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Study on Degradation Process of Procyanidins

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To improve the utilization rate and biological activity of procyanidins, the hydrolysis of procyanidins took palce at 60 °C for 90 min with 5 % sodium bisulfite, the volume ratio is 1:9. The average polymerization degree of proanthocyanidins polymer dropped to 2.3 from 4.6 and its IC_{50} (mg/L) value dropped from 2.88 to 2.18, but the antioxidant activity improved.

Keywords: Proanthocyanidins, Hydrolysis, Antioxidant activity.

INTRODUCTION

Grape seed proanthocyanidins is one of the most powerful antioxidants in scavenge free radicals, which can prevent a variety of diseases caused due to free radicals, including heart disease, cancer and arthritis. Based on procyanidins's biochemistry, pharmacology, procyanidins have been widely used in medicine, health products, food, beverages and cosmetics and other fields. According to the size of the polymerization degree, 2-4 polymers usually called oligomer procyanidins (abbreviated opc, water-soluble substance), is easily absorbed. More than five polymers is called polymer procyanidins (referred PPC), the feature of polymer procyanidins is a lower of water-soluble and difficult to be absorbed. The antioxidant activity and free radical scavenging capacity of proanthocyanidins depends on the activity of phenolic hydroxyl groups in the molecule and the polymerization degree will inevitably affect its physiological activity[1]. With the increase of polymerization degree , procyanidins molecule is more and more big, the molecules of a growing number of phenolic hydroxyl formed intramolecular hydrogen bond, leading to the same amount of phenol hydroxyl gradually reduce, radical scavenging ability is also weakened[2]. The high polymeric procyanidins content accounts for over 50 % of the total amount antioxidant activity of procyanidins in grape seed extract, so as to improve the biological activity and utilization rates of grape seed proanthocyanidins. The polymer procyanidins hydrolysis into oligomer procyanidins has important significance.

Currently, reported in the literature, hydrolysis methods of polymer procyanidins are mainly the following: (1) oxidative degradation method[3] which is a certain concentration of hydrogen peroxide or potassium chlorate as oxidant to degrada condensed tannins; (2) acidic degradation method[4] with hydrochloric acid, study of procyanidins high polymer hydrolysis process, determine the strong acid resin as the best medium, Junying *et al.*[5] use sodium sulfite as hydrolysis medium, under optimal conditions, the results show procyanidin have an average degree of polymerization of 7.06 down to 4.3; (3) alkaline degradation method; (4) hydrogenated degradation method, for example[6] by hydrogenation methods to reduce the degree of proanthocyanidins, identified the optimal condition, the average degree of proanthocyanidins from 6.3 down to 2.2. Other workers reports the microbiological degradation[7-9] and benzyl mercaptan degradation[10], *etc*.

The method of oxidative degradation use hydrogen peroxide and potassium chlorate as an oxidant, it is easy to decompose in use and during storage stable, resulting in low catalyst efficiency, consuming large and easily lead to a phenolic hydroxyl group is oxidized to a carboxyl group resulting product inactivation. Hydrogenated degradation method requires high temperature and pressure, the operation is relatively complicated.

In this paper, we use sodium bisulfite as the hydrolysis medium, sodium bisulfite is a common nucleophile, polymer portion of C4-C8 keys of polymer proanthocyanidin to breaking under the attack of the nucleophilic reagent, so as to the purpose of degradation in acidic conditions.

EXPERIMENTAL

(t)-Catechin and ()-epicatechin were obtained from National Institutes for Food and Drug Control; sodium bisulfite, vanillin, AR, Beijing Chemical Plant; Grape seed extract (proanthocyanidins content of 85 %), Shaanxi Senfo Technology Co., Ltd.

Preparation of materials: Grape seed extract by macroporous resin of AB-8 for purification, use deionized water, 30 and 60 % ethanol elution successively, 60 % ethanol elution rotary evaporation to aqueous phase as hydrolysis raw materials. Proanthocyanidins could not extract from grape seed directly, but from commercially purchased proanthocyanidin extract. The proanthocyanidins bought from factory is conscious removed part of the high polymer proanthocyanidins, so the average polymerization degree of the above of hydrolyzed raw was not very high, but does not affect the test fumble.

