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The Influences of Concentrate Extract Properties and Ethanol Addition Amount on the Ethanol Precipitation Process of Salvia Miltiorrhiza

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Abstract

In this work, 10 batches of Salvia miltiorrhiza concentrate were prepared and purified with ethanol precipitation process. Dry matter content, pH value, conductivity and water content of the concentrates and supernatants were all determined. When more ethanol was used in ethanol precipitation, the pH value of the supernatant generally increased, but dry matter content, water content, and the conductivity decreased. Multivariate linear models were built with the most determination coefficient values greater than 0.7. More than 80% of stachyose was removed in the ethanol precipitation process. The removal rate of fructose, raffinose and sucrose were all higher than 30%. When ethanol addition amount increased, the purity of phenolic acids in the supernatant increased, but the retention of lithosperimic acid and salvianolic acid B decreased. The conductivity and pH value of concentrated extract show relatively small influences on ethanol precipitation indices. When fructose, raffinose, or stachyose contents in the concentrated extract were high, the retention rate of phenolic acids tends to be low on most occasions. The purity and retention rate of phenolic acids in the supernatants were also affected by the purity of phenolic acids in the concentrated. The sugar contents in the concentrate are suggested to be monitored in industry because they significantly affect ethanol precipitation process indices.

Keywords

Ethanol Precipitation Process, Salvia Miltiorrhiza, Nature of Concentrate, Sugars, Stepwise Regression

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1. Introduction

Ethanol precipitation is a commonly used purification process in Chinese medicine industry [1]. The advantages of ethanol precipitation process include simple operation, safe solvent, and high impurity removal rate [2]. The ethanol precipitation process mainly realizes the impurity removal by reducing the solubility of impurities by adding ethanol. Impurities mainly include water-soluble sugars, proteins, and salts [3], etc. Increasing the relative density of concentrate, increasing the amount of ethanol, and decreasing the refrigeration temperature are conducive to improve the removal rate of water-soluble sugars, such as glucose, sucrose, maltose, raffinose [3] [4] [5], etc.

However, ethanol precipitation process will also lead to the loss of active ingredients [6] [7]. By optimizing the parameters of ethanol precipitation process, such as system pH, ethanol amount, ethanol adding speed and ethanol concentration [8] [9], refrigeration temperature [10], and refrigeration time [11], it is possible to reduce the loss of active ingredients. In addition, controlling the properties of concentrated extract before ethanol precipitation also affects the loss of active ingredients [12]. For example, Yan *et al.* [13] found that the contents of Danshensu, caffeic acid and salvianolic acid B in Guanxinning concentrate can significantly affect the retention of active ingredients after ethanol precipitation. Zhang *et al.* [14] found that the pH value and caffeic acid content of salvia miltiorrhiza concentrate can significantly affect the retention rate of phenolic acids in ethanol precipitation. However, the studies mentioned above did not investigate the effects of sugars in concentrated extract on the purification of ethanol precipitation.

Salvia miltiorrhiza is a medicinal herb for promoting blood circulation and removing blood stasis. Its main active ingredients are phenolic acids and tanshinones. Salvia miltiorrhiza extract is usually purified by ethanol precipitation process. In this study, taking the ethanol precipitation process of Salvia miltiorrhiza extract as an example, multiple batches of Salvia miltiorrhiza concentrate were treated with ethanol precipitation process. The contents of sugars and phenolic acids before and after ethanol precipitation were determined. Some other material properties including dry matter content, pH value, and conductivity were also determined. Mathematical models were built to investigate the influence of the composition of the concentrate and the ethanol addition amount on the ethanol precipitation process. Finally, the critical indices of Salvia miltiorrhiza concentrate were determined.

2. Materials and Methods

2.1. Experimental Reagents

Ethanol (analytical pure) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. Methanol (Chromatographic pure) was purchased from Merck Life Sciences (Shanghai) Co., Ltd. Acetonitrile (chromatographic purity) was

purchased from Merck Life Sciences (Shanghai) Co., Ltd. Fructose (batch No. A0282904) was obtained from ACROS organics, Belgium. Glucose (batch No. 20141121) reference substance was purchased from Sinopharm Chemical Reagent Co., Ltd. Sucrose (batch No. WXBB0906V) was purchased from sigma Aldrich company of the United States. Danshensu sodium (batch No. 160120), protocatechuic aldehyde (batch No. 160125), caffeic acid (batch No. 180103), rosmarinic acid (batch No. 150901), lithospermic acid (batch No. 190530), salvianolic acid B (batch No. 200107), raffinose (batch No. 180923) and stachyose (batch No. 171129) were purchased from Shanghai Ronghe Pharmaceutical Technology Co., Ltd. Deionized water. Anhydrous methanol (80080418) was purchased from Sinopharm Chemical Reagent Co., Ltd. Karl Fischer Reagent (k820873) was purchased from Hangzhou ChenTong Materials Co., Ltd. Deionized water was produced with an ultra-pure water machine (Milli-q, Millipore Company).

2.2. Preparation of Concentrate Samples

Salvia miltiorrhiza of 200 g was put into the decocting pot (FT-30H, Zhushuixi Electric). Then water was added with liquid-solid mass ratio of 8:1. Salvia miltiorrhiza was soaked for 30 min before decoction. Salvia miltiorrhiza was then decocted for 30 min and the extract was collected. After that, Salvia miltiorrhiza was decocted again to obtain aqueous extract. The extracts were combined. After concentration, Salvia miltiorrhiza concentrate was obtained with a relative density of 1.15 - 1.25. The weight and volume of a concentrate were both measured to obtain the density value of the concentrate. Ten different batches of Salvia miltiorrhiza concentrates were prepared.

2.3. Preparation of Supernatant Samples

Each batch of Salvia miltiorrhiza concentrate was put into conical bottles. A 95% (v/v) ethanol solution was added drop wisely with a pump (YZ15, Changzhou weixier Fluid Technology Co., Ltd.) under magnetic stirring (HJ-2A, Xicheng Xinrui Instrument Factory). The mass ratio of ethanol solution to concentrated extract (ECR) was 1.2, 1.5, 2.0 and 3.0, respectively. After adding ethanol, stirring was kept for 20 minutes and the conical bottles were sealed with sealing film. In pre-experiments, it is found that supernatant composition changed little after refrigeration for more than 12 hours. Therefore, the conical bottles were refrigerated for more than 12 hours at 4°C. After that, the supernatant was collected.

2.4. Determination of Dry Matter, Conductivity and pH of Concentrated Extract and Supernatant

A weighing bottle was dried in a drying oven (XMTD-8222, Shanghai Jinghong Experimental Equipment Co., Ltd., IBAO-250, stukai instrument equipment (Shanghai) Co., Ltd.) at 105°C for 1 h. Then its weight (m_1) was determined with

an electronic balance (AB204-N, METTLER TOLEDO Shanghai Co., Ltd.). An appropriate amount of concentrated extract was added. The total weight of the bottle and concentrated extract was weighed (m_2). The weighing bottle was dried in the oven at 105°C for 3 h. Then it was taken out and weighed. The total weight of the bottle and dried extract was m_3. After that, the weighing bottle was dried in the oven at 105°C for 1 h and weight again. The new weight was m_4. If the difference between m_3 and m_4 was less than 5 mg, the drying was stopped. The dry matter can be calculated with Formula (1). Dry matter value was determined three times in parallel.

$$\text{Dry matter} = \frac{m_4 - m_1}{m_2 - m_1} \tag{1}$$

0.50 g concentrated extract sample was weighed and diluted with deionized water to 50 mL. Its conductivity was measured with a conductivity meter (DDBJ-350, Hangzhou Qiwei Instrument Co., Ltd.). The sample conductivity of ethanol precipitation supernatant can be directly measured by the conductivity meter. The pH of supernatant and concentrate were measured by a pH meter (SevenMulti series, METTLER TOLEDO Shanghai Co., Ltd.).

