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Extraction of Antioxidant Total Phenol from Lees of Xiaoqu Spirits

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Abstract

By the Soxhlet extraction, ethanol is used as extraction solvent of lees to determinate the total phenol content of Xiaoqu spirits lees. Folin-phenol method is handled to extract and to determine the reducing ability of extraction. The total phenolic content in the most suitable condition is determinated by the Folin-phenol method and the optimum extraction technological condition of total phenol is determinated by the experiment. The experimental results show that the optimization conditions of the determination methods of total phenolic content are determined by 3.5 mL for the Folin phenol reagent, 1:1 for the 10% Na_2CO_3 ratio and 30 min for the chromogenic reaction at room temperature. The optimum extraction technological condition of total phenol from lees is as following: solid-liquid ratio 1:12, ethanol concentration 100%, extraction temperature 80˚C, extraction time 10 h.

Keywords

Xiaoqu Spirits, Lees, Extraction, Total Phenols

1. Introduction

Antioxidants are a material that can delay or prevent the oxidation of the substrate at low concentrations and are widely used in food, medicine, cosmetics and other fields [1] [2]. For a long time, people have used the synthetic antioxidants in order to preserve and prevent oxidation. In recent years, research about seeking natural antioxidants from nature has attracted scientists' attention from various countries, the natural antioxidants that have been developed or are studying mainly include spice extracts, polyphenols, natural flavonoids, vitamins, proteins and enzymes, phytic acid, herbal extracts, etc. [3]-[5]. The small liquor distiller's grains were used as raw material in this experiment to extract the natural antioxidants total phenolic, and measure its reducing ability. This

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topic provides a theoretical basis for the development and application of natural antioxidants, and plays an active role in the utilization of wine lees byproduct and environmental protection.

1.1. Materials and Methods

1.1.1. Experimental Materials

Ditty lees were provided by Tanks Wine Co. in Sichuan.

1.1.2. Laboratory Equipment and Instruments

HH-S temperature water bath, Jiangsu Province is based Instrument Co., Ltd. Jintan; DHG-9140A electric oven, Infineon Technologies Limited in Shanghai; JA5003 electronic balance, Scientific Instrument Co., Ltd. Shanghai Heng Ping in Shanghai; DK-98-II type universal furnace, Instrument Co., Ltd., Tianjin; T6 New Century UV-V spectrophotometer, Beijing Purkinje General Instrument Co., Ltd.; LG10-2.4A high-speed centrifuge, Beijing Medical Centrifuge Plant. Soxhlet extraction tube, condenser, flat-bottomed flasks, beakers, flasks, test tubes, pipettes, etc., are conventional biochemical laboratory instruments.

1.1.3. Experimental Drugs

Ethanol, sodium dihydrogen phosphate dihydrate, twelve water disodium hydrogen phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, anhydrous sodium carbonate, gallic acid, sodium tungstate, sodium molybdate, lithium sulfate, concentrated sulfuric acid, concentrated hydrochloric acid and liquid bromine were of analytical grade, and these drugs were provided by Chengdu Kelong Chemical Reagent Factory.

1.2. Methods

1.2.1. Extraction of Antioxidant Total Phenol from Lees of Xiaoqu Spirits (Soxhlet Extraction)

Putting the lees into blast drying oven at 85˚C for 2 h, weighing 16 g dried raw material in the distillers and Soxhlet extraction tube, connecting the test device, adding 160 mL of ethanol to a round bottom flask of the Soxhlet extracted tube, the temperature magnetic heating stirrer was used to hear it, maintaining 80˚C ~ 85˚C and refluxing fir 8 h, till extract was cooled to room temperature, and voluming to 250 mL with boiled distilled water to become the sample solution.

1.2.2. Lowry Method for the Determination of Total Phenolic Content of Extracts [6]

Let gallic acid concentration (μg/mL) as the abscissa, absorbance at 760 nm value as the vertical axis, then we make gallic acid standard curve. Taking 1 mL sample solution in 100 mL graduated test tube with stopper, joining Folin phenol reagent 3.5 mL, adding 10% Na_2CO_3 3.5 mL after 7 to 8 minutes, after reaction at room temperature for 1 h, voluming to 100 mL with distilled water, then shaking it. Distilled water is as the reference solution, by adjusting the wavelength of 760 nm, measuring the absorbance, we could get the total phenol concentration in the solution from the standard curve of total phenolic content, resulting in total phenolic content of the sample. Isolating on standard curves corresponds to the amount of total phenols, calculating formula of the total phenolic content in the sample was as follows:

$$\text{Total phenol}/\% = 0.00375G/W \times 100\% \quad (1)$$

G is the total amount of phenol (μg) from the standard curve, W is the sample weight (g).

