of orthogonal experiment were shown in Table 1.

Table 1: Orthogonal experiment and its level

item	dosage enzyme A	of B	solid-liquid ratio	microwave power C	duration D/min	temperature E/°C
1	1.0%		1:15	400	13	55
2	1.2%		1:20	500	15	60
3	1.4%		1:25	600	17	65
4	1.6%		1:30	700	20	70

2.3.2 Investigation of pigment stability

The changes in the absorbance of pigment are measured under 370 nm. Besides, effect of pH value, temperature, illumination, citric acid, vitamin C, sodium benzoate, oxidizing agent, metal ion, glucose and starch on pigment stability were researched.

2.3.3 Vitro antioxidant experiments

(1) Scavenging activity on DPPH

Refer to research of Fan [13] with some adjustments, 2 mL of samples with different concentration for mass respectively were put into 10 mL colorimetric tube before 2 mL liquor of 0.2 mmol/L DPPH added in. After mixing, the mixture was placed in dark before used for reaction at room temperature for 30 min. The sample of blank group was replaced by distilled water. Besides, DPPH was replaced by ethyl alcohol and distilled water was taken as a reference. Absorbance of sample A, blank A and reference A were investigated under a wave length of 517 nm.

DPPH clearance rate% = $[(\text{blank A} - \text{sample A} + \text{reference A}) / \text{blank A}] \times 100$

which blank A is 2 mL DHHP added with 2 mL water, reference A is 2 mL absolute ethyl alcohol added with 2 mL water, sample A is 2 mL DPPH added with 2 mL sample for test.

(2) Measurement of reducing power

Referring to research, with some improvements, 1 mL of fluid with different concentration of mass were put into 2.5 mL of phosphate buffer (0.2 mol/L, PH=6.6) and 2.5 mL of 1% aqueous solution of potassium ferricyanide, respectively [14]. After mixing and cooling in thermostatic water bath at 50 °C for 20 min, 1 mL of solution for trichloroacetic acid (10%) was put into the mixture before centrifuged for 10 min at 500 r/min. 2.5 mL of supernate was put into another test tube firstly. Then 2.5 mL of distilled water and 0.5 mL of liquor ferritrichloridi (0.1%) were

added in before mixed and stood for 10 min. Absorbance A was measured under 700 nm with ethanol solution (50%) to be blank control. Solution of sample will not be added into the blank group, which can carry out a measurement of absorbance A_0 .

Pigment reduction force = $A - A_0$

(3) Elimination to nitrite

Based on a study, with some adjustments, 1 mL of fluid to be checked with different concentration of mass were taken before 2.5 mL of solution of NaNO_2 (10 $\mu\text{g/mL}$) added in [15]. Then the reaction was carried out in thermostatic waterbath at 37 °C for 30 min. After the above operations, 0.5 mL of solution of sulfanilic acid (0.4%) was added in immediately. Then 0.25 mL of 0.2% solution of Naphthalene hydrolorideis added in after mixing and standing for 15 min. Finally, 7 mL of distilled water was added in before mixed and stood for 15 min. With 50% ethyl alcohol used to be blank control, the absorbance value A_i is measured under 538 nm.

Nitrite clarity rate% = $[(A_0 - A_i + A_j) / A_0] \times 100$

which A_i is meant for absorbance of fluid under test, A_j is meant for test solution background value, A_0 is meant for comparison with no fluid under test.

3. RESULT AND DISCUSSION

3.1 The result of orthogonal experiment

16 portions of dry FRDP were weighed and taken for each 2.0 g. The conditions of extraction were made according to the table of orthogonal test. After the extraction, enzyme was killed in condition of 90 °C for 30 s before filtrated with filter liquor which diluted by 50% methyl alcohol before constant volume. The absorbance was measured under 370 nm. The result was shown in Table 2.

Table 2: The result and analysis of orthogonal experiment

Experiment number	A	B	C	D	E	absorbance
1	1	1	1	1	1	0.462
2	1	2	2	2	2	0.467
3	1	3	3	3	3	0.473
4	1	4	4	4	4	0.468
5	2	1	2	3	4	0.466
6	2	2	1	4	3	0.468
7	2	3	4	1	2	0.467
8	2	4	3	2	1	0.471
9	3	1	3	4	2	0.447
10	3	2	4	3	1	0.447
11	3	3	1	2	4	0.447
12	3	4	2	1	3	0.449
13	4	1	4	2	3	0.447
14	4	2	3	1	4	0.444
15	4	3	2	4	1	0.448
16	4	4	1	3	2	0.449
K ₁	0.4675	0.4555	0.4565	0.4555	0.457	
K ₂	0.468	0.4565	0.4575	0.458	0.4575	
K ₃	0.4475	0.45875	0.45875	0.45875	0.45925	
K ₄	0.447	0.45925	0.45725	0.45775	0.45625	
R	0.021	0.00375	0.00225	0.00325	0.003	

According to the results in Table 2, the order of effects of factors on the extraction for pigment was $A > B > D > E > C$. Therefore, it could be summarized that proper dosage of compound of enzyme has the greatest effect to extraction followed by solid-liquid ratio, extracting time, extracting temperature in turns. Finally, microwave power has the poorest impact to extraction. After comprehensive analysis, the best condition for extraction were $A_2B_4C_3D_3E_3$. In summary, the dosage of compound of enzyme was 1.2% of quality for FRDP. Solid-liquid ratio was selected as 1:30. Microwave power was selected to be 600 W. Time for extraction was 17min and extracting temperature was 65 °C. The type of enzyme was a compound enzyme which is made of cellulose and pectase.