According to a certain ratio of solid and liquid, adding a certain concentration of sodium bisulfite to proanthocyanidins hydrolysis, degradation at certain temperature, then cool to room temperature, the average polymerization degree was measured. The effect on the degradation like liquid ratio, the concentration of sodium hydrogen sulfite, temperature, hydrolysis time were also investigated.

Proanthocyanidins degraded material was cooled, centrifuged, alcohol precipitation and concentrated, purified with the AB-8 resin, eluting with water to remove the sodium hydrogen sulfite and finally with 60 % ethanol to obtain the target product.

Determination of the average polymerization degree: Determination of average polymerization degree use the modified method of vanillin[9], using acetic acid as a solvent, vanillin only have condensation reaction with the end of flavan-3-alcohol, so we can determine the molar amount of proanthocyanidins, procyanidins concentration determined using conventional vanillin, combination of both to obtain an average polymerization degree of proanthocyanidins[11].

$$DP = m/(n \times M)$$

DP is the average polymerization degree; m is the mass concentration of proanthocyanidins, μg/mL; n is the molar amount of substance concentration, μmol/mL; M is the molecular weight of the monomer catechin, g/mol.

Determination of antioxidant activity of proanthocyanidins: (1) Take the sample of before and after degradation of procyanidins each 12.54 mg constant volume to 100 mL volumetric flask, the concentrations were respectively 0.14 mg/mL, 0.137 mg/mL. Diluted into a series of concentrations of free radical scavenging test done.

(2) 95 % ethanol as the solvent, prepare 50 μmol/L of DPPH o solution.

(3) Take a certain concentration of sample solution 2 mL in 10 mL test tube, then add 2 mL 50 μmol/L DPPH solution reacted at room temperature for 0.5 h, then poured it into 1 cm light path cuvette, recording the absorbance value at 517 nm as A1 (with 2 mL 95 % ethanol and 2 mL sample solution zero to deduct the color of the sample itself) added 2 mL corresponding sample solvent to 2 mL DPPH solution, record the absorbance value as A2. According to the formula obtained sample clearance rate η.

The formula of clearance rate:

$$\text{Clearance rate } \eta\ (\%) = (A1\text{-}A2) / A1 \times 100$$

(4) Draw the curve of clearance rate and proanthocyanidins concentration, according to the curve equation to calculate the IC_{50} values[10,11].

Analysis the sample by HPLC: Weighed sample of about 10 mg and methanol diluted to 10 mL, 0.45 μm membrane filter before injection.

Chromatographic conditions: Mobile phase is acetonitrile: water (1000 mL water containing 3 mL of acetic acid) = 10: 90 (pH = 3.10); flow rate: 0.8 mL/min; column temperature: 35 °C; detection wavelength = 280 nm; column: zorbax (5 μm, 4.6 mm × 250 mm); 2498 detector; injection volume: 10 μL.

RESULTS AND DISCUSSION

Dosage of sodium bisulfite: 1 mL degradation material were added to five cuvettes respectively, then 5-10 mL of 3 % solution of sodium hydrogen sulfite were added, reaction for 90 min at 80 °C, cooled to room temperature with cold water, the average polymerization degree of proanthocyanidins was measured.The dosage of sodium bisulfite effects on the average polymerization degree as shown in Fig. 1.

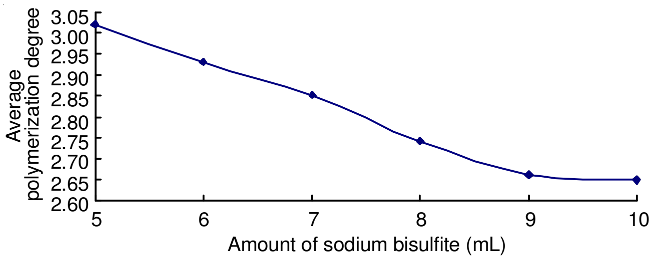

Fig. 1. Dosage of sodium bisulfite effects on the average degree of polymerization

Fig. 1 showed that with the increase of dosage of sodium bisulfite, procyanidins average polymerization degree gradually decline, when each 1 mL degradation material added to 9 mL sodium bisulfite, the average polymerization degree of polymerization reached the maximum and basically no longer change.

