Asian Journal of Agriculture and Food Sciences (ISSN: 2321 – 1571)
Volume 03 – Issue 06, December 2015

Studies on Extraction Process of Total Polyphenols from Galanga (*Alpinia officinarum* Hance) Rhizomes

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ABSTRACT—*The effects of different factors on the yield of total polyphenols were discussed, and the extraction conditions of total polyphenols from galanga rhizomes based on single-factor test and orthogonal experiment were studied in this paper. The results showed that, ethanol concentration had the great effects on the yield of total polyphenols, fllowed by extraction temperature and extraction time, and that ethanol concentration and extraction temperature have significant difference (p<0.05). The optimum extraction conditions were ethanol concentration 60%, extraction temperature 30 ^{o}C, extraction time 1.0 h and ratio of liquid to material 15:1. Under the optimized conditions, the yield of total polyphenols from galanga rhizomes was 13.43 mg/g.*

Keywords—Galanga rhizomes, Polyphenols, Extraction process

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1. INTRODUCTION

Galanga (*Alpinia officinarum* Hance) is a pungent and aromatic rhizome, which is a species of the family Zingiberaceae widely cultivated in southern China. The rhizomes of this plant have been used as a traditional Chinese medicine for relieving stomach ache, treating colds, invigorating the circulatory system and reducing swelling (Liu *et al*., 2014). Pharmacological research found that Galangal had anti-inflammatory, antioxidant, antibacterial, anti-proliferative, anticancer, antitumor, anti-arthritic, antiemetic, anti-nociceptive, anti-psychiatric, antalgic and immunomodulatory effects in oriental medicine (Lee *et al*., 2009; Köse *et al*., 2015; Juntachote & Berghofer, 2005; Zhang *et al*., 2010; An *et al*., 2008; Mayachiew & Devahastin, 2008).

In the chemical studies of the rhizomes, some bioactive chemical constituents in galanga including volatile oil (Jirovetz *et al*., 2003), phenylpropanoids (Ly *et al*., 2002), diarylheptanoids, and Flavonoids (Tao *et al*., 2006; Zhao *et al*., 2010) have been reported so far. However, as one of important secondary metabolites and bioactive substances in plant, total polyphenols from galanga rhizomes has little been reported.

In this study, extraction technology of the extraction process of total polyphenols in galanga rhizomes are studied based on orthogonal design, which is also expected to provide technical support for industrial production and comprehensive development and utilization.

2. MATERIALS AND METHODS

2.1 Materials and regents

The dried galanga rhizomes were purchased from the local market. Gallic acid was from Sigma. Other chemicals used were all of analytical grade.

2.2 Extraction process

The dried galanga rhizomes 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 10 min at 4 ^{o}C and 4000 g. After centrifugation, the supernatants were obtained as total phenolic extracts from galanga rhizomes.

2.3 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 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).

2.4 Single factor experiments

In order to develop an optimum extraction condition of total polyphenols from galanga rhizomes, influences of four factors including ratio of solid to liquid, extraction temperature, extraction time and ethanol concentration on yield were investigated.

2.5 Orthogonal Experiments

On the basis of the single factor experiment, an orthogonal experiment design was used to optimize extraction condition of total polyphenols from galanga rhizomes. The independent variables, including ethanol concentration (A), extraction temperature (B) and extraction time (C) at three levels in the extraction process were investigated.

3. RESULTS AND DISCUSSION

3.1 Effects of solid to liquid ratio on polyphenols yield

As shown in Figure 1, the yield of total polyphenols increased remarkably with the increased ratio of liquid to solid from 1:5 to 1:15. 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 total 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 total polyphenols. Accordingly, in view of achieving more polyphenols with less solvent and cost consumption, ratio of liquid to solid should be 15:1.

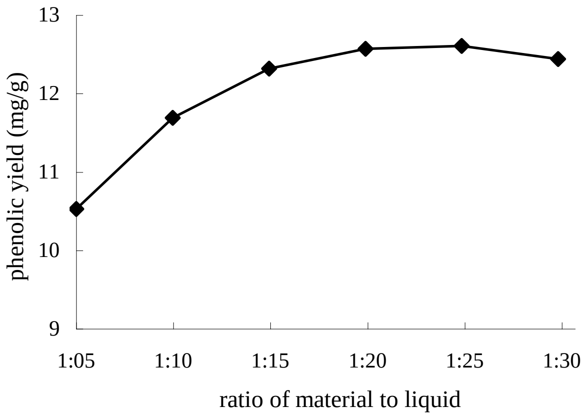

Figure 1. Effect of solid to liquid ratio on total polyphenols yield

3.2 Effects of ethanol concentration on polyphenols yield

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 2.

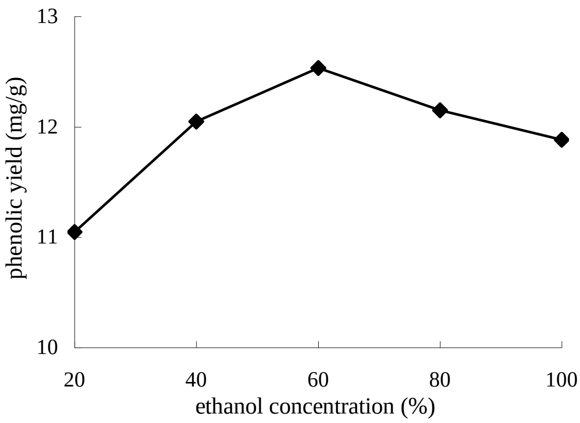

Figure 2. Effect of ethanol concentration on total polyphenols yield

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 2, 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 galanga rhizomes.

