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Effect of Drying Method on Total Flavonoids in Red Jujube

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To study the effects of various drying methods on total flavonoids in red jujube, high performance liquid chromatography is used, the crude flavonoids red dates obtained from the tests are analyzed to understand its main ingredients, and further study on the influence of serum lipid levels in mice and the antioxidant activities of crude flavonoids red dates is made. Through the experiment, the following results are obtained: the red total flavonoids extraction and purification technology average recovery was 99.4%, RSD=1.34%, which meets the accuracy requirements of the test. LSA-20 macroporous resin used for purification of flavonoids had the best effect. The adsorption rate was 98.6%, the elution rate of 97.6%, and the relative obtaining rate was 96.23%. The total flavonoid content of fresh jujube (with rutin as a standard) is 296.6mg/100g. When microwave drying power was 0.245W, drying time is 10min and unit sample volume is 50g/dm^2, the total flavonoids content of red jujube was the highest. The sequence of influencing factors is: microwave power>drying time>unit volume sample size. Based on the above findings, it is concluded that the effect of microwave power and microwave drying time on total flavonoids content of red jujube was very significant. In addition, the amount of total flavonoids in red jujube was significantly affected by the amount of sample per unit area.

1. Materials and methods

1.1 Experimental materials

The raw materials are Shaanxi Jiaxian jujubes, picked in October 2002, which was stored in low temperature freezer after being picked.

1.2 Experimental animals

CIR mice, which are purchased from Xi'an Jiao Tong University School of Medicine, with average weight of 22 ±2g.

1.3 Chemical reagents for testing

Sodium hydroxide, potassium hydroxide, sodium nitrite, hydrochloric acid, aluminum nitrate, chloroform, methanol, ethanol, petroleum ether, carbon tetrachloride, sodium chloride, ethyl ether were all analytical reagents, purchased from Xi'an chemical glass supply station.
Macroporous adsorption resin (LSA 5, LSA 8, and LSA 20): Xi'an Lanshen Resin Research Institute.
Rutin standard: Shanghai Biochemical Reagent Factory of China Pharmaceutical Group.
Serum total Cholesterol Reagent box: Shanghai Rongsheng Biotechnology Co., Ltd.
Serum glycerin Kit: Zhejiang Eastern European Biological Engineering Co., Ltd.
Altimetry density lipoprotein Kit: Shanghai Rongsheng Biotechnology Co., Ltd.
Superoxide dismutase (SOD) Kit: Nanjing Institute of Biological Engineering.
Catalase (CAT) (ultraviolet spectrophotometry) Kit: Nanjing Institute of biological engineering.
MDA Kit: Nanjing Institute of Biological Engineering (Wojdyło, et al., 2016).
Basic feeds: School of Medicine, Xi'an Jiao Tong University.
Cholesterol: Shanghai Chemical Reagent Second Factory.
Pig suet: pig suet purchased in the market is self-boiled, filtered, and cooled.

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High fat diet: basic diet supplemented with 1% cholesterol and 10% lard mixed.

1.4 Red jujube total flavones extraction process

Red jujube-Cleaning-removing core-cutting and crushing-drying-beating machine for crushing-methanol immersion-ethanol reflux extraction-concentration-petroleum ether degreasing-extraction.
Note:
1. Rinse with flowing water and drain naturally after washing.
2. Hand shearing, snunx10nun in particle size.
3. Liquid volume ratio of methanol soaking of 1:1.
4. The extraction volume of anhydrous ethanol was 1:15, the extraction temperature was 80, and the time was 6 hours.
5. Rotary evaporator reduced concentration, temperature of 40 DEG C.

2. Red jujube total flavones determination methods

2.1 Draw standard curve

Take the rutin standard to dry at 105 DEG C with hot air at constant quality and cool in the dryer to room temperature. Accurately weigh standard 5.0mg, dissolve in methanol, and move into 50mL capacity bottle. The beaker is washed three times with methanol and fixed into the volumetric flask. The constant volume is added to the standard rutin solution of 10mL. Imbibe the rutin standard solution 0.00mL, 0.50mL, 1.00mL, 2.00mL, 3.00mL, and 4.00mL, which is equivalent to that 0μg, 50μg, 100μg, 200μg, 300μg, and 400μg rutin contained are moved into 10mL volumetric flask, add 30% (ethanol) solution to 5.00mL. Add 5% (sodium nitrite) solution 0.30mL, shake evenly and put for 5min, then add 10% (aluminum nitrate - Monohydrate) solution 0.30mL, shake and place for 6min (Kamiloglu, et al., 2016). Then add 1.0mol/L of sodium hydroxide solution 2.0mL, 30% (ethanol) solution to volume to zero. With pipe blank, use 1cm color cup, determine the absorbance in the wavelength of 510nm, draw the standard curves of rutin content and absorbance, and obtain the regression equation.