Effect of hydrolysis temperature: 9 mL of 5 % sodium bisulfite solution and 1 mL of degradation solution were added to five cuvettes respectively, in a water bath 50-90 °C reaction for 1 h. Then cool to room temperature, the average polymerization degree was measured. Hydrolysis temperature effect on the average polymerization degree is shown in Fig. 2.

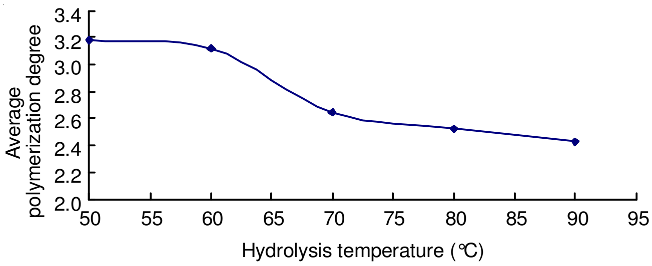

Fig. 2. Hydrolysis temperature effect on the average degree of polymerization

As can be seen from Fig. 2, the most dramatic changes is in the reaction at 60-70 °C and with the increas of temperature, the average polymerization degree of proanthocyanidin is gradually decreased and still decreased at 90 °C. However, considering the procyanidins is active substances, high temperature will reduce the activity of a phenolic hydroxyl group. So choose the best hydrolysis temperature for 80 °C.

Effect of sodium bisulfite concentration: 1 mL of degradation material were added to five cuvettes, then 10 mL of 0.5, 1-7 % sodium hydrogen sulfite solution were added, reaction for 90 min at 70 °C, cooled to room temperature water taken, cool to room temperature, the average polymerization degree was measured. The effect of sodium bisulfite concentration on the average polymerization degree was shown Fig. 3.

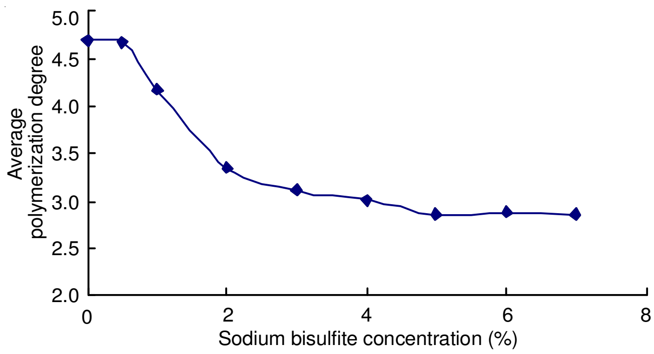

Fig. 3. Effect of sodium bisulfite concentration on the average polymerization degree

From Fig. 3, with the increase of concentration of sodium bisulfite, the average polymerization degree of proanthocyanidin is gradually decreased, highest drop in 1 % and become slow after than, when sodium bisulfite reached 5 %, procyanidins average degree of polymerization is basically no longer change.

Effect of hydrolysis time: 1 mL of degradation material were added to six cuvettes, then 9 mL of 5 % solution of sodium bisulfite solution were added, respectively reaction for 15, 30, 60, 90, 120, 150 min at 70 °C, cooled to room temperature and the average polymerization degree was measured. The effect of hydrolysis time on sodium bisulfite concentration was shown in Fig. 4.

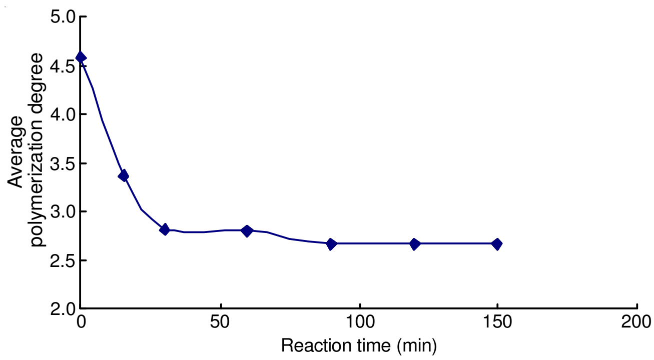

Fig. 4. Effect of hydrolysis time on sodium bisulfite concentration

Fig. 4 showed that the extension of reaction time gradually, the average polymerization degree of procya-nidins decreased and within 0.5 h before amplitude is large, then slow degradation, 90 min after the average polymerization degree is basically no longer change.