Figure 1: The effect of pH value on pigment stability

Figure 2: The effect of temperature on pigment stability

3.2.2 The effect of temperature on pigment stability

The extracting solution for pigment in section 2.2.1 were transferred into water bath kettle at 30, 40, 50, 60, 70, 80, 90, 100 °C respectively. The result was shown in Figure 2 that temperature for extraction had a certain effect on stability of pigment.

Figure 3: The effect of citric acid, Vc, sodium benzoate on pigment stability

3.2.3 The effect of citric acid, Vc, sodium benzoate on pigment stability

The result was shown in Figure 3. With the increase of added citric acid, Vc and sodium benzoate, the absorbance of pigment tended to be stable, which explained the poor effect of citric acid, Vc sodium benzoate on pigment stability

Figure 4: The effect of oxidizing agent on pigment stability

3.2.4 The effect of oxidizing agent on pigment stability

As shown in Figure 4, the addition of hydrogen peroxide changed the absorbance of pigment a little, which explained the poor effect of oxidizing agent on stability pigment.

3.2.5 The effect of glucose on pigment stability

The result was shown in Figure 5. With the addition of glucose solution in different concentration, the curve appeared stable as a whole, which explained glucose had no effect on pigment stability.

Figure 5: The effect of glucose on pigment stability

Figure 6: The effect of starch on pigment stability

3.2.6 The effect of starch on pigment stability

The result was shown in Figure 6. With the addition of starch solution in different concentration, the curve appeared stable as a whole. Ignoring the experimental error, starch had no effect on pigment stability.

3.2.7 The effect of illumination on pigment stability

All the experiments were conducted in room temperature. The result was shown in Table 3. The absorbance of pigment had a little change with different strength of illumination, which explained its poor effect.

Table 3: The effect of illumination on pigment stability

illumination	indoor light	sunlight	UV-light	place away from sunlight
absorbance	0.286	0.288	0.297	0.319

3.2.8 The effect of metal ion on pigment stability

The result was shown in Table 4. The addition of solution of sodium chloride, calcium chloride and manganese sulfate did not change the

absorbance for pigment obviously. While it benefited a lot from the ferric trichloride. That meant Na^+ , Ca^{2+} and Mg^{2+} had little effect on stability of pigment of pigment, while Fe^{3+} had a relatively larger impact.

Table 4: The effect of metal ion on pigment stability

0.01 mol/L solution	metal ion	blank	sodium chloride	calcium chloride	manganese sulfate	ferric trichloride
24h		0.279	0.259	0.272	0.284	2.171
48h		0.230	0.212	0.236	0.226	2.052

3.3 In Vitro Antioxidant Experiment of Pigment

As shown in Figure 7, the clearance rate to DPPH increased up to about 90% with the growing of concentration of mass when concentration of pigment ranged from 0.01 mg/mL to 0.1 mg/mL. When concentration of pigment was over 0.1 mg/mL, the increase of concentration of pigment

had almost no influence on the clearance rate to DPPH. With the growing concentration of pigment, clearance to nitrite for pigment increased. This positive correlation indicated that pigment had a certain clearance to nitrite. According to Figure 8, the curve tended to rise when the concentration of pigment of samples increased, which indicated that reducing capacity of pigment gained when the concentration increases.

Figure 7: The measurement of pigment antioxidant capacity

Figure 8: The measurement of pigment reducing capacity

4. CONCLUSION

Using FRDP as raw material, microwave coenzyme was applied to extract pigment. The best extracting process was determined by orthogonal design and single factor experiment. With the stability and inoxidizability of pigment be explored, the best method to extract pigment was as follows: 50% methyl alcohol was used as extractant. The complex phosphoesterasum formed by cellulase and pectinase was used as type of enzymes. Dosage of enzyme was based of 1.2% quality of dry FRDP and solid-liquid ratio was selected as 1:30. Microwave power was 600 W and pH=6.0. The process of extraction was carried out at 65 °C for 17 min. The pigment was stable under acidic condition and illumination, temperature, oxidizing agent, citric acid, Vc, sodium benzoate, dextrose solution, starch solution had almost no effect on the stability. Na^+ , Ca^{2+} , Mg^{2+} had a small impact on the stability of pigment. While Fe^{3+} had a big influence on stability. Pigment possesses a certain clearance to DPPH and nitrite, besides, it possessed strong reducing capacity. This indicated that pigment had a certain in vitro oxidation resistance, which with the increase growth of concentration of mass for pigment. Pigment is a kind of natural pigment with high economic value and was expected to be exploited. It can be widely used in the development of healthy food and drinking. However, the specific functions need further exploration.

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