Extraction of Plant Sterols from Jatropha Cake

SHUYU LIU^{1,*}, CHAO LIU² and HUI WANG¹

¹Shanghai University of Engineering Science, Shanghai 201620, P.R. China

²Pharmacy Department of Medical Institute of Nanchang University, Nanchang 330002, P.R. China

*Corresponding author: E-mail: shuyuli1219@yahoo.com.cn

(Received: 24 March 2012;

Accepted: 21 December 2012)

AJC-12592

Solvent extraction technique was applied for the extraction of plant sterols from jatropha cake. The optimum conditions for the lab scale solid liquid extraction were obtained at temperature at 55 °C, extraction time of 2 h, ratio of liquid to solid of 6/1 (v/m) and ligroin (30-60 °C) as a solvent. Under the optimal conditions, the yield of plant sterols was 5.84 %. The plant sterols of Jatropha cake was 0.232 %.

Key Words: Jatropha cake, Extraction, Plant sterols.

INTRODUCTION

Plant sterols, also referred to as phytosterols (PSs), are essential constituents of plant cell membranes because of their membrane stabilizing effect¹. Plant sterols are well-known for their cholesterol lowering properties². Normally, solid liquid extraction is dependent on the nature of the solvent and oil, reaction time between solvent and seeds, temperature of the process and the ratio of solvent to the meal. The objectives of the present study were to investigate the factors affecting plant sterols extraction from jatropha cake using organic solvents and find suitable extraction parameters for plant sterols.

EXPERIMENTAL

Jatropha seeds were purchased from Guizhou province of China. Jatropha cake were residue of Jatropha seeds after the oil extraction.

Ligarine (30-60 °C), ligarine (60-90 °C), diethyl ether, dehydrated alcohol, acetone, acetidin and *n*-hexane were purchased from the Nan Jing Jian Chen Biotechnology Lt. Cod. These reagents used were of analytical grade. β -Sitosterol (95 %) was purchased from Sigma.

All experiments were performed using a WFJ2000 ultraviolet-visible spectrophotometer. The green matter of plant sterols with phosphorus iron pyrites developer had the maximum adsorption at the wave length of 680 nm. The optimal coloration condition of spectrophotometry was 2 mL ethanol, 2 mL phosphorus iron pyrites developer and reacting for 15 min at 50 °C. The UV mode resulted in a determination

coefficient of $R^2 = 0.9983$ at a concentration range of 0.01-0.05 mg/mL. The linear equation was $A = 2.133C - 0.0919$.

Effect of extraction parameters on the yield of the extract

Effect of solvents on the yield of the extract: 25 g, Jatropha cake were placed in 250 mL round bottom flasks. Then 100 mL solvents (ligarine (30-60 °C), ligarine (60-90 °C), diethyl ether, acetone, dehydrated alcohol, acetidin and *n*-hexane) were, respectively added into the flasks. The extracts were in the flasks in a heated water bath shaker (DKS-12; Jiaying Zhongxin Medical Instruments Co., Ltd., Jiaying City, China) for extraction (50 °C, 1 h) and vacuum filtered to remove solvents. Combined filtrate was vacuum evaporated to recover the solvent at 25 °C. Evaporation of the organic solvent under reduced pressure gave the plant sterols extraction, which was weighed to give the yield of the extract.

Yield of the extract (%) = [Plant sterols extraction (g)/Jatropha cake (g)] $\times$ 100 %.

Effect of volume of solvent on the yield of the extract: Volume of solvent was set at 100, 125, 150, 175 and 200 mL, respectively. Ligarine (30-60 °C) was chosen as solvent for extraction (50 °C, 1 h).

Effect of temperature on the yield of the extract: Extraction temperature was set at 35, 40, 45, 50 and 55 °C, respectively. Ligarine (30-60 °C) was chosen as solvent for extraction (100 mL, 1 h).

Effect of extraction time on the yield of the extract: Extraction time was set at 1, 1.5, 2, 2.5 and 3 h, respectively. Ligarine (30-60 °C) was chosen as solvent for extraction (50 °C, 175 mL).