Orthogonal reach: Iinvestigating the dosage of sodium bisulfite, hydrolysis temperature, hydrolysis time, sodium bisulfite concentration, design orthogonal tests, optimize hydrolysis conditions, the various levels of factors as shown in Table-1.

TABLE-1
ORTHOGONAL EXPERIMENT L (4^3) HORIZONTAL FACTOR TABLE

Level	Factors			
	Temp. (°C) A	Time (min) B	Dosage of sodium bisulfite (mL) C	Sodium bisulfite concentration (%) D
1	60	30	7	3
2	70	60	8	4
3	80	90	9	5

Single factor results show that the dosage of sodium bisulfite, concentration of sodium bisulfite, hydrolysis time and temperature have a great influence on the average polymerization degree. This article designed the four factors three levels orthogonal experiment, with the average polymerization degree as the most optimal index, the optimum hydrolysis conditions were determined (Table-2).

TABLE-2
HYDROLYSIS ORTHOGONAL TEST RESULTS AND ANALYSIS

Test number	Temp. (°C) A	Time (min) B	The dosage of sodium bisulfite (/mL) C	Sodium bisulfite concentration (%) D	The average polymerization degree
1	1	1	1	1	3.11
2	1	2	2	2	2.79
3	1	3	3	3	2.53
4	2	1	2	3	2.68
5	2	2	3	1	2.62
6	2	3	1	2	2.51
7	3	1	3	2	2.55
8	3	2	1	3	2.47
9	3	3	2	1	2.46
K1j	2.81	2.78	2.70	2.73	–
K2j	2.60	2.62	2.59	2.61	–
K3j	2.49	2.5	2.57	2.56	–
Rj	0.32	0.28	0.13	0.17	–

By orthogonal test and extreme difference analysis, three factors impact on the average polymerization degree size, the order is A > B > D > C. Temperature > time > bisulfite concentration>the dosage of sodium bisulfite. Optimal combination of A3B3C3D3, namely the reaction temperature 80 °C, reaction time 1.5 h, solid-liquid ratio of 1:9, sodium bisulfite concentration of 5 %, in this condition the average polymerization degree of the sample from 4.6 down to 2.3.

DPPH clearance test: Comparison of the degradation by DPPH radical scavenging capacity procyanidins are shown in Fig. 5.

We can see from Fig. 5, after degradation of DPPH radical scavenging is more activity than procyanidins before degradation proanthocyanidins. According curve equation, calculate the IC_{50} values in Table-3.

Table-3 summarizes that after degradation of procyanidins relative to before degradation of procyanidins has better ability of removing DPPH , the IC_{50} (mg/L) value dropped

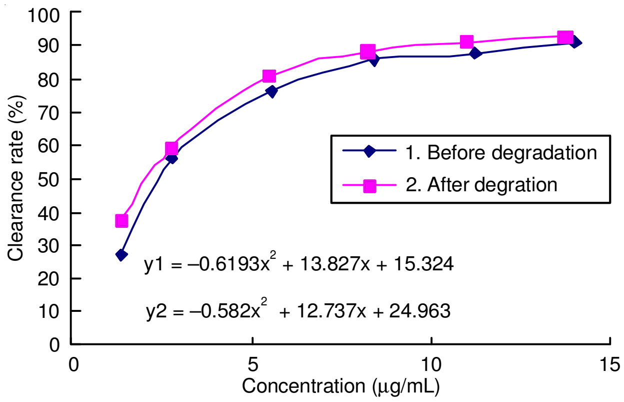

Fig. 5. DPPH radical scavenging capacity procyanidins

TABLE-3
IC_{50} VALUE OF PROCYANIDINS REMOVE DPPH

Sample	IC_{50} (mg/L)
Before hydrolysis procyanidins	2.88
After hydrolysis procyanidins	2.18

to 2.18 from 2.88 shows that sodium bisulfite degradation method is scientific and feasible in theory and practice.

(+)-Catechin and (-)-epicatechin monomer as examining index, characterized by the difference of liquid chromatogram hydrogenated similarities and differences between before and after hydrogenation procyanidins results are as follows.