## 2.5. Determination of Phenolic Acid Contents in Concentrated Extract and Supernatant

Preparation of test solution: a 10 mL volumetric flask was taken. An appropriate amount of concentrated extract or supernatant was added. The sample was diluted with 70% methanol solution (V/V) and shaken well. The concentrated extract sample was then centrifuged at 12,000 rpm for 10 min. Then it was filtered with a 0.22 μm filter membrane to obtain the test solution. The ethanol precipitation supernatant sample can be directly filtered with a 0.22 μm filter membrane to obtain the test solution.

Preparation of reference solution: a 10 mL volumetric flask was taken. 2.65 mg, 0.88 mg, 1.89 mg, 1.18 mg, 4.58 mg and 8.49 mg of Danshensu sodium, protocatechuic aldehyde, caffeic acid, rosmarinic acid, lithospermic acid and salvianolic acid B were accurately weighed with analytical balances (AB204-N, AE240, METTLER TOLEDO Shanghai Co., Ltd.), respectively. 70% methanol solution (V/V) was added to dissolve the references. The solution was shaken well to prepare a mixed stock solution.

Phenolic acid contents were determined with an HPLC system with a UV detector (FL5090, Zhejiang Fuli Analytical Instrument Co., Ltd.; Agilent 1100, Agilent Technology Co., Ltd.). Chromatographic conditions and detector parameters were taken from literature [15]. An Extend-C18 (250 mm) × 4. 6 mm, 5 μm) chromatographic column was used. The mobile phase was composed of 0.1% formic acid in water (A) and acetonitrile (B). Elution gradient was shown in Table 1. Column temperature was 25°C. The flow rate was 1 ml/min and the detection wavelength was 281 nm. Injection volume was 5 μL. The typical chromatogram obtained was shown in Figure 1.
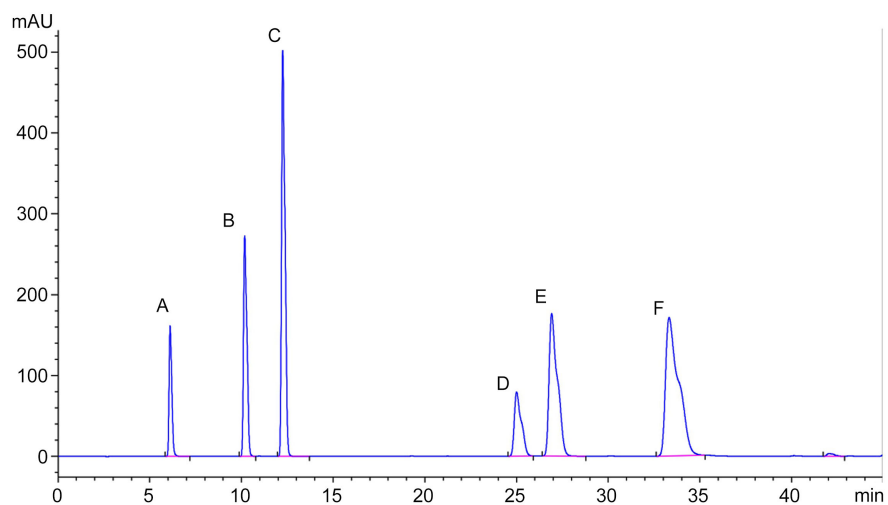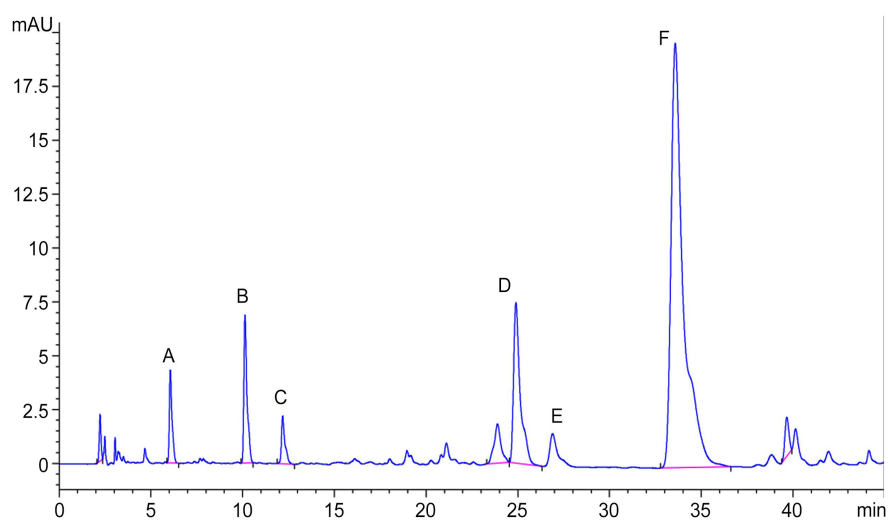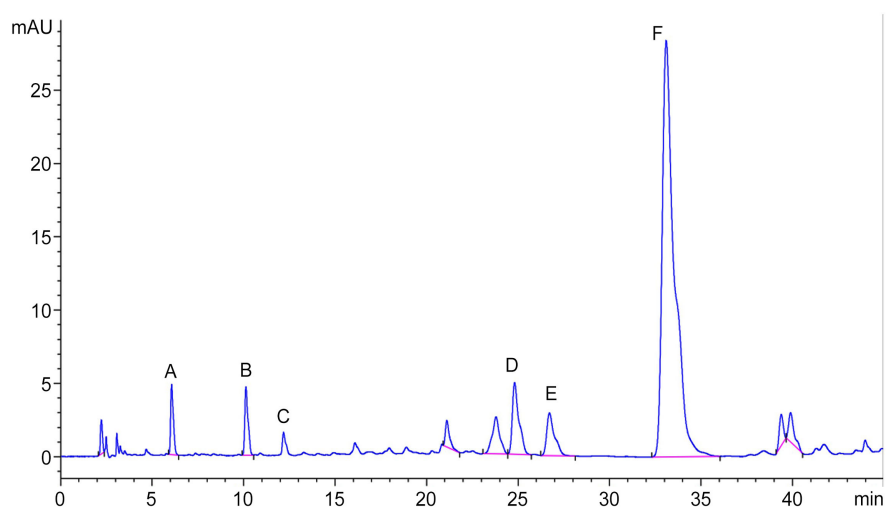
Figure 1. HPLC chromatogram of phenolic acids. A: Danshensu; B: Protocatechuic aldehyde; C: Caffeic acid; D: Rosmarinic acid; E: Lithospermic acid; F: Salvianolic acid B.

