European Journal of Food Science and Technology
Vol.3, No.4, pp.52-58, September 2015
Published by European Centre for Research Training and Development UK (www.eajournals.org)

OPTIMIZATION OF EXTRACTION PROCESS FOR TOTAL POLYPHENOLS FROM ADLAY

Yun-Xin Liu and Qing-Ping Hu*
College of Life Sciences, Shanxi Normal University, Linfen 041004, China

ABSTRACT: *The single-factor experiment and an orthogonal experiment design were adopted to optimize the extracting technology of polyphenols from adlay. The results showed that, the impact order of the influence factors was ethanol concentration > extraction time > extraction temperature, and that ethanol concentration and extraction time have significant difference (p<0.05). The optimum extraction conditions were ethanol concentration 60%, extraction time 1.5 h, extraction temperature 40°C and ratio of liquid to material 15:1. Under the optimized conditions, the yield of total polyphenols from adlay was 2.84 mg/g*.

KEYWORDS: Adlay, Polyphenols, Optimization, Extraction process

INTRODUCTION

Adlay (*Coix lacryma-jobi* L. var. *ma-yuen* Stapf) is an annual or perennial crop that has widely cultivated in China. The matured seed in adlay which is commonly named Chinese pearl barley or soft-shelled Job's tears has long been consumed in traditional Chinese medicine (Lu *et al*., 2008) and as a nourishing food due to its high nutritional value and special biological and functional effects on the human body (Wu *et al*., 2007).

Numerous recent reports have confirmed that the consumption of adlay is really beneficial to the human body. It was reported that adlay has various activities, such as anticancer (Lee *et al*., 2008), antiallergic effects (Chen *et al*., 2010), anti-inflammatory (Chen *et al*., 2011) and antioxidant (Tseng *et al*., 2006; Wang *et al*., 2013) activities and has a great high value in food nutrition and pharmaceutical. In general, the polyphenols in adlay can be exploited as novel antioxidants and have the potential to be used as a functional factor used in food, cosmetic and pharmaceutical industries. However, although the extraction of adlay bran and the pharmacological activities of adlay have been thoroughly investigated, little work has been done regarding the phytochemicals of adlay. In this regard, detailed work is needed to carry out the extraction process of polyphenols in adlay which would not only contribute to the sustainable use of adlay agricultural resource, but also a base for further studies of industrial use of biochemicals in adlay.

MATERIALS AND METHODS

Materials and Regents: The adlay was purchased from the local market. Gallic acid was

ISSN 2056-5798(Print), ISSN 2056-5801(online)

from Sigma. Other chemicals used were all of analytical grade.

Extraction Process: Dried dehulled adlay was grounded and passed through a 40 mesh screen. Adding a certain mount of different concentration ethanol to extract phenolics for some time under certain temperature. Then, the mixtures were centrifuged for 15 min at 4 ^{o}C and 5000 g. After centrifugation, the supernatants were obtained as phenolic extracts from adlay.

Determination of Total Phenolic Content: Total phenolic content was determined using the Folin-Ciocalteu colorimetric method as described by Xu et al. (2010) with slight modifications. An aliquot (0.1 mL) of diluted extracts, 2.8 mL of deionized water and 0.1 mL Folin-Ciocalteu reagent were mixed and stirred. After 8 min, 2 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured at 765 nm wavelength after incubation for 2 h at room temperature. Total phenolic content was expressed as milligram gallic acid equivalent per gram dried weight (mg GAE/g DW).

Single Factor Experiments: In order to develop an optimum extraction condition of polyphenols from adlay, influences of four factors including ratio of solid to liquid, extraction temperature, soaking time and ethanol concentration on yield were investigated.

Orthogonal Experiments: On the basis of the single factor experiment, an orthogonal experiment design was used to optimize extraction condition of polyphenols from adlay. The independent variables, including ethanol concentration (A), extraction time (B) and extraction temperature (C) at three levels in the extraction process were shown in Table 1.