1.2.3. Reducing Ability Determination of Grains Extract [7] [8]

Taking 1 mL extract (blank tube by adding an equal volume of 80% ethanol), adding 0.2 mol/L, pH 6.6 sodium phosphate buffer 1.0 mL and 1% potassium ferricyanide 1.0 mL, rapid cooling after reaction at 50˚C water bath for 20 min, adding 10% trichloroacetic acid 1.0 mL, adding distilled water to a final volume of 10 mL, 3000 r/min centrifugal 10 min, supernatant 2.5 mL, adding 0.1% ferric chloride solution 0.5 mL, voluming to 5 mL with distilled water after mixing, measuring the 700 nm absorbance after 10 min. Calculated formula was as follows:

$$\text{Reducing power} = \left(\text{A sample} - \text{A blank}\right)/\text{A blank} \times 100\% \quad (2)$$

1.2.4. The Study of Total Phenol Content Lowry Assay Conditions

The optimum conditions of Folin phenol method that was used to measure the total phenolic content were determined through determining the reagent, reaction temperature and reaction time.

1.2.5. The Research of Total Phenol Extraction Process Conditions from Grains

Through single factor and orthogonal experiments, we measure alcohol concentration, extraction temperature, solid-liquid ratio and extraction time on the heat reflux extraction and determine the optimum extraction conditions of total phenols.

2. Results and Analysis

2.1. The Optimum Conditions for Total Phenolic Content of Lowry Assay

2.1.1. Determination of the Amount of Reagent

The steps of determination of Folin phenol reagent and 10% Na_2CO_3 proportional is: taking 0.5 mL gallic acid standard solution, adding Folin phenol reagent 3.5 mL, adding different volumes of 10% Na_2CO_3 solution according to **Table 1** after 7 ~ 8 min, voluming to 100 mL and measuring absorbance values after reaction at room temperature for 1 h, the measurement results are shown in **Table 1**.

Table 1 shows that when adding 10% Na_2CO_3 3.5 ml, ratio of Folin phenol reagent and Na_2CO_3 is 1:1, the absorbance value is the maximum, so chromogenic reaction is complete when ratio of Folin phenol reagent and Na_2CO_3 is 1:1.

The determination of Folin phenol reagent dosage: the amount of Folin phenol reagent are 0.5, 1.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0 mL, adding equal volume of 10% Na_2CO_3 solution, other conditions and methods remain unchanged. The measurement results are shown in **Table 2**.

As apparent from **Table 2**, when the Folin phenol reagent addition is 3.5 mL, the absorbance is the maximum.

2.1.2. The Determination of the Reaction Temperature

3.5 mL Folin phenol reagent, 3.5 mL 10% Na_2CO_3 solution, the reaction temperature are set to 5˚C, 10˚C, 25˚C, 35˚C, 45˚C, other conditions and methods remain unchanged. The measurement results are shown in **Table 3**.

As apparent from **Table 3**, the absorbance difference is not obvious when the temperatures are 10˚C, 25˚C, 35˚C, the absorbance is the maximum value at 25˚C. Visible under normal temperature conditions, temperature has little effect on the absorbance values, illustrating that the determination of total phenolic content can be carried out at room temperature.

2.1.3. Determination of the Reaction Time

3.5 mL Folin phenol reagent, 3.5 mL 10% Na_2CO_3 solution, react at room temperature, other conditions and methods remain unchanged. Measuring absorbance value at a certain time interval, the measurement results are shown in **Table 4**.

Table 1. Effect of 10% Na_2CO_3 quantity on extinction value.

Ratio of Folin phenol reagent and Na_2CO_3	4:1	3:1	2:1	1:1	1:2	1:3	1:4
Absorbance	0.126	0.133	0.138	0.209	0.205	0.201	0.192

Table 2. Effect of Folin phenolic reagent quantity on extinction value.

Folin phenol reagent/mL	0.5	1.5	1.0	2.0	2.5	3.0	3.5	4.0
Absorbance	0.191	0.211	0.216	0.221	0.230	0.240	0.247	0.215

Table 3. Effect of reagent ration on extinction value.

Temperature/˚C	5	10	25	35	45
Absorbance	0.156	0.166	0.168	0.167	0.159

As apparent from **Table 4**, the absorbance value added to a certain value after 30 min, and no significant effect increased with time. Therefore, the best reaction time was 30 min.