Table 1. Elution gradient for the determination of salvianolic acids.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0 - 10	93 - 83	7 - 17
10 - 16	83 - 79	17 - 21
16 - 32	79 - 71	21 - 29
32 - 40	71 - 65	29 - 35
40 - 44	65 - 28	35 - 72
44 - 45	28 - 25	72 - 75

2.6. Determination of Sugar Content in Concentrated Extract and Supernatant [16]

Preparation of test solution: a 10mL volumetric flask was taken. An appropriate amount of concentrated extract or supernatant was added. The sample was diluted with 80% acetonitrile solution (V/V) and shaken well. Then it was filtered with a 0.22 μm filter membrane to obtain the test solution.

Preparation of reference stock solution: 4.99 mg, 4.60 mg, 4.06 mg and 5.51 mg of fructose, sucrose, raffinose and stachyose were accurately weighed with analytical balances (AB204-N, AE240, METTLER TOLEDO Shanghai Co., Ltd.) respectively into a 5mL volumetric flask. A small amount of deionized water was added to dissolve them. Then the volume was fixed with deionized water and shaken well to obtain the mixed reference stock solution.

Sugar contents were determined with an HPLC system with an ELSD detector (Agilent 1260, Agilent Technology Co., Ltd.). A Carbohydrate ES (250 mm × 4.6 mm, 5 μm) Chromatographic column was used. The mobile phase was composed of water (A) - acetonitrile (B). Elution gradient was shown in Table 2. Column temperature was 25°C. Injection volume was 5 μL. Flow rate was 0.6 mL/min. Evaporative light scattering detector atomizer (ELSD) temperature was 70°C. Drift tube temperature was 60°C. Nitrogen flow rate was 1.0 L/min. The typical chromatogram obtained was shown in Figure 2.

2.7. Model Building

The effects of ethanol precipitation were characterized with phenolic acid purity in the supernatant and phenolic acid retention after precipitation. They were calculated with Formulas (2) and (3) and considered as process indices.

$$\text{Phenolic acid purity} = \frac{\text{Phenolic acid content}\left(\text{mg/g materials}\right)}{\text{Dry matter}\left(\text{mg/g materials}\right)} \times 100\% \quad (2)$$

$$\begin{aligned}\text{Phenolic acid retention} =\\ \frac{\text{Phenolic acid content in supernatant} \times \text{Supernatant weight}}{\text{Phenolic acid content of concentrate} \times \text{Concentrate weight}} \times 100\%\end{aligned} \quad (3)$$

The quantitative models were established by Formula (4) to correlate concentrated extract indices, ethanol addition amount, and process indices.

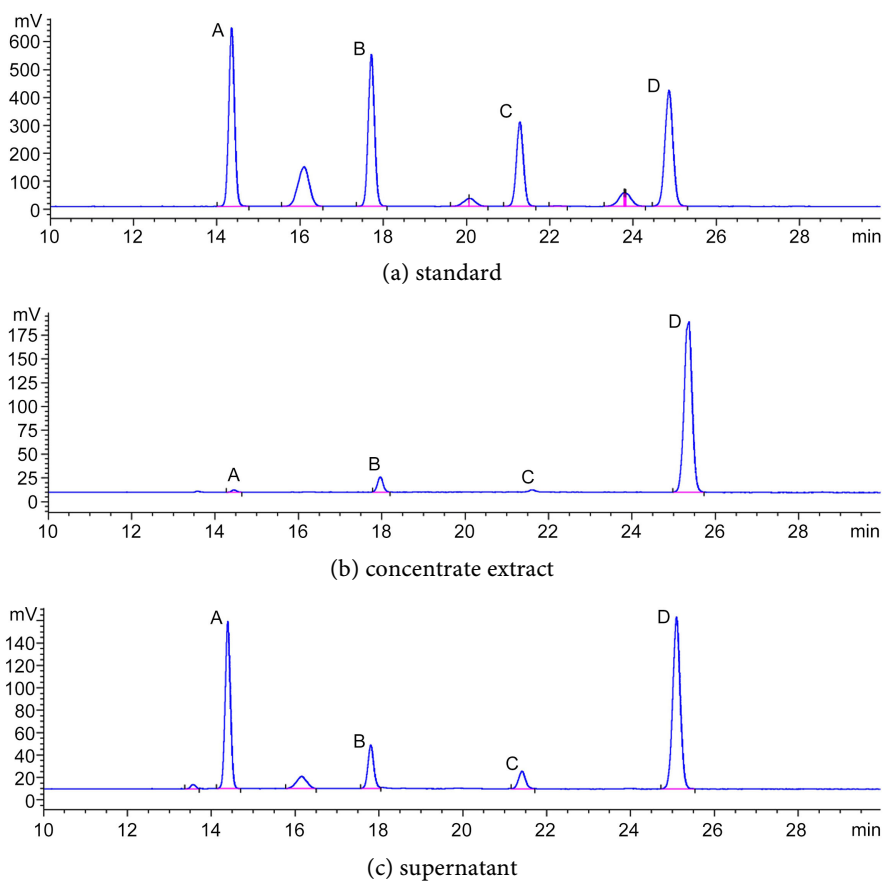$$Y = b_0 + \sum_{i=1}^{n} b_i z_i + cx \quad (4)$$

Figure 2. HPLC-ELSD chromatogram of sugars. A: Fructose; B: Sucrose; C: Raffinose; D: Stachyose.

Table 2. Elution gradient for the determination of seven sugars.

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0 - 5	20 - 25	80 - 75
5 - 10	25 - 35	75 - 65
10 - 28	35 - 50	65 - 50
28 - 29	50 - 80	50 - 20
29 - 39	80	20
39 - 40	80 - 20	20 - 80

where y was the process indices, b_0 was the constant term, b_i and c were the partial regression coefficients, Z_i was a concentrate index, n was the number of concentrate indices, and X represent ECR. The model was simplified by stepwise regression, and the P values of moving in and moving out a term were both 0.05.

3. Experimental Results

3.1. Quality Index of Concentrate

The dry matter content, pH value, and conductivity of ten batches of Salvia miltiorrhiza concentrate are shown in **Table 3**. Most of the dry matter content were

more than 40.0%. The pH value varied from 4.5 - 6.0. The conductivity was between 4 and 8 mS/cm.

Phenolic acid purity of each batch of concentrated extract is shown in **Table 4**. Salvianolic acid B was the component with the highest purity, and the purity were all more than 4%. Followed by Danshensu and lithospermic acid, and their purity values were between 0.2% - 0.6%. The purity value of protocatechuic aldehyde and caffeic acid were lower than 0.1%. The purity of rosmarinic acid varied from 0.1% to 0.8%.

Sugar content of concentrated extract was calculated with Formula (5) and shown in **Table 5**. The content of stachyose was the highest, mostly more than 30.0%. The contents of fructose and raffinose were low, mostly less than 1.0%. The content of sucrose in each group varied greatly, ranging from 0.2% to 2.0%.

$$\text{Sugar content} = \frac{\text{Sugar concentration}\,(\text{mg/g materials})}{\text{Dry matter}\,(\text{mg/g materials})} \times 100\% \qquad (5)$$

Table 3. Determination results of concentrate mass, dry matter, pH and conductivity.