3.3 Effects of extraction time on polyphenols yield

As seen in Figure 3, the yield of total 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 2.5 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.

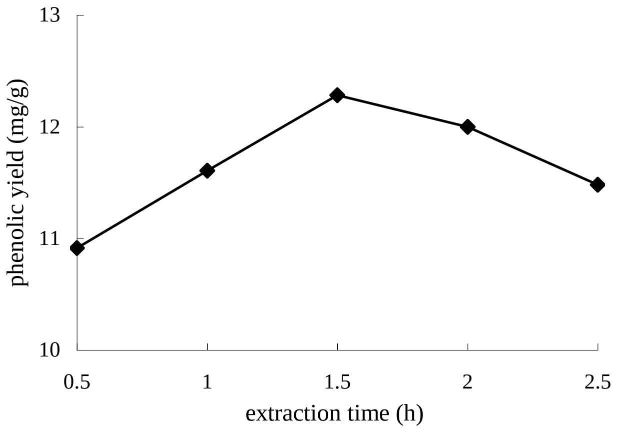

Figure 3. Effect of extraction time on total polyphenols yield

3.4 Effects of extraction temperature on polyphenols yield

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 are depicted in Figure 4. As revealed in Figure 4, the yield significantly increased with the improvement of temperature from 20 to 40^{o}C. While the extraction temperature rose continually, the yield decreased apparently. That is because a too high temperature also caused the hydrolyzation and aggregation of polyphenols which would decrease the extraction yield of polyphenols (Li *et al*., 2005). Therefore, in order to obtain the maximum extraction yield, 40 ^{o}C was chosen for the suitable temperature.

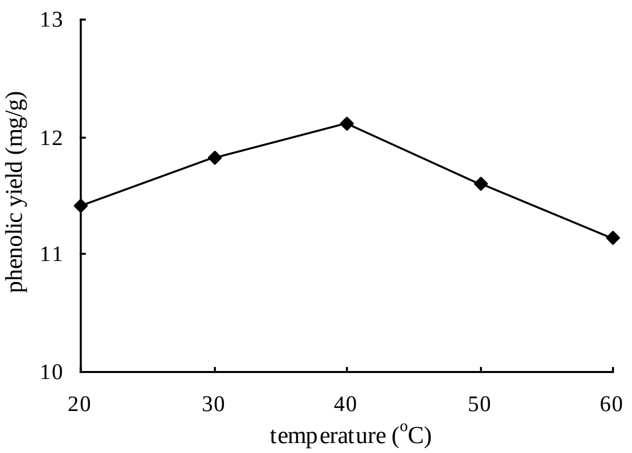

Figure 4. Effect of extraction temperature on total polyphenols yield

3.5 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 1. The influence of extraction factors on the extraction yield of polyphenols from galanga rhizomes could be classified in the following decreasing order: ethanol concentration > extraction temperature > extraction time. According to extreme difference analysis, the optimum condition for polyphenols extraction would be the combination of $A_2B_1C_1$. Consequently, the maximum yield of 13.43 mg/g was achieved at ethanol concentration 60%, extraction temperature 30 ^oC, extraction time 1.0 h and ratio of liquid to solid 15:1.

Table 1: Results of orthogonal experiment

No.	A ethanol concentration/%	B temperature/^oC	C extraction time/h	Vacant column	Yield (mg/g)
1	1 (50)	1 (30)	1 (1.0)	1	11.82
2	1	2 (40)	2 (1.5)	2	12.15
3	1	3 (50)	3 (2.0)	3	10.69
4	2 (60)	1	2	3	13.45
5	2	2	3	1	12.24
6	2	3	1	2	11.71
7	3 (70)	1	3	2	11.78
8	3	2	1	3	11.71
9	3	3	2	1	11.09
K_1	11.55	12.35	11.75	11.72	
K_2	12.47	12.03	12.23	11.88	
K_3	11.53	11.16	11.16	11.95	
R	0.94	1.19	1.17		

3.6 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 temperature had a great influence on the yield of the total polyphenols. No significant differences (p>0.05) was found for Factor C, indicating that the extraction time in the three level of orthogonal design had no significant effects on the yield of total polyphenols (Table 2). 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 $A_2B_1C_1$, namely liquid/material ratio 15:1, extraction time 1.0 h, 60% ethanol, extraction temperature 30 ^oC. Under this condition, the yield of total polyphenols from galanga rhizomes was 13.43 mg/g.

Table 2: Variance analysis of orthogonal experiments

Sources of variation	Quadratic sum	Degree of freedom	Mean square	F value	*p* value
A	1.7185	2	0.8592	19.9773	0.0477
B	2.2654	2	1.1327	26.3345	0.0366
C	0.7004	2	0.3502	8.1423	0.1094
D	0.0860	2	0.0430		
Error	0.0960	2	0.0430		
Sum	4.7703				

4. CONCLUSION

Based on the single factor experiment and orthogonal design, the important factors on the total polyphenols yield of galanga rhizomes was ethanol concentration > extraction temperature > extraction time, and ethanol concentration and extraction temperature have significant difference (p<0.05). The optimum extraction conditions were ethanol concentration 60%, extraction time 1.0 h, extraction temperature 30 ^{o}C and ratio of liquid to material 15:1. The yield of total polyphenols from galanga rhizomes was up to 13.43 mg/g under this condition.

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