2.2 Determination of samples

The total flavonoids of the red jujube were separated and purified, and then the absorbance was determined by spectrophotometry. The total flavone content expressed in rutin was determined by substituting in the regression equation. The total flavonoid content in the raw sample was obtained by the following formula:
Total flavonoids content of raw materials:

$$(\%)=\frac{CV1V3}{WV2V4}\times10^{-6}\times100\% \quad (1)$$

C: Total flavonoids in the sample solution (μg)
V_1: Volume of crude extract (mL)
V_2: Macroporous resin sample volume (mL)
V_3: Dissolved volume of the eluted component (mL)
V_4: Sampling liquid volume during color determination (mL)
W: Sample size of red jujube (g)

3. Test index determination method

The determination of total flavonoids in samples (rutin as standard) is calculated such as the formula proposed in the method 2.2.

3.1 Method for determination of serum indexes in mice

Serum preparation: mice eyes were enucleated, naturally blooding to a blood test tube. Natural coagulation at room temperature, and after solidification, the blood clot was peeled along with the tube wall with bamboo. As a result, the serum was extracted as soon as possible and then in 2500-300orpm, make centrifugal for 15min, and then serum was poured into another clean blood test tube, cryo preservation for standby.
(1) Triglyecrides determination (cholesterol oxidase - peroxidase), wavelength 546nm, specific shade diameter 1cm, reaction time 5~10min, and reaction temperature 37 DEG C.
Results: the content of glycerin:

$$(mmol/L)=\frac{Ar}{As}\times 2.26 \quad (2)$$

Unit conversion formula: (mg/dL)X0.01129=mmol/L

(2) Determination of serum total cholesterol

Vinegar samples free cholesterol and cholesterol forms cholesterol, and cholesterol and H_2O, under the action of cholesterol enzymes, form cholesterol and fatty acid. While cholesterol and O_2 and H_2O, under the action of cholesterol oxidase, form Δ^4 cholestene -3- ketone and H_2O_2. Then, H_2O_2 and 4- amino antipyrine and phenols substances, under the action of peroxidase, forms water and quinoneimine (Benahmed, et al., 2017). Ketimine produced by the reaction can determine the absorbance at 500nm by spectrophotometric method. And according to the change of absorbance, we can calculate the content of cholesterol, shade diameter 1cm, reaction time 10min, reaction temperature 37 DEG C.

Calculate:

$$\text{Cholesterol}(\text{mg}/\text{dl})=\frac{\det er\min edA}{s\tan dardA}\times 200 \quad (3)$$

Unit conversion: cholesterol mmol/L=mg/dl×0.0258

(3) Determination of serum high density lipoprotein (precipitation)

Human serum low density lipoprotein (DLL), very low density lipoprotein (VLDL) and polyethylene glycol -6000 act and produce precipitation reagent. After centrifugation, high density lipoprotein cholesterol (HDL-CHOL) in the supernatant can be measured by enzyme method.

The experimental parameters were: high density lipoprotein separation centrifugal force of 1500r/min, centrifugation time of 10min, determination temperature of 37 DEG C, and wavelength of 500unl. The sample and reagent are placed at room temperature for the balance of 15 minutes. Take the samples of 200μL and 200μL precipitant mixed for 15 minutes, make the centrifugal with centrifugal force of 1500r/min for 10 minutes, take 50μL supernatant, add 1500μL working reagent for mixing, and determine the absorbance (A) after 37 DEG C water bath for 10min.

Calculate:

$$HDL\text{-}C(\text{mmol}/L)=\frac{\text{determined}\quad\text{absorbance}}{s\tan dard\quad absorbance}\times s\tan dard\quad solution\quad concentration\times 2 \quad (4)$$

Unit conversion: High density lipoprotein cholesterol mmol/L=mg/dL×0.0258

3.2 Determination of antioxidant indices in mice

Plasma preparation: blood test tube was added into heparin anticoagulant and dried at low temperature. After taking blood, shake it well and put it back in the ice bath immediately. It is cooled at 3000r/min and centrifuged for 5min. The plasma is separated, and then the plasma is poured into another clean test tube to keep it at low temperature.