Medicinal Material Batch	Dry matter content (%)	pH	Conductivity (mS/cm)
200525	43.84	5.290	4.88
180927	41.50	5.377	4.16
181001	41.91	4.659	6.47
180901	42.66	5.132	5.98
200501	41.70	5.354	4.22
200502	43.36	5.283	5.08
200503	40.17	5.564	5.98
200504	36.27	5.432	4.96
200505	45.01	5.541	7.37
200506	46.07	5.213	4.32

Table 4. Determination results of phenolic acid purity of concentrated extract.

Medicinal Material Batch	Danshensu (%)	Protocatechuic Aldehyde (%)	Caffeic Acid (%)	Rosmarinic Acid (%)	Lithospermic acid (%)	Salvianolic Acid B (%)
200525	0.451	0.052	0.035	0.291	0.449	9.38
180927	0.532	0.055	0.033	0.178	0.442	5.31
181001	0.558	0.026	0.020	0.241	0.393	7.51
180901	0.467	0.029	0.022	0.088	0.338	4.29
200501	0.397	0.051	0.020	0.210	0.356	6.16
200502	0.418	0.056	0.023	0.193	0.352	6.33
200503	0.403	0.054	0.032	0.780	0.313	5.91
200504	0.431	0.055	0.020	0.206	0.273	6.22
200505	0.447	0.071	0.029	0.316	0.282	6.11
200506	0.408	0.038	0.029	0.218	0.322	6.23

Table 5. Determination results of sugar content in concentrated extract.

Medicinal Material Batch	Fructose (%)	Sucrose (%)	Raffinose (%)	Stachyose (%)
200525	0.195	1.937	0.310	34.45
180927	0.700	0.166	0.532	40.50
181001	1.123	0.359	0.408	31.68
180901	0.912	0.168	0.205	25.38
200501	0.278	0.417	0.446	48.91
200502	0.376	1.647	0.519	38.90
200503	0.318	0.286	0.400	31.38
200504	0.355	1.659	0.337	30.44
200505	0.282	1.775	0.368	34.88
200506	0.324	1.197	0.388	43.92

3.2. Quality Index of Supernatant

Dry matter content, pH value, conductivity and water content of the ethanol precipitation supernatants were determined and shown in Figure 3. Dry matter content, conductivity and water content decreased as ethanol addition amount increased. Supernatant pH value slightly increased as ethanol addition amount increased, and varied from 5.0 and 6.0. The conductivity ranged from 3 to 20 mS/cm. Dry matter content of a supernatant was less than 12%. Water content of a supernatant was between 20% - 35%.

The phenolic acid purity values of ethanol precipitation supernatant samples are shown in Figure 4. Phenolic acid purity values increased as ethanol addition amount increased. Salvianolic acid B was the component with the highest purity, which ranged from 5.0% to 20%. Most of other phenolic acid purity values were less than 2%. Compared with that of concentrated extract, phenolic acid purity increased after ethanol precipitation. This phenomenon agreed well with those observed in previous works. The main reason is that a large amount of dry matter was removed in the ethanol precipitation process [6] [17].

Figure 5 shows the retention rate of phenolic acids. The retention rate of salvianolic acid B and lithospermic acid slightly decreased as ethanol addition amount increased. The retention rate of protocatechuic aldehyde and caffeic acid varied from 45.0% to 90.0%. Most of rosmarinic acid retention rate values were above 50.0%. Most of lithospermic acid, Danshensu and salvianolic acid B retention values were below 50%.