2.2. The Optimum Extraction Conditions of Total Phenols

2.2.1. Single Factor Test Results

1) The Effect of Ethanol Concentration on the Heat Reflux Extraction

Dried distillers grains as raw material, each weighed 16 g, solid-liquid ratio was 1:10, adding 40%, 50%, 65%, 80%, 95% ethanol solution, heating with temperature magnetic heating stirrer, maintaining 80˚C ~ 85˚C and refluxing for 8 h, till extract was cooled to room temperature, voluming to 250 mL with boiled distilled water, then determining total phenolic content and reducing power of extracts, the measurement results are shown in **Figure 1**.

As can be seen from **Figure 1**, the ethanol concentration has a significant influence on the thermal reflux extraction. When the ethanol concentration is 95%, the total phenolic content and reducing power of extracts are high. Therefore, we could determine that the optimal concentration of ethanol is 95%.

2) The Effect of Extraction Temperature on the Heat Reflux Extraction

Ethanol concentration was 95%, extraction temperatures were set to 80˚C, 85˚C, 90˚C, 95˚C, 100˚C, other conditions and methods remained unchanged. Measurement results of the total phenolic content and reducing power of the extracts are shown in **Figure 2**.

Figure 2 shows the total phenolic content of the extract and reducing power decreases as the temperature rises. Because of the process of the solvent extraction mass transfer, temperature affects both dissolution rate of the solute in a solvent, but also affects the speed of the outward diffusion of solute; temperature has a great influence on the extraction of heat reflux extraction. Temperature increases, mass transfer rate increases, the diffusion speed, extraction rate will accelerate, thus heating could help improve material yield of the crude extract. But too high temperature will affect the activity of the active substance, even make it completely inactivated. Phenols are more sensitive to heat, it will accelerate the oxidation or decomposition when exposed to heat, so total phenolic content of the crude extract will vary with the temperature decrease. But warming also could improve the solubility and diffusion coefficient of phenolic compounds overall, therefore, decline in total phenolic content is relatively flat. Considering that, the optimum extraction temperature is determined to 80˚C.

Table 4. Result of stability test.

Time/min	5	10	15	20	25	30	35	40	45	50	55	60
Absorbance	0.132	0.164	0.175	0.203	0.219	0.246	0.246	0.247	0.248	0.248	0.249	0.249

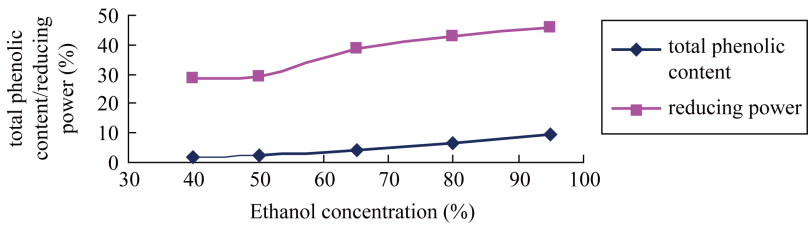

Figure 1. Effect of ethanol concentration on extraction rate.

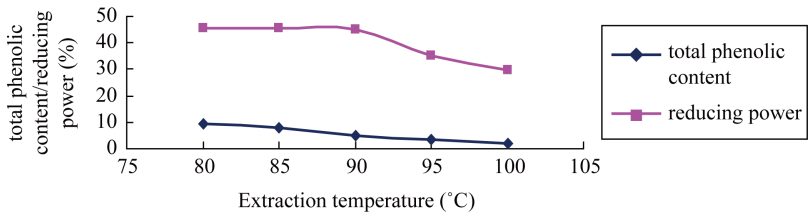

Figure 2. Effect of temperature on extraction rate.

3) The Effect of Liquid Ratio on the Heat Reflux Extraction

Ethanol concentration was 95%, extraction temperature was 80˚C, solid-liquid ratio were set to 1:6, 1:8, 1:10, 1:12, 1:15, other conditions and methods remain unchanged. Measurement results of the total phenolic content and reducing power of the extracts shown in **Figure 3**.

Figure 3 shows, the increasing of solid-liquid ratio has an impact on both total phenolic content and reducing power in crude extracts, increasing of the solid-liquid ratio can improve the rate of diffusion and shorten the time of equilibrium concentration, improve the extraction yield, however, a large amount of solvent can affect the processing (such as distillation) burden, will also greatly increase the cost. Considering that the best solid-liquid ratio was determined to 1:12.

4) The Effect of Extraction Time on the Heat Reflux Extraction

Solid-liquid ratio was 1:12, the ethanol concentration was 95%, extraction temperature was 80˚C, extraction time were set to 4 h, 6 h, 8 h, 10 h, 12 h, other conditions and methods remain unchanged. Measurement results of the total phenolic content and reducing power of the extracts shown in **Figure 4**.