Optimization of the experimental conditions: On the basis of single-factor test, an attempt was made to optimize three parameters, which are confirmed to significantly affect the yield of the extract, as volume of solvent, temperature, extraction time to obtain good yield of plant sterols by orthogonal test. Each of these parameters was varied at three levels: volume of solvent at 150, 175 and 200 mL, temperature at 45, 50 and 55 °C and time of extraction at 1.5, 2.0 and 2.5 h.

RESULTS AND DISCUSSION

Assay of effect of different solvents on the yield of the extract: As shown in Fig. 1, the maximum extraction yield differed with different solvents and was in turn 5.82 % when using ligarine (30-60 °C) as the solvent, 5.77 % when using acetidin, 5.26 % when using acetone, 3.72 % when using *n*-hexane, 3.32 % when using ligarine (60-90 °C), 2.78 % when using diethylether, 1.72 % when using dehydrated alcohol. Result indicate that maximum yield of the extract was achieved when ligarine (30-60 °C) was used. Therefore, ligarine (30-60 °C) was used as the solvent for plant sterols production in the experiment.

Fig. 1. Effect of solvent on the yield of the extract

Assay of effect of solvent volume on the yield of the extract: The yield of plant sterols with increasing volume and reached a peak value at 175 mL (Fig. 2). The plant sterols yield was invariable after this optimum volume value. Therefore, 175 mL was chosen as optimum extraction volume.

Fig. 2. Effect of solvent volume on the yield of the extract

Assay of effect of extraction temperature on the yield of the extract: The yield of plant sterols increased with increasing temperature and reached a peak value at 45 °C (Fig. 3). The percent plant sterols yield inversely decreased after this optimum temperature value. Therefore, 45 °C was chosen as optimum extraction temperature.

Fig. 3. Effect of temperature on the yield of the extract

Assay of effect of extraction time on the yield of the extract: It can be observed that the yield of the extract was found to increase from 2.74-2.97 % when the extraction time increased from 1.0-1.5 h (Fig. 4). The yield of the extract no longer increased with further increase in extraction time, suggesting that when *Jatropha* cake was extracted for 1.5 h, remanent plant sterols contained in powder particles had been dropped to a minimum value. According to the obtained results, extraction time was chosen between 1.5 and 2.5 h for later orthogonal optimisation tests.

Fig. 4. Effect of time on the yield of the extract

Optimization of extraction technology of plant sterols: Based on the analytical data obtained in Table-1, the optimal extraction condition was determined as A3B2C1 when all levels of the three factors were considered. Therefore, the optimal parameters combinations are as following: 150 mL solvent, temperature 55 °C, extraction time 2 h. Further

TABLE-1
OPTIMIZATION OF EXTRACTION
PARAMETERS OF PHYTOSTEROLS

No.	Factors			Extraction rate (%)
	Temperature (°C)	Time (h)	Solvent volume (mL)	
1	1	1	1	3.8124
2	1	2	2	4.9692
3	1	3	3	4.5504
4	2	1	2	3.5024
5	2	2	3	4.3504
6	2	3	1	4.4092
7	3	1	3	3.7836
8	3	2	1	5.8276
9	3	3	2	4.5444
K1	1.111	0.925	1.171	
K2	1.022	1.262	1.085	
K3	1.180	1.125	1.057	
R	0.158	0.337	0.114	

variance analysis shows that the decreasing order of effect of the three factors on extraction yield of plant sterols were B >

A > C. The plant sterols mean extraction yield of three batches of oil was 5.84 % under the optimal extraction condition. The plant sterols of Jatropha cake was 0.232 %.

Conclusion

Four main operating parameters affecting the extraction of plant sterols were optimized. The optimum conditions for the lab scale extraction were obtained 150 mL solvent, temperature 55 °C, extraction time 2 h and ligarine (30-60 °C) as a solvent. The decreasing order of effect of the three factors on extraction yield of plant sterols were time > temperature > solvent volume. Under the optimal conditions, the yield of plant sterols was 5.84 %. The plant sterols of Jatropha cake was 0.232 %.

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