Sugar content in dry matter is shown in Figure 6. With the addition of ethanol, fructose content and sucrose content generally increased, but stachyose content significantly decreased. At most occasions, stachyose content was much higher than that of other sugars. After ethanol precipitation, fructose content and sucrose content were less than 10% and 15%, respectively. Raffinose content was less than 3%. Stachyose content was always higher than 10%.
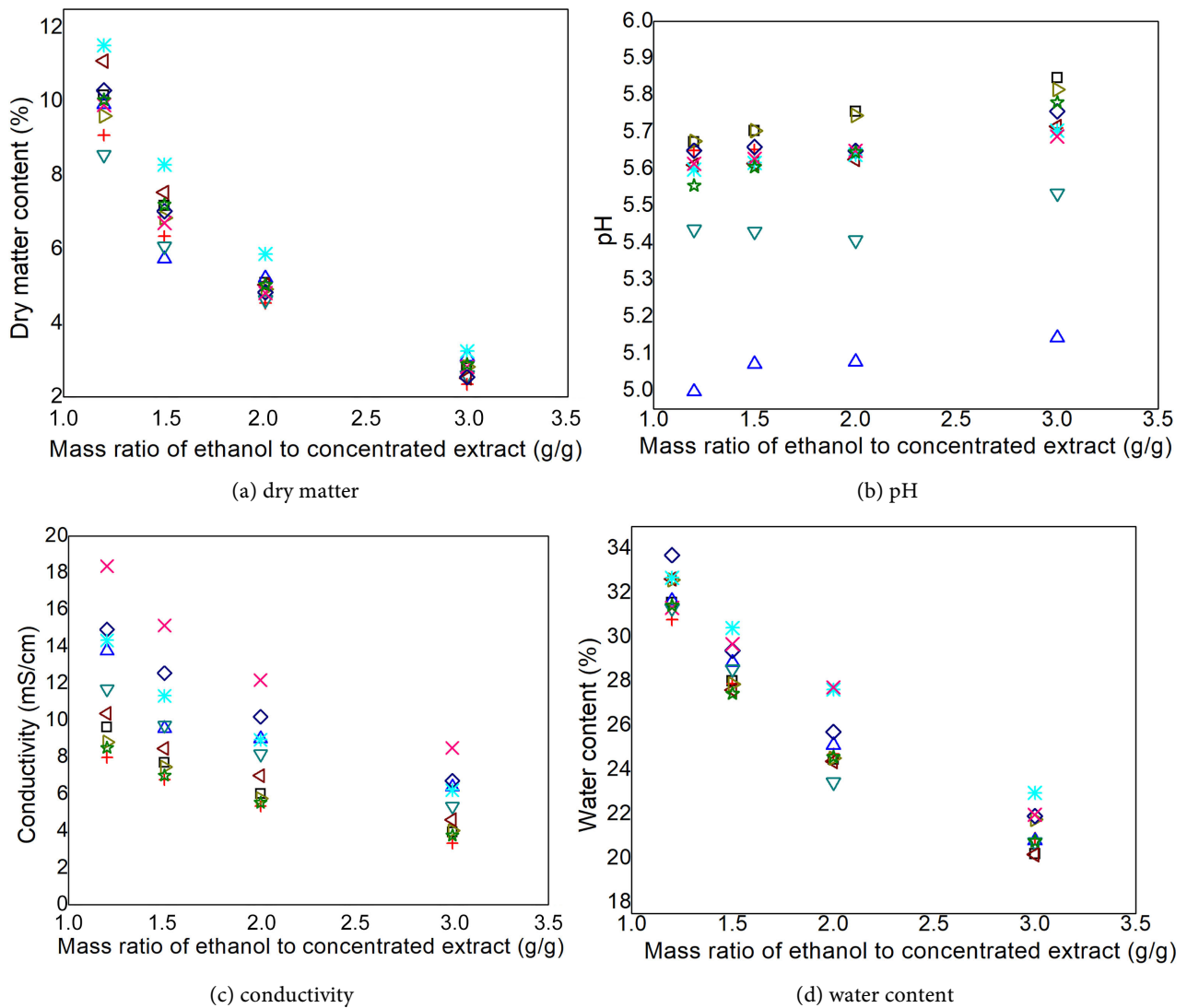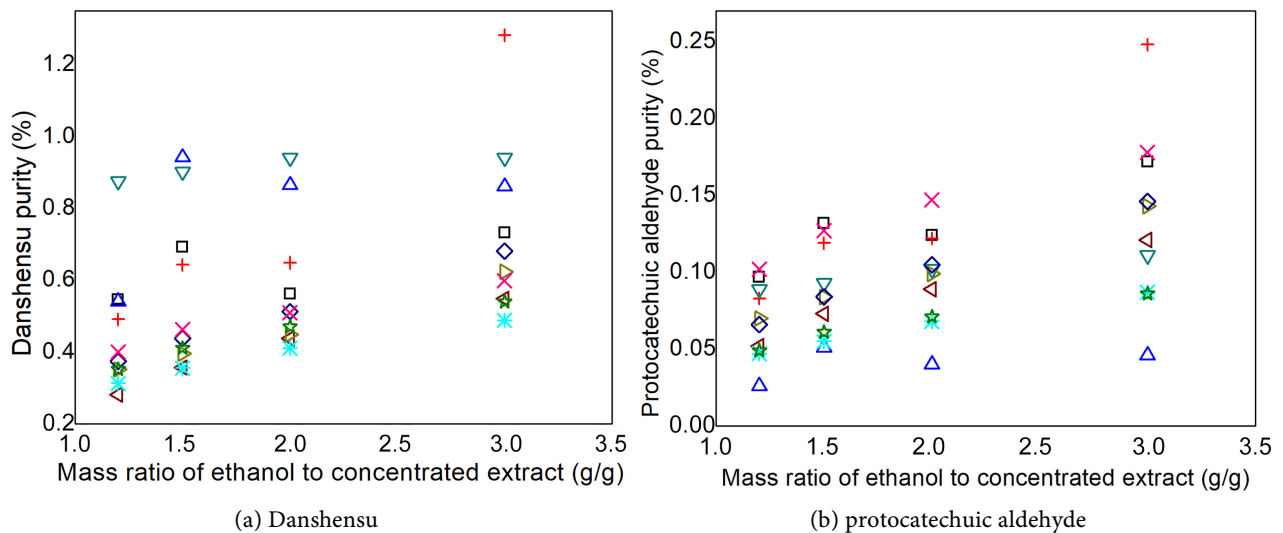
Figure 3. Dry matter content, pH value, conductivity and water content results of supernatant. □ 200525; + 180927; △ 181001; ▽ 180901; ◁ 200501; ▷ 200502; ◇ 200503; ✳ 200504; ✕ 200505; ☆ 200506.
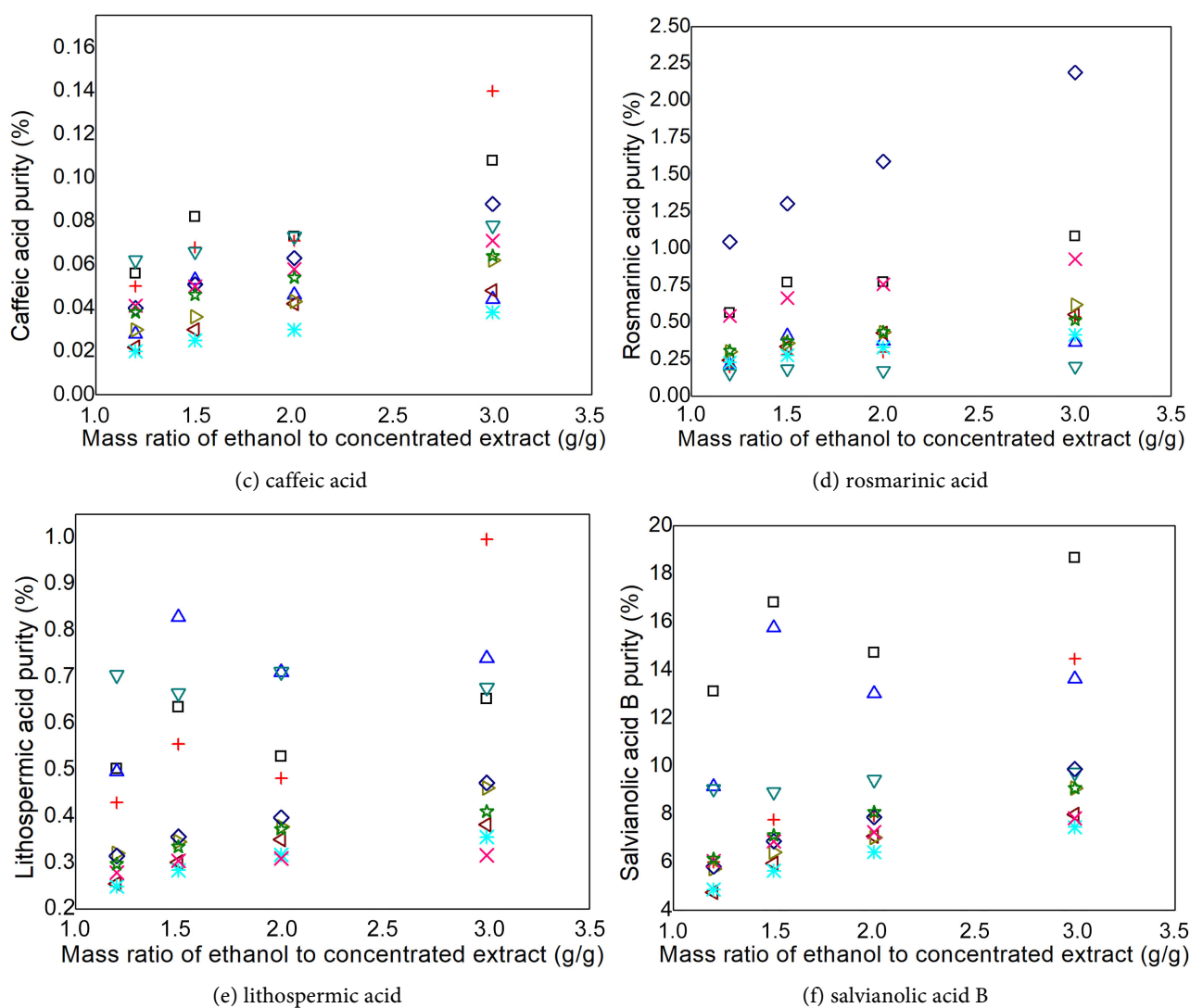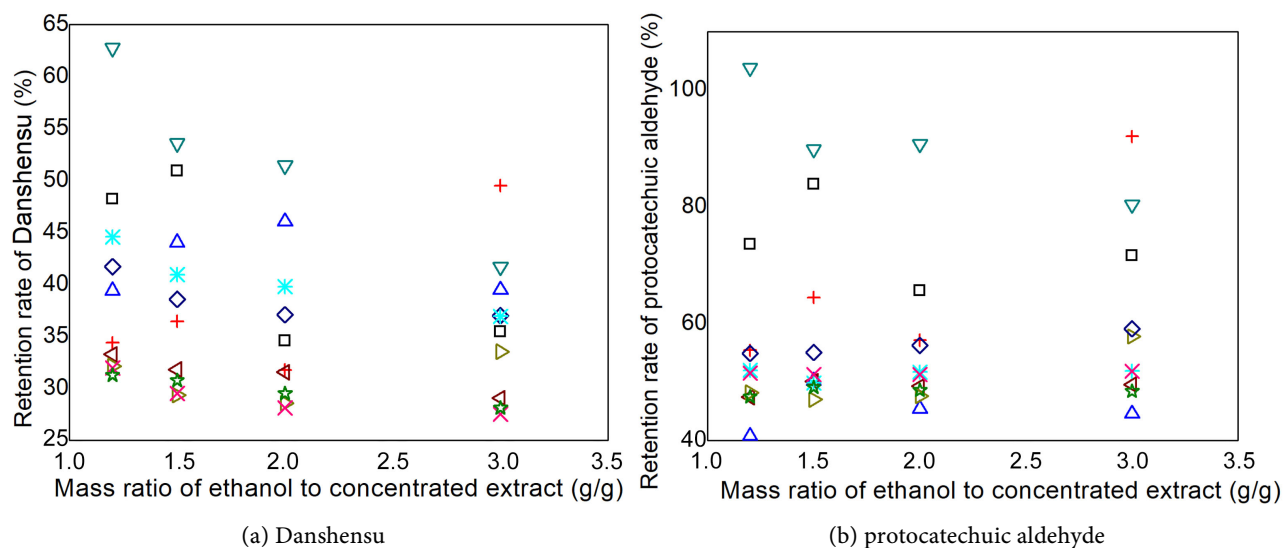
Figure 4. Phenolic acid purity of supernatant. □ 200525; + 180927; △ 181001; ▽ 180901; ◁ 200501; ▷ 200502; ◇ 200503; * 200504; ✕ 200505; ☆ 200506.

