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Full Length Research Paper

Microwave-assisted extraction and antihyperlipidemic effect of total flavonoids from corn silk

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The process of microwave-assisted extraction (MAE) of total flavonoids from corn silk and the hypolipidemia in animal models were studied. Influence of solvent concentration, microwave power, extraction time and dose of solvent were investigated and then, the orthogonal experiments were performed. Animal models of hyperlipidemia induced by high-fat diet were established. The serum levels were respectively measured, including total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). The optimum extraction parameters were determined as follows: the ethanol concentration (60%); the power (600 W); the extraction time (16 min); the ratio of plant material to solvent (M/S) (1:20), and the yield of extract (4.55%). Corn silk total flavonoids (CSTF) significantly lowered the serum TC, TG and LDL-C levels. The serum lipid level was decreased by CSTF in hyperlipidemic animal models in a dose dependent manner.

Key words: Corn silk, total flavonoids, microwave-assisted extraction (MAE), antihyperlipidemia.

INTRODUCTION

Corn silk is the style and stigma of *Zea mays* Linne. Currently, only a small amount is used as medicine and most is thrown away as waste. Flavonoids from stigma maydis were reported to scavenge hydroxyl, regulate lipid metabolism in mice, and have anti-aging and anti-fatigue effect. In order to take full advantage of the resources, the comprehensive utilization of agricultural and by-products should be developed. The extraction of corn silk total flavonoids (CSTF) was studied for the development of corn silk as natural food antioxidants and natural medicine. It has an important economic value and social significance. Single factor experiments were carried out under the conditions of different concentration of organic solvent, time, the ratio of plant material and solvent (M/S) and microwave power, and then, the orthogonal experiments were performed. The total flavonoid content is reported that CSTF has many pharmacological effects, such as antioxidative and hypoglycemic effects.

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Antihyperlipidemic activities of CSTF were was determined as an indicator of the optimal process. It mainly investigated in the experiment. Hyperlipidemia is mainly shown as a result of lipid metabolism and lipoprotein peroxide disorders. Hyperlipo- proteinemia is a cardinal risk factor that induces atherosclerosis (AS), coronary heart disease (CHD) and other cardiovascular diseases. Consequently, to control hyperlipo-proteinemia, the key method is to prevent or treat AS and CHD. We observed the effect of CSTF on metabolism of cholesterol and lipoprotein-cholesterol in the experimental hyperlipidemia rats, to prove if CSTF is beneficial to prevent or treat AS.

MATERIALS AND METHODS

Corn silk was collected in the suburbs of Jilin city in 2010 and identified with Sweet sticky No. 1 by Wang Guang- shu from Jilin University. Anhydrous ethanol, methanol, aluminum chloride, concentrated hydrochloric acid, petroleum ether, ethyl acetate and n-butanol were all of analytical grade. The content of rutin was checked by high performance liquid chromatography and was

Table 1. Factors and levels of the L_9 (3^4) orthotropic experiments for MAE.

Level	Concentration of alcohol (%)	Time (min)	M/S (g : ml)	Power (w)
1	60	8	1:15	500
2	70	16	1:20	600
3	80	24	1:25	700

not less than 99.9%. Wistar rats weighing 200 to 240 g which were supplied by Experimental Animal Center of Jilin University were used in all the experiments.

Extractions of the plant materials

Single-factor exploration

The basic parameters of the effect of four factors on the yield of MAE were alcohol concentration (70%) (volume ratio); microwave time (16 min); M/S, (1:20) and power (600 W). The single factor experiments were carried out by changing a factor, fixing another three factors and repeated three times, respectively. About 2.0 g of the powdered sample was placed in an extraction vessel with a 100 ml bottom flask.

Orthogonal experiment

According to the result of single-factor experiments, the orthotropic experiment table of four-factor and three-level was used in order to optimize conditions of extraction. The results are shown in Table 1.

Verification test

The optimal condition of the exaction orthotropic experiments was used to verify the experiment.