Figure 4 shows, the total phenolic content and reducing power of extract increased with time, but only showed a significant increase state within the first 8 h, 8 ~ 10 h relatively gentle rise, decreased significantly after 10 h. The solvent extraction process will take some time, refluxed for longer, the raw materials and extraction have more adequate contact, the active ingredient can be better dissolution. But reflux for too long, resulting in material heating time is long, Phenols easily oxidized at high temperatures, the polymerization, degradation and loss, so the total phenolic content will appear after the first rise in decline. Considering that the optimum extraction time is determined to 8 h.

2.3.2. Orthogonal Test Results

Ethanol concentration, solid-liquid ratio, extraction time, extraction temperature was four factors, set 3 levels and L9 (34) orthogonal experiment [9], in order to determine the optimum extraction conditions of total phenols. L9 (34) factor levels are shown in **Table 5**, the test results shown in **Table 6**.

Analysis of table poor R 6, affecting the factors of the extraction rate of total phenols in descending order as follows: extraction temperature > ethanol concentration > extraction time > solid-liquid ratio. Optimum extraction conditions of the total phenols can be derived from K value was A3B2C3D1, ethanol concentration was 100%, solid-liquid ratio was 1:12, extraction time was 10 h, extraction temperature was 80˚C.

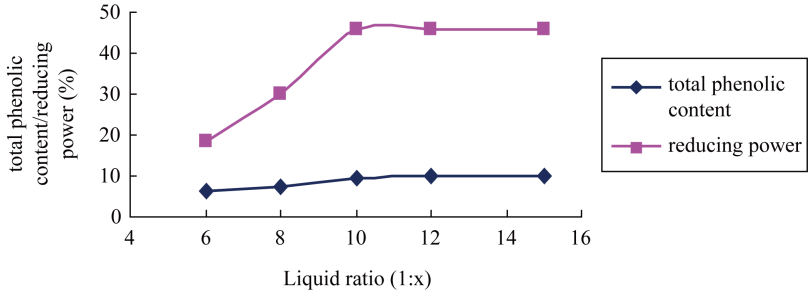

Figure 3. Effect of solid-liquid ratio on extraction rate.

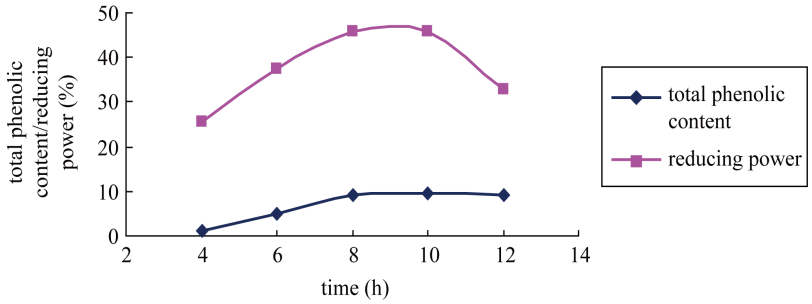

Figure 4. Effect of time on extraction rate.

Table 5. The factor and level of orthogonal tests.

Level	Factor			
	A-ethanol concentration/%	B-liquid ratio	C-extraction time	D-extraction temperature
1	90	1:10	6	80
2	95	1:12	8	85
3	100	1:14	10	90

Table 6. The result and analysis of orthogonal tests.

Test No	Factor				Total phenol content/%
	A	B	C	D	
1	1	1	1	1	8.57
2	1	2	2	2	8.10
3	1	3	3	3	7.45
4	2	1	2	3	6.55
5	2	2	3	1	9.47
6	2	3	1	2	9.01
7	3	1	3	2	9.23
8	3	2	1	3	9.11
9	3	3	2	1	9.72
k_1	8.04	8.12	7.68	9.25	
k_2	8.34	9.23	8.12	8.78	
k_3	9.35	8.73	8.90	7.70	
R	1.31	1.11	1.22	1.55	

3. Conclusions

In this study, ethanol was used as the solvent in Soxhlet extraction to extract natural antioxidants total phenols from the small liquor grains, and determine its total phenolic content and the reducing ability. The conclusions were as follows:

1) Optimum conditions of Folin phenol method determines total phenolic content: Folin phenol reagent is 3.5 mL, with 10% Na_2CO_3 ratio of 1:1, and the color reaction is 30 min at room temperature.

2) Factors of affecting the extraction rate of total phenols in descending order are as follows: Extraction temperature > ethanol concentration > extraction time > solid-liquid ratio. Optimum extraction conditions of Lees total phenols were as follows: Liquid ratio 1:12, 100% ethanol concentration, extraction temperature 80˚C, extraction time 10 h.

3) In this study, providing a theoretical basis for the development and application of natural antioxidants and wine by-product, which plays an active role in environmental protection.

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