(c) caffeic acid

(d) rosmarinic acid
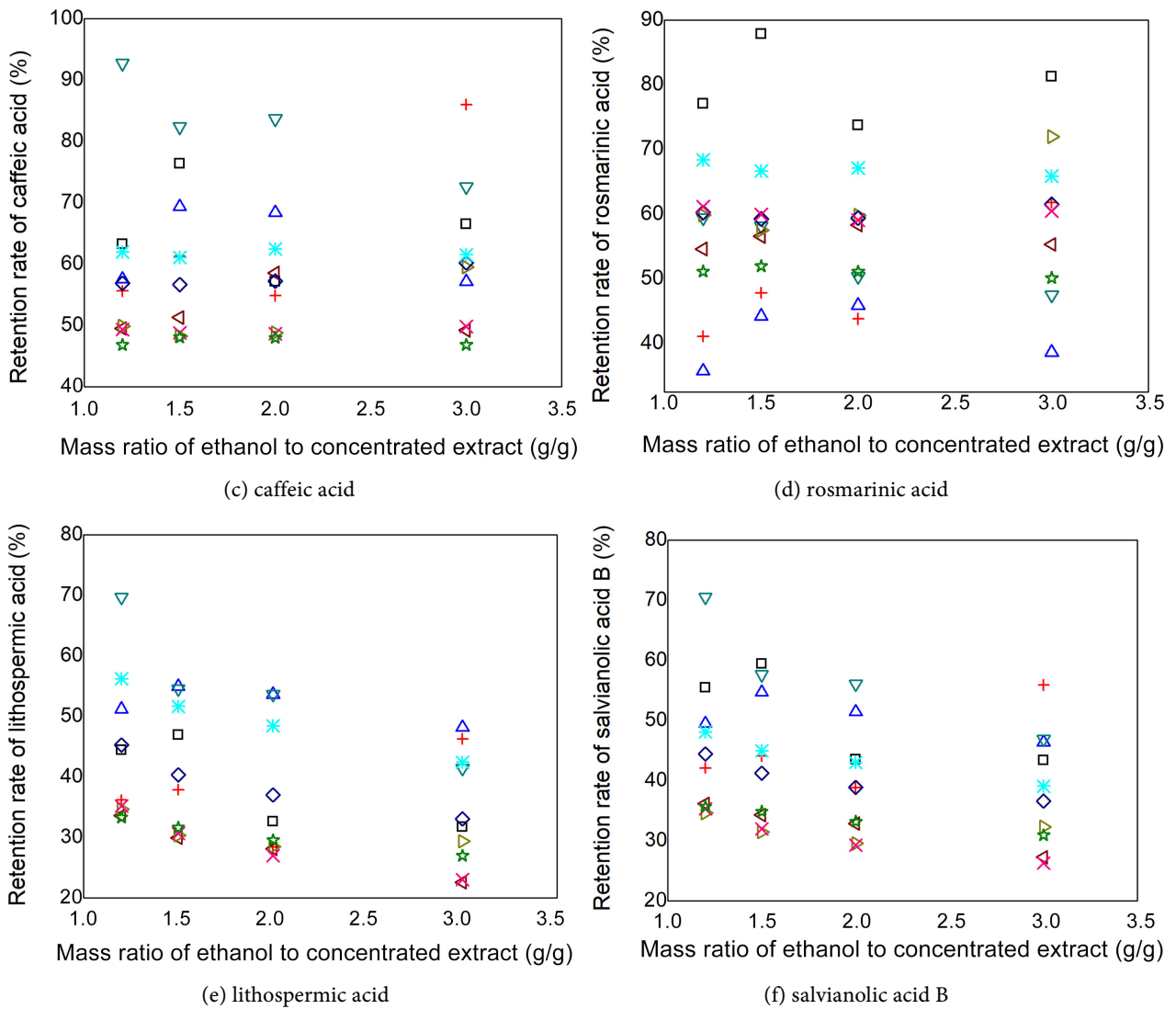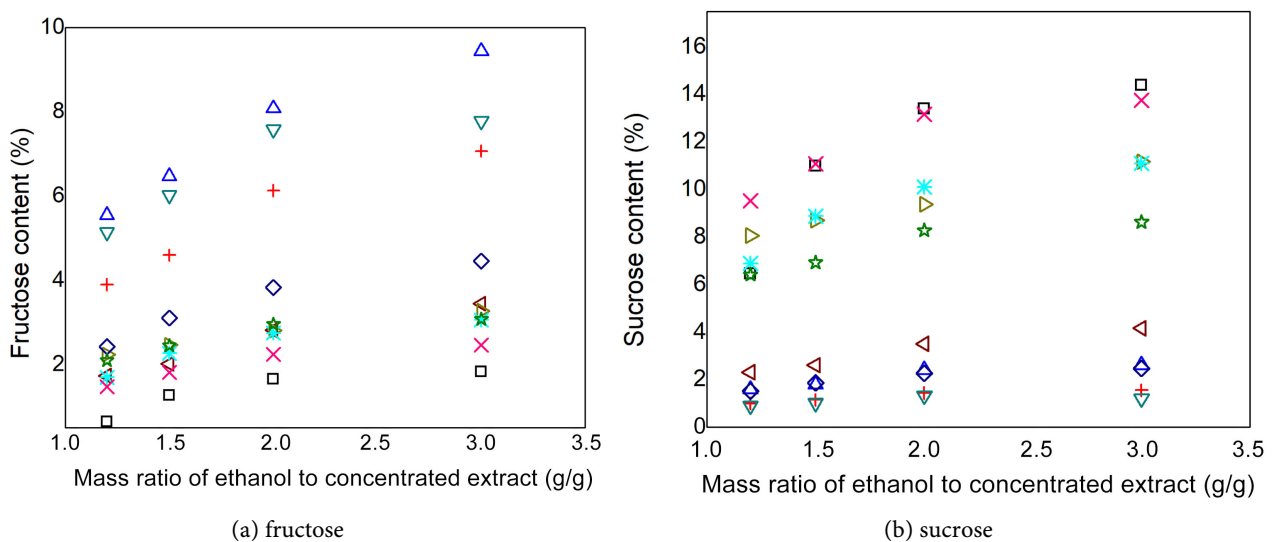