Analytical methods

UV analysis was applied to determine the content of flavonoids according to Ren and Ding(2004). The method was $AlCl_3$ chromogenic reaction, using rutin as the calibration standard. A good linear relationship was obtained over the range of 0.006 to 0.045 mg·mL$^{-1,}$ and the relationship was expressed as C = 20.7A + 0.3 (r = 0.9998), where A, is the absorbance at 272 ± 2 nm, C is the concentration of flavonoids (mg·mL^{-1}) and r is the regression coefficient. The average recovery rate was 99.6% with RSD = 0.731% (n = 5).

Antihyperlipidemia experiment

Hyperlipidemia model was conducted according to previously described techniques. The rats were fed with hyperlipidemia feed containing cholesterol (2 g/100 g weight), propylthiouracil (0.2 g/100 g weight), sodium cholate (0.3 g/100 g weight), fat (7.5 g/100 g weight) and ordinary feed (90 g/100 g weight) at 7:00 to 8:00 pm for 20 days. The rats were assigned to the following experimental groups: group 1, rats with the ordinary feed were treated for 20 days with normal solvents (2 ml/kg, ig); group 2, rats with hyperlipidemia feed were treated for 20 days with normal solvents (2 ml/kg, ig); groups 3 to 5, rats with hyperlipidemia feed were treated for 20 days with CSTF (200, 400 and 800 mg/kg, ig); group 6, rats with hyperlipidemia feed were treated for 20 days with lovastatin (10mg/kg, ig). After 24 h of the last administration, the rats were anaesthetized (sodium pentobarbital, 30 mg/kg ip). Blood was collected through aortaabdominalis and serum was centrifuged. Triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined by biochemistry analyzer. All the results were expressed as $\bar{x}$ ±s. Statistical analysis was performed using the paired and unpaired student's t-test. Comparison among multiple groups was made by analysis of variance.

RESULTS AND DISCUSSION

As shown in Table 2, the sequence of factors affected yield as follows: alcohol concentration > microwave power > M/S ≈ irradiation time. The optimum condition was $A_1B_2C_2D_2$, which was 60% ethanol, 600 W microwave power, 16 min extraction time and M/S ratio of 1:20.The MAE of $A_1B_2C_2D_2$ was done three times with parallel experiments and the average yield was 4.55%. The results indicated that the yield of MAE was higher than that of hot reflux extraction, which was 70% ethanol, 80°C extraction temperature, 2 h extraction time and M/S ratio is of 1:30.

Serum TG and TC levels of the model group were higher than those in the control group, the differences were statistically significant ($P<0.01$), and the hyperlipidemia model succeeded. In contrast to the model group, the levels of serum TC, TG and LDL-C in middle and high-dose groups were lower, and the difference were statistically significant ($P<0.05$) (Table 3). In comparison with the model, the levels of HDL-C in the three dose groups had no statistical difference ($P>0.05$).

The sequence of factors of orthogonal optimizing experiment of MAE was A > D > B ≈ C; namely the ethanol concentration was 60%, the power was 600 W, the extraction time was 16 min, M/S ratio was 1:20, and the extract yield was 4.55%. Moreover, CSTF significantly reduced the level of the serum TC, TG and LDL-C in the hyperlipidemia rats.

The sequence of factors of orthogonal optimizing experiment of MAE was A > D > B ≈ C. The optimum condition was $A_1B_2C_2D_2$; the ethanol concentration was 60%, the power was 600 W, the extraction time was 16 min, M/S ratio was 1: 20 and the extract yield was 4.55%. MAE is highly efficient and selective, and it can be simply operated, it has high yield, fewer by-products and the

Table 2. Results of the orthotropic experiments for MAE.

Number	Concentration of alcohol (%) A	Time (h) B	M/S (g/ml) C	Power D	Extract yield (%)
1	1	1	1	1	3.177
2	1	2	2	2	4.554
3	1	3	3	3	3.639
4	2	1	3	2	3.243
5	2	3	2	1	2.979
6	2	2	1	3	3.221
7	3	1	3	2	2.840
8	3	2	1	3	2.686
9	3	3	2	1	2.774
K1	11.371	9.261	9.085	8.931	
K2	9.444	10.462	10.308	10.638	
K3	8.301	9.393	9.722	9.546	
k1	3.790	3.087	3.028	2.977	
k2	3.148	3.487	3.436	3.546	
k3	2.767	3.131	3.241	3.182	
R	0.642	0.400	0.408	0.569	

Table 3. The effect of different treatments on TC, TG , HDL-C and LDL-C ($\bar{x}$ ± s, n = 10).