Table 1. Factors and levels of the orthogonal tests

Variable	Levels		
	1	**2**	**3**
Ethanol concentration	50	60	70
Extraction time (h)	1.0	1.5	2.0
Extraction temperature	40	50	60

RESULTS AND DISCUSSION

Effects of Ethanol Concentration on Yield of Polyphenols: In this work, the concentration of ethanol was set at 20%-100%, respectively, to investigate its effect on the yield of polyphenols and the results were shown in Figure 1.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

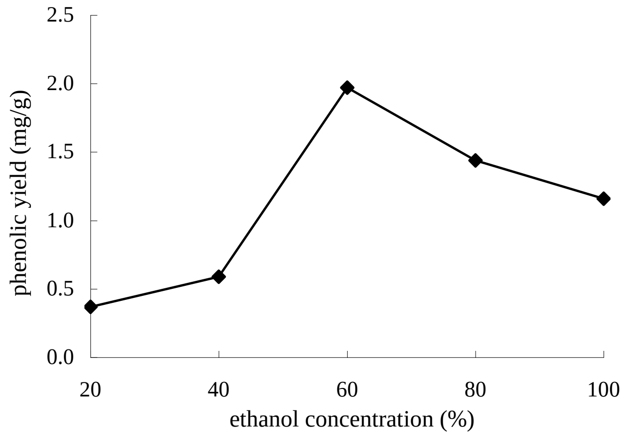

Figure 1. Effect of ethanol concentration on yield of polyphenols from adlay

In general, solvent is considered as an important parameter and the polarity of polyphenol was generally associated with the presence of phenolic hydroxyl groups (Cheok *et al*., 2012; Rebey *et al*., 2012). Thus, the ethanol concentration would affect the polarity of extraction solvent and further influence the solubility of polyphenols during the extraction process. As shown in the Figure 1, the yield of total polyphenols increased gradually with the increase of ethanol concentration in the broad ranges of 20%-60%, and reached the maximum yield at 60%. Thereafter, the polyphenols yield started to reduce slightly with increasing ethanol concentration. Therefore, the 60% ethanol was most suitable for the extraction of total polyphenols from adlay.

Effects of Extraction Time on Yield of Polyphenols: As seen in Figure 2, the yield of polyphenols rose significantly with the increase of extraction time from 0.5 to 1.5 h. After stepping for 1.5 h, extraction yield increased to the peak. That is because longer extraction time could make the release and diffusion of the polyphenols easier into extraction solvent. However, when the extraction time extended from 1.5 to 3.0 h, the yield of polyphenols decreased obviously. It can be explained that when the polyphenols were already sufficiently extracted, excessively lengthening extraction time will induce the condensation of polyphenols or oxidized by oxygen in air which would lead to the decrease of extraction yield. Therefore, after the maximum extraction yield was achieved, longer time of extraction was not necessary and the point of 1.5 h was selected as the optimum extraction time.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

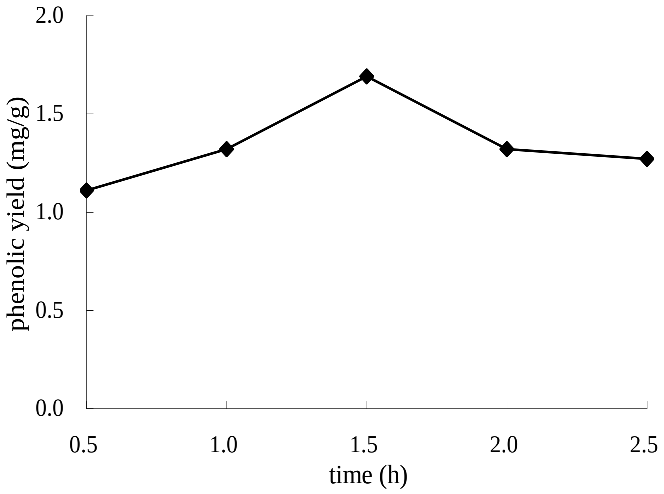

Figure 2. Effect of extraction time on yield of polyphenols from adlay

Effects of Solid to Liquid Ratio on Yield of Polyphenols: As shown in Figure 3, the yield of polyphenols increased remarkably with the increased ratio of liquid to solid from 5:1 to 15:1. When ratio of liquid to solid was more than 15:1, the yield no longer apparently increased. The possible reason is that a larger volume of solvent can make the diffusion of polyphenols occurred more quickly and more polyphenols molecules could dissolve in solvent, which results in a higher yield. But after 15:1, the curve slightly leveled off, meaning that further increase of solid to liquid ratio would not increase the extraction yield of polyphenols. That is because larger volume of solvent will result in more wastage of polyphenols during the procedure. Accordingly, in view of achieving more polyphenols with less solvent and cost consumption, ratio of liquid to solid should be 15:1.

