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Optimization of the extraction of essential oils from *Areca catechu* L. flowers using response surface methodology

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ABSTRACT

The present work was to optimize a water vapor distillation procedure to extract essential oils from *Areca catechu* L. Flowers. A three-variable, three-level Box-Behnken experimental design (BBD) was conducted to analyse the effects of soaking time, distillation time and solid to liquid ratio on the extraction efficiency. The experimental data were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed using the appropriate statistical methods. The optimisation suggested that extraction with the soaking time for 4.8h, distillation time for 5.0h and the ratio of solid to liquid 1:2.7 were the best solutions for this combination of variables, under the optimum operating conditions, the extraction yield was 0.0196%. © 2014 Trade Science Inc. - INDIA

KEYWORDS

Areca flower (*Areca catechu* L.);
Essential oils;
Water vapor distillation;
Response surface analysis (