Tissue plasma preparation: apply the wet tissue plus saline by W:V=I:5, under the condition of the ice bath, tissue homogenizer was homogenized, and 3000r/min centrifugal is carried out for 10min, and the supernatant was poured into the clean tube cryopreservation standby.

(1) Measurement of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) produces superoxide radicals by xanthine oxidase and Suoyin yellow voice response system. The latter one oxides the light amine and forms the nitrite, shown as purple in color agent. When the measured samples contain SOD, there is the specific inhibitory effects on superoxide anion free radical, so the formed nitrite reduces (Figiel and Michalska, 2016).

Calculate: SOD activity in plasma $NU/\text{mL}=\frac{OD1-OD2}{OD1}\div 50\%\times\text{Dilution factor}$

SOD activity in tissue homogenates:

$$NU/\text{mgprot}=\frac{OD1-OD2}{OD1}\div 50\%\times\text{Dilution factor}\div\text{Protein content in tissues}$$

$OD_1=\text{Absorbance of control tube}$

$OD_2=\text{Absorbance of determination tube}$

(2) Determination of catalase (CAT) (ultraviolet method)

Hydroxyl radical (OH-) is the active oxygen with the most active chemical properties. It almost acts with each kind of organic compounds within the cell, such as sugars, amino acids, lecithin, nucleic acid and organic acid. Its destructive is extremely strong, but it can be decomposed by peroxided enzyme, and catalase (CAT) of red blood cells or a tissue can directly decompose the substrate hydrogen peroxide (H_2O_2) under certain conditions, so the H_2O_2 concentration in the reaction solution gradually decreased and the corresponding absorbance also decreased.

Take the samples after pretreatment for 0.02mL, add into bottom cuvette, and set the pre temperature to 25 DEG C. For the 3mL substrate solution with OD value between 0.5~0.55, directly pipette quickly into the cuvette with 5mL or 10mL solution, and at 240nm, immediately measure the absorbance and write down the value of OD_2. The cuvette is not removed, 1 minute later, measure the absorbance once and write down the value of OD_1 (Siriamornpun, et al., 2016). The unit definition and calculation formula are as follows:

Calculation formula:

$$CAT \quad \text{activity} = \log\frac{OD1}{OD2} \times \frac{2.303}{60s} \times A \div B$$

Concentration is positively related to absorbance. OD_1 is the absorbance at 240nnl at zero second, and OD_2 is the absorbance at 240nnl at 60 seconds.

When 2.303 is converted from the natural logarithm to the common logarithm log, it needs to multiply by 2.303.

A is the dilution times of hemoglobin or tissue protein (including dilution times before the sampling test and dilution times of the sampling amount in the reaction fluid).

B is the amount of hemoglobin per milliliter in a sample, or the tissue protein per milliliter.

4. Results and analysis

4.1 Standard curve and regression equation

According to the experiments, the absorbance of rutin standard solution at each point is determined, as shown in Table 1.

Table 1: The absorbance of rutin standard solution

Standard solution (mL)	0.0	1.0	2.0	3.0	4.0
Absorbance (A)	0.0000	0.1082	0.2241	0.3327	0.4080
Flavonoid content (μg)	0.0	100.0	200.0	300.0	400.0

The standard curve obtained is shown in Figure 1

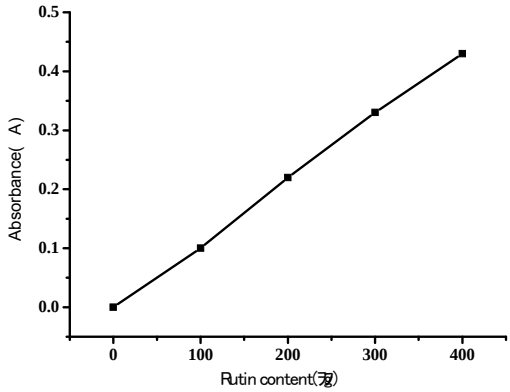

Figure 1: Rutin standard curve

Its regression equation is: Y=0.001X+0.0065, R=0.9974

4.2 Determination of total flavonoids in fresh jujube

Based on the above experiment methods, the absorbances of fresh juijube determined are: 0.3601, 0.3605, and 0.3617, and the average value is 3608.

In accordance with the regression equation, flavonoids in fresh jujube juice is determined as C=354.3μg.