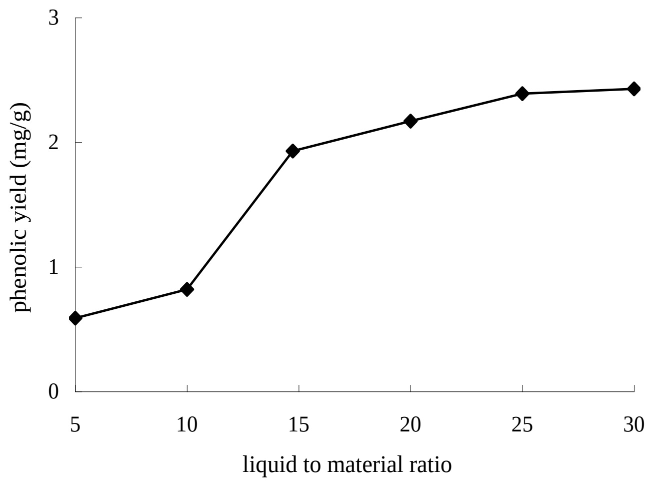

Figure 3. Effect of solid to liquid ratio on yield of polyphenols from adlay

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Effects of Extraction Temperature on Yield of Polyphenols: Generally speaking, temperature shows significant effect on the extraction efficiency and a suitable temperature is such important for polyphenols extraction. Higher temperature would increase the solubility of components in plant tissues and reduce the solvent viscosity, which resulted in the increase of mass transfer and promoted the extraction of polyphenols. In this study, the extraction yields at different temperature (20, 30, 40, 50 and 60°C) are depicted in Figure 4. As revealed in Figure 4, the yield significantly increased with the improvement of temperature from 20 to 60°C. While the extraction temperature rose continually, the yield no longer apparently increased. That is because a too high temperature also caused the hydrolyzation and aggregation of polyphenols which would decrease the extraction yield of polyphenols from adlay (Li *et al.*, 2005). Therefore, in order to obtain the maximum extraction yield, 50 °C was chosen for the suitable temperature.

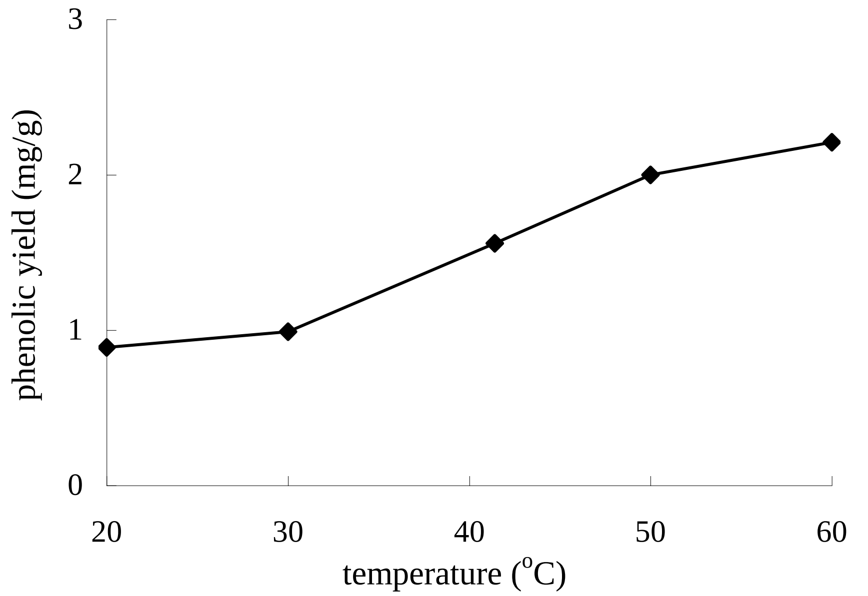

Figure 4. Effect of extraction temperature on yield of polyphenols from adlay

Orthogonal Test: On the basis of the above single factor experiments, an orthogonal test was designed to further optimize extraction parameters. The results of orthogonal test were given in Table 2. The influence of extraction factors on the extraction yield of polyphenols from adlay could be classified in the following decreasing order: ethanol concentration > extraction time > extraction temperature. According to extreme difference analysis, the optimum condition for polyphenols extraction would be the combination of $A_2B_2C_3$. Consequently, the maximum yield of 2.86 mg/g was achieved at ethanol concentration 60%, extraction temperature 60 °C, extraction time 1.5 h and ratio of liquid to solid 15:1.