(e) lithospermic acid

(f) salvianolic acid B

Figure 5. Phenolic acid retention results. □ 200525; + 180927; △ 181001; ▽ 180901; ◁ 200501; ▷ 200502; ◇ 200503; * 200504; ✕ 200505; ☆ 200506.
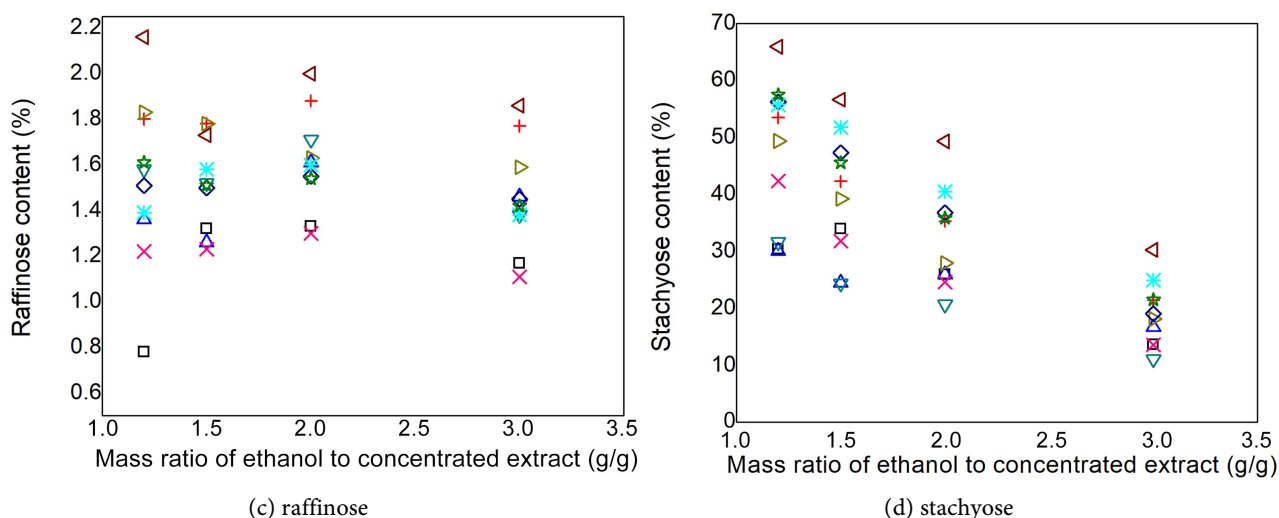

(a) fructose

(b) sucrose

Figure 6. Determination results of sugar content in supernatant. □ 200525; + 180927; △ 181001; ▽ 180901; ◁ 200501; ▷ 200502; ◇ 200503; * 200504; ✕ 200505; ☆ 200506.

Sugar removal rate was also calculated and shown in **Figure 7**. All the sugars were removed partly. The removal rate of raffinose and stachyose increased as ethanol addition amount increased. Stachyose removal rate was more than 80%. The removal rate of raffinose or sucrose was more than 50%. Fructose removal rate varied among batches, ranging from 30.0% to 80.0%.

3.3. Modeling Analysis

The quantitative models of process indices were established with Formula (4). The regression coefficients and variance analysis results of the models are shown in **Table 6**. The determination coefficient (R^2) values of most models are between 0.70 - 0.90, indicating that the model can explain most of the data variation.

Ethanol addition amount significantly affected most of process indices except the retention of protocatechuic aldehyde, caffeic acid and rosmarinus acid. The increase of ethanol addition amount can improve the purity of phenolic acids in the supernatant, but reduce the retention of lithosperimic acid and salvianolic acid B. The conductivity and pH value of concentrated extract affected a small number of process indices.