Group	Dose (mg kg^{-1})	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control		2.16±0.11	0.69±0.35	0.86±0.06	1.01±0.04
Model		12.93±2.09$^{\triangle\triangle}$	0.98±0.15	1.56±0.23	10.67±0.64$^{\triangle\triangle}$
Lovastatin	10	7.46±1.25**	0.32±0.11*	1.03±0.12*	6.40±1.40*
Low-dose	200	9.93±1.69	0.44±0.12	1.41±0.04	9.64±0.10
Middle-dose	400	8.28±1.63*	0.32±0.03*	1.33±0.19	7.02±1.05*
High-dose	800	7.72±1.25*	0.30±0.02*	1.18±0.26	6.84±0.54*

$^{\triangle}$P<0.05; $^{\triangle\triangle}$ P<0.01 in comparison with control; * P<0.05,** P<0.01 in comparison with model.

product is also easier to purify. Microwave extraction breaks the bottleneck of traditional extraction methods, becoming one of the most advanced extraction technology.

Hyperlipidemia resulting from lipid metabolism disorder in the body is a frequently-occurring disease, and it is a risk factor leading to arteriosclerotic cardiovascular disease, which is seriously harmful to human health. With the improvement of living standards, the population of hyperlipidemia is increasing. Hence, the development of hypolipidemic agents has good social and economic benefits. A considerable number of researches show that extract from corn silk (such as CSTF) have a better effect on antioxidative activation (ref). However, the study of its hypolipidemic properties is rarely reported in literatures. The results showed that CSTF significantly reduced the level of the serum total cholesterol, triglyceride and LDL-C in hyperlipidemia mice. It provides the experimental data for further study on hyperlipidemic active ingredients in corn silk.

REFERENCES

Brites FD, Bonavita CD, Cloes M, Yael MJ, Fruchart JC, Castro GR, Wikinski RW (1998). VLDL compositional changes and plasma levels of triglycerides and high density lipoprotein. Clin. Chim. Acta. 269: 107-124.

Chen Y, Wang, J, Wan D (2010). Determination of total flavonoids in three Sedum crude drugs by UV-Vis spectrophotometry. Pharmacogn. Mag. 6:259-263.

Cibin TR, Devi DG, Abraham A (2010). Chemoprevention of skin cancer by the flavonoid fraction of Saraca asoka. Phytother. Res. 24:666-672.

El-Ghorab A, El-Massry KF, Shibamoto T (2007). Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of Egyptian corn silk (*Zea mays* L.). J. Agric. Food Chem. 55: 9124-9127.

Fang SC, Hsu CL, Lin HT, Yen GC (2010). Anticancer effects of flavonoid derivatives isolated from Millettia reticulata Benth in SK-Hep-1 human hepatocellular carcinoma cells. J. Agric Food Chem. 58: 814-820.

Johnston TP, Zhou X (2007). Oxidation of low-density lipoprotein cholesterol following administration of poloxamer 407 to mice results from an indirect effect. J. Cardiovasc Pharmacol. 49:246-252.
Kao ES, Wang CJ, Lin WL, Yin YF, Wang CP, Tseng TH (2005). Anti-inflammatory potential of flavonoid contents from dried fruit of *Crataegus pinnatifida in vitro* and *in vivo*. J Agric Food Chem 53:430-436.
Kohlman-Trigoboff D (2005). Lipid management in patients with vascular disease. J. Vasc. Nurs. 23:72-76.
Ren SC, Ding XL (2004). Study on determination methods of flavonoids from corn silk. J. Food Sci., 24: 139-142.
Westhuyzen J (1997). The oxidation hypothesis of atherosclerosis: an update. Ann. Clin. Lab. Sci. 27:1-10.
Yang X, Kang SM, Jeon BT, Kim YD, Ha JH, Kim YT, Jeon YJ (2011). Isolation and identification of an antioxidant flavonoid compound from citrus-processing by-product. J. Sci. Food Agric. 91:1925-1927.