ISSN 2056-5798(Print), ISSN 2056-5801(online)

Table 2. Results of orthogonal experiment

No.	A	B	C	Vacant	Yield
1	1	1 (1.0 h)	1 (40	1	1.26
2	1	2 (1.5 h)	2 (50	2	1.65
3	1	3 (2.0 h)	3 (60	3	1.78
4	2	1	2	3	2.37
5	2	2	3	1	2.86
6	2	3	1	2	2.49
7	3	1	3	2	1.61
8	3	2	1	3	1.75
9	3	3	2	1	1.86
K_1	1.56	1.75	1.83	1.99	
K_2	2.57	2.09	1.96	1.92	
K_3	1.74	2.04	2.08	1.97	
R	1.01	0.34	0.25	0.07	

Variance Analysis of Orthogonal Experiment: The results of variance analysis showed that the factors, A, B have significant difference (p<0.05), indicating that the ethanol concentration and extraction time on the yield of the total polyphenols had a great influence. No significant differences (p>0.05) was found for Factor C, indicating that the extraction temperature in the three level of orthogonal design had no significant effects on the yield of total polyphenols, therefore, the levels can be selected according to the actual situation (Table 3). Based on this analysis, extraction efficiency, the cost of energy and the feasibility of experiment, the optimal conditions of total polyphenols extraction were therefore determined the combination of $A_2B_2C_1$, namely liquid/material ratio 15:1, extraction time 1.5 h, 60% ethanol, extraction temperature 40 ^oC. Under this condition, the yield of total polyphenols from adlay was 2.84 mg/g.

Table 3. Variance analysis of orthogonal experiments

Sources of variation	Quadratic sum	Degree of freedom	Mean square	F value	p value
A	1.7458	2	0.8729	192.0758	0.0052
B	0.2055	2	0.1027	22.6088	0.0424
C	0.0938	2	0.0469	10.3154	0.0884
D	0.0091	2	0.0045		
Error	0.0091	2	0.0045		
Sum	2.0541				

ISSN 2056-5798(Print), ISSN 2056-5801(online)

CONCLUSION

Based on the single factor experiment and orthogonal design, the important factors on the yield of polyphenols of adlay was ethanol concentration > extraction time > extraction temperature, and ethanol concentration and extraction time have significant difference (p<0.05). The optimum extraction conditions were ethanol concentration 60%, extraction time 1.5 h, extraction temperature 40 ^{o}C and ratio of liquid to material 15:1. The yield of total polyphenols from adlay was up to 2.84 mg/g under this condition.

REFERENCES

Chen, H.-J., Chung, C.-P., Chiang, W. and Lin Y.-L. (2011). Anti-inflammatory effects and chemical study of a flavonoid-enriched fraction from adlay bran. Food Chemistry, 126: 1741–1748.

Chen, H.J., Shih, C.K., Hsu, H.Y. and Chiang, W. (2010). Mast cell-dependent allergic responses are inhibited by ethanolic extract of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) testa. Journal of Agricultural and Food Chemistry, 58: 2596–2601.

Lee, M.Y., Lin, H.Y., Cheng, F., Chiang, W. and Kuo, Y.H. (2008). Isolation and characterization of new lactam compounds that inhibit lung and colon cancer cells from adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) bran. Food and Chemical Toxicology, 46: 1933–1939.

Li, L., Wang, Y. and Yang X. (2005). Study on optimization of extraction technology and antioxidant activity of *Coix lachrymajobi* L. polyphenols. Journal of Xiangnan University, 36(2):23-257.

Lu, Y., Li, C.S. and Dong, Q. (2008). Chinese herb related molecules of cancer-cellapoptosis: A minireview of progress between Kanglaite injection and related genes. Journal of Experimental & Clinical Cancer Research, 27: 31.

Rebey, I.B., Bourgou, S., Debez, I.B.S., Karoui, I.J., Sellami, I.H. and Msaada, K. (2012). Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (*Cuminum cyminum* L.) seeds. Food and Bioprocess Technology, 5: 2827–2836.

Wu, T.-T., Charles, A.L. and Huang, T.-C. (2007). Determination of the contents of the main biochemical compounds of Adlay (*Coxi lachrymal-jobi*). Food Chemistry, 104:1509–1515.

Xu, J.G., Hu, Q.P., Wang, X.D., Luo, J.Y., Liu, Y. and Tian, C.R. (2010). Changes in the main nutrients, phytochemicals, and antioxidant activity in yellow corn grain during maturation. Journal of Agricultural and Food Chemistry, 58: 5751–5756.

Wang, L., Chen, J., Xie, H., Wang, Y., Shi, J., Liang, Z., Liu R., Ju, X. and Yuan, J. (2013). Cytotoxicity and anti-proliferation effect on HepG2 cells and cellular antioxidant activity(CAA) of adlay polyphenols. Scientia Agricultura Sinica, 2013, 46(14): 2990-3002.

ISSN 2056-5798(Print), ISSN 2056-5801(online)