Sucrose content was removed in model building. The increase of fructose content in the concentrated extract can improve the purity of Danshensu and lithospermic acid in the supernatant, but reduce the retention rate of rosmarinic acid in the supernatant. The increase of raffinose content in the concentrated extract can reduce the purity of protocatechuic aldehyde, lithospermic acid and salvianolic acid B in the supernatant, and also reduce the retention rate of protocatechuic aldehyde and caffeic acid. The increase of stachyose content in concentrate reduced the retention of caffeic acid, rosmarinic acid, lithospermic acid, and salvianolic acid B. It can be concluded that higher content of fructose, raffinose, and stachyose in the concentrated extract generally led to lower retention rate of phenolic acids.
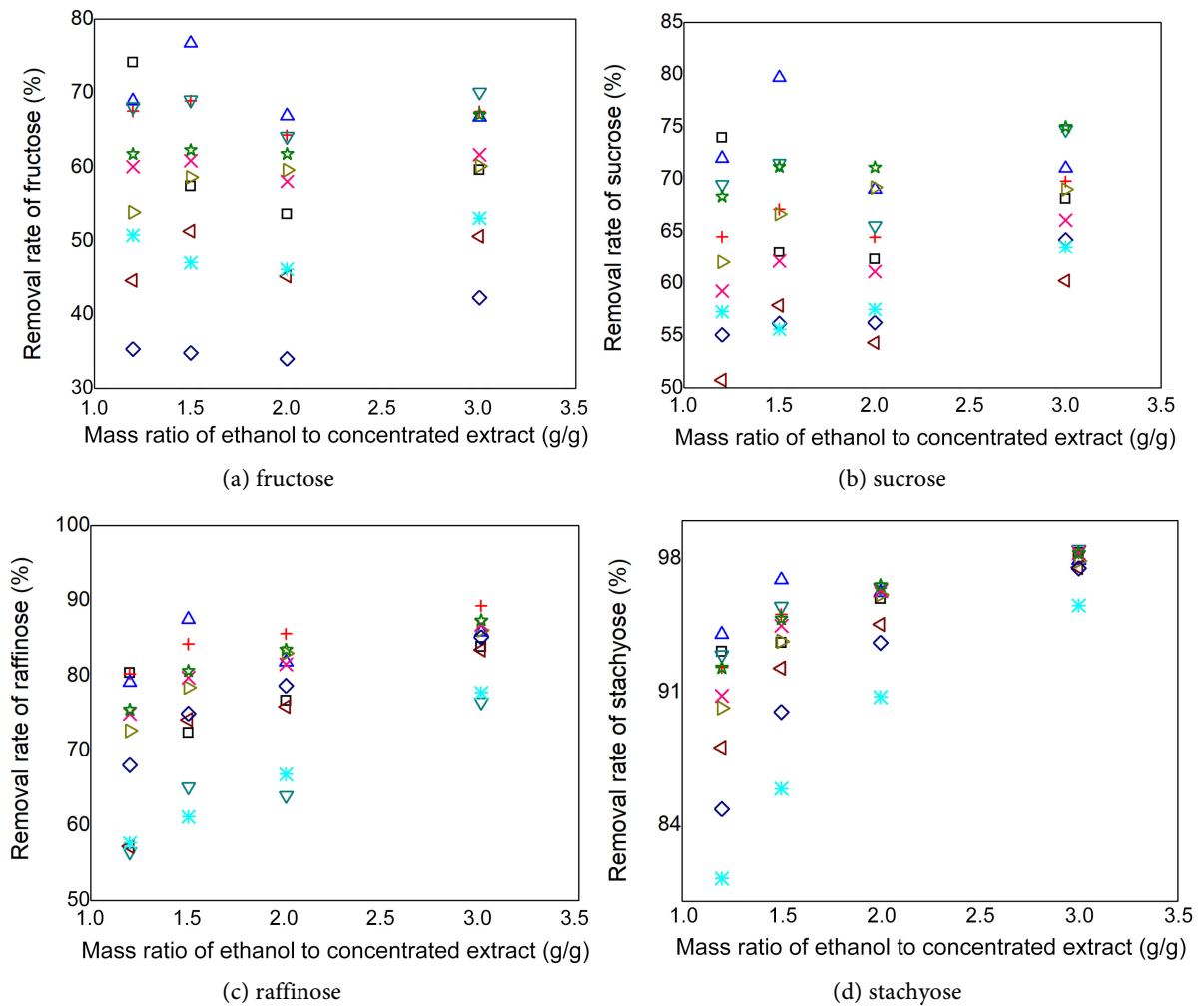
(a) fructose

(b) sucrose

(c) raffinose

(d) stachyose

Figure 7. Results of carbohydrate removal rate of supernatant. □ 200525; + 180927; △ 181001; ▽ 180901; ◁ 200501; ▷ 200502; ◇ 200503; * 200504; ✕ 200505; ☆ 200506.

Table 6. Regression coefficient and analysis of variance results.

Process index		Constant	ECR	Conductivity	pH	Fructose content	Raffinose content	Stachyose content	Purity of Danshensu	Purity of protocatechuic aldehyde	Purity of Caffeic acid	Purity of Rosmarinic acid	Purity of Lithospermic acid	Purity of Salvianolic acid B	R^2	R^2_{adj}
Purity of Danshensu	Regression coefficient	1.051	0.140			0.467					0.618					0.7436	0.7222
	P value		<0.0001			<0.0001					0.0004						
Purity of protocatechuic aldehyde	Regression coefficient	0.051	0.035					−0.113			0.144			0.099	−0.047	0.8185	0.7918
	P value		<0.0001					<0.0001			<0.0001			<0.0001	0.0020		
Purity of Caffeic acid	Regression coefficient	0.093	0.018					−0.017			0.103			0.050	−0.043	0.8163	0.7893
	P value		<0.0001					0.0024			<0.0001			<0.0001	<0.0001		
Purity of Rosmarinic acid	Regression coefficient	1.251	0.188								0.562	0.932				0.8973	0.8888
	P value		<0.0001								0.0033	<0.0001					
Purity of Lithospermic acid	Regression coefficient	0.329	0.078			0.236	−0.271							0.404		0.8121	0.7907
	P value		0.0002			<0.0001	0.0011							<0.0001			

Continued

Purity of Salvianolic acid B	Regression coefficient	7.245	1.811	6.463			−6.010							9.837	4.460	0.8614	0.8410
	P value		<0.0001	0.0040			<0.0001							<0.0001	<0.0001		
Retention of Danshensu	Regression coefficient	25.43			11.23				−14.66		−18.35			14.08		0.6643	0.6259
	P value				0.0483				<0.0001		0.0015			0.0011			
Retention of Protocatechuic aldehyde	Regression coefficient	25.663			18.333				−63.747					54.449	−29.880	0.7865	0.7621
	P value				0.0015				<0.0001					<0.0001	<0.0001		
Retention of Caffeic acid	Regression coefficient	43.616						−23.776		−15.319				31.777	−18.420	0.7006	0.6664
	P value							0.0053		0.0010				<0.0001	0.0004		
Retention of Rosmarinic acid	Regression coefficient	14.384				−37.991			−27.229			−23.281	−5.649	27.796		0.8509	0.8290
	P value					<0.0001			<0.0001			0.0154	0.0272	<0.0001			
Retention of Lithospermic acid	Regression coefficient	10.850	−5.035	−25.966					−23.430	23.250	−10.815	−13.268				0.8328	0.8024
	P value		<0.0001	0.0060					<0.0001	0.0066	0.0078	0.0399					
Retention of Salvianolic acid B	Regression coefficient	29.590	−3.669						−17.910		−13.144		19.925			0.7769	0.7514
	P value		0.0036						<0.0001		0.0007		<0.0001				

Higher salvianolic acid B purity in the concentrate can result in lower retention rate of protocatechuic aldehyde and caffeic acid, lower purity of protocatechuic aldehyde and caffeic acid, but higher purity of salvianolic acid B purity in the supernatant. Higher lithospermic acid purity in the concentrate led to higher purity and retention rate of multiple phenolic acids. Higher purity of caffeic acid, rosmarinic acid, and protocatechuic aldehyde can lead to higher phenolic acid purity in the supernatant, but at most occasions can result in lower phenolic acid retention rate. Higher Danshensu purity in the concentrate can result in higher retention rate of lithospermic acid.

4. Conclusion

In this work, multiple batches of Salvia miltiorrhiza concentrate were prepared and treated with ethanol precipitation. Dry matter content, pH value, conductivity and water content of the concentrates and supernatants were measured. Most of stachyose was removed in the ethanol precipitation process. Fructose, raffinose and sucrose were also significantly removed. With the increase of ethanol addition amount, the purity of phenolic acids in the supernatant increased, but the retention of lithosperimic acid and salvianolic acid B decreased. The conductivity and pH value of concentrated extract have a relatively small effect on ethanol precipitation indices. With a higher fructose, raffinose, or stachyose content in the concentrated extract, a lower retention rate of phenolic acids will be obtained on most occasions. Sucrose content in the concentrated extract showed little effect on process indices. The purity of phenolic acids in the concentrated will affect the purity and retention rate of phenolic acids in the supernatants. The results of this work indicated that the sugar content in the concen-

trate needs to be monitored in industry due to their influences on ethanol precipitation process indices.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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