Flavonoids content in fresh jujube $(\%) = \frac{CV1C3}{WV2V24} \times 10^{-6} \times 100\% = 0.2966\%$

4.3 Sample recovery test results

The sample recovery test results are shown in the Table 2:

Table 2: Recovery of Rutin in red jujube samples

Sampling of fresh jujube (g)	Rutin addition (mg)	Flavonoids content should be measured (mg)	Measured flavonoids content (mg)	Recovery rate (%)
20	5	64.32	63.20	98.26
20	10	69.32	70.16	101.21
20	15	74.32	72.96	98.17
20	20	79.32	78.57	99.05
20	25	84.32	84.63	100.37

The average recovery rate of 5 times samples addition was 99.1%, and the relative standard deviation was RSD=1.34%. It is proved that the extraction process can meet the requirement of test accuracy.

4.4 Comparison of adsorption and elution properties of macroporous adsorption resins for Flavonoids

From Table 3, it is known that the strongest absorbance capacity (98.6%) of three macroporous resins for rutin is LSA-20 (Valadez-Carmona, et al., 2017). While for ellusion rate, the three macroporous have small difference, but LSA-50 is the highest (98.4%). Comprehensively considering, in the whole absorption - ellusion process, the relative ontaining rate of LAS-20 to rutin is the highest (23%), so LSA-20 is the most suitable resin for the experiment.

Table 3: Comparison of adsorption desorption ability of three resins to rutin

Macroporous resin model	amount of rutin added (μg)	Static adsorption capacity (μg)	Adsorption rate (g)	Elution content (μg)	Elution rate (μg)
LSA-5	3000	2840	94.6	2810	98.4
20	3000	2870	95.6	2810	97.9
20	3000	2960	98.6	2890	97.6

4.5 Microwave drying test results

Table 4: Microwave drying orthogonal two times repeated test results

Processing number	A power (W)	B drying time (min)	C Sample size (g/dm2)	D Empty column	Total flavonoids content of red jujube (mg/100)
1	1 (0.07)	1(10)	1(50)	206.6	211.4
2	1 (0.07)	2(20)	2(100)	191.3	190.2
3	1 (0.07)	3(30)	3(150)	186.1	187.6
4	2 (0.245)	1(10)	3(150)	227.0	211.1
5	2 (0.245)	2(20)	1(50)	209.6	204.3
6	2 (0.245)	3(30)	2(100)	197.1	196.4
7	3 (0.350)	1(10)	2(100)	187.2	186.9
8	3 (0.350)	2(20)	3(150)	175.6	176.2
9	3 (0.350)	3(30)	1(50)	170.1	175.1
K_1	1173.20	1230.20	1177.10	1163.30	
K_2	1245.50	1147.20	1149.10	1164.80	
K_3	1071.10	1112.40	1163.60	1161.70	
K_1	195.53	205.03	196.18	193.88	
K_2	207.58	191.20	191.52	194.13	
K_3	178.52	185.40	193.93	193.62	
R	29.07	19.63	4.67	0.52	

From the analysis of Table 4, it is known that the optimal process combination of microwave drying is A and C, namely the microwave power of 0.245W, drying time of 10min, and unit volume sampling of 50g. At the same time, the effect of each factor on total flavonoids content of red jujube is ordered as A>B>C according to the primary and secondary relation, namely microwave power>drying time>unit volume sampling load.

5. Conclusion

(1) Verification of the extraction and purification process of total flavonoids in red jujube:
The average yield of adding sample was 99.4%, and relative standard deviation was 1.34%, which met the requirement of test precision.
(2) Selection of macroporous adsorption resin:
LSA-5 adsorption rate was 94.6%, elution rate was 98.9%, relative yield rate was 93.5%; LSA-8 adsorption rate was 95.6%, elution rate was 97.9%, and relative rate was 93.59%; LSA 20 adsorption rate was 98.6%, elution rate was 97.6%, and relative rate was 96.23%. As a result, LSA-20 was selected as the purified resin.
(3) Content of total flavonoids in fresh jujube (rutin as standard) was 296.6mg/100g.
(4) Orthogonal test of microwave drying:
The range analysis concluded that: in the condition of microwave power of 0.245w, microwave drying time of 10min, unit volume sample load of 509, the content of total flavonoids in the microwave drying red jujube got up to 275.2mg/100g; and the influence factors order is: microwave power> microwave drying time>unit volume sampling load. The analysis of variance showed that microwave power and microwave drying time had an extremely significant influence on total flavonoids content of red jujube, and the unit volume sample load had significant influence on total flavonoids content of red jujube.

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