As can be seen from Fig. 6, the retention time of catechin, epicatechin were 18.794, 30.186 min, Figs. 7 and 8 show that catechin and epicatechin content were increased 3.1-fold and 1.8 times after degradation respectively and new peaks appeard at 10.942, 12.864, 24.150, 25.833 min. It is likely to be the new hydrolysis product. In Fig. 7 liquid 72.069 min disappeared after hydrolysis, there may be decreased degradation. HPLC analysis results strongly indicate that, after the process of degradation of sodium hydrogen sulfite, grape seed high polymer proanthocyanidins cleavage of oligomeric proanthocyanidins, further evidence of the feasibility of this condition.

Conclusion

By investigating the concentrations of $NaHSO_3$, the dosage of $NaHSO_3$, reaction time, reaction temperature on the influence of the average polymerization degree of procyanidins, it is

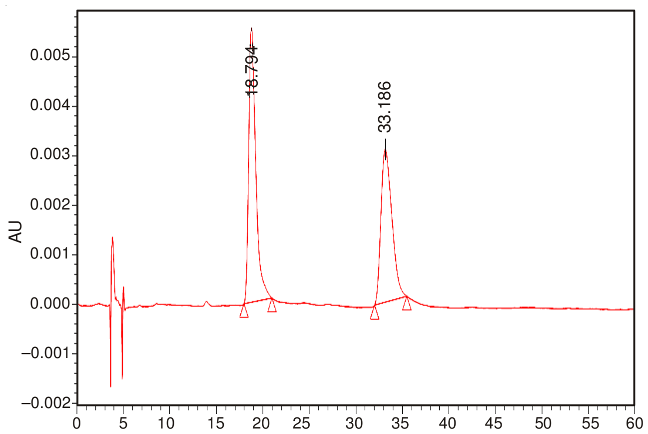

Fig. 6. Hybrid reference substance by HPLC chromatogram

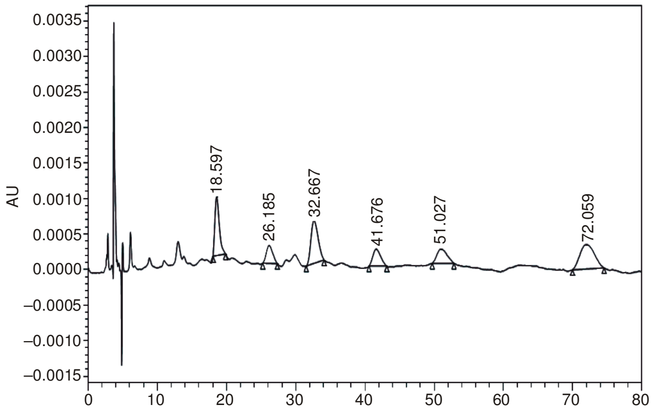

Fig. 7. Before hydrolysis proanthocyanidins by HPLC chromatogram

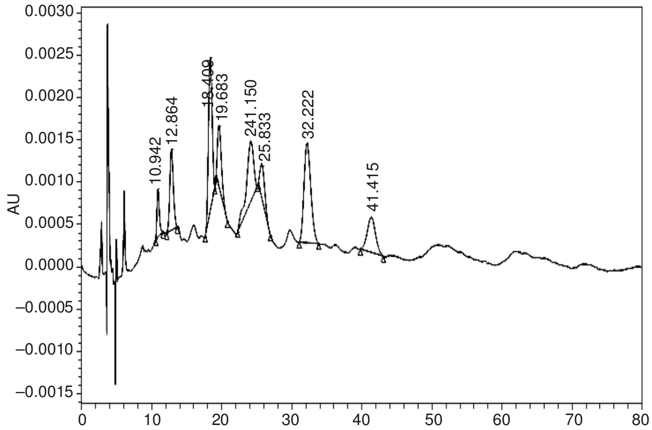

Fig. 8. After hydrolysis proanthocyanidins by HPLC chromatogram

concluded that the optimal hydrolysis conditions: reaction temperature 80 °C, reaction time 1.5 h and solid-liquid ratio of 1:9, 5 % of $NaHSO_3$, under the condition the average polymerization degree of reaction samples from 4.6 to 2.3, the IC_{50} (mg/L) value dropped to 2.18 from 2.88, the antioxidant activity to enhance and the operation is simple, low cost, easy to operate, sodium bisulfite is easy to remove and suitable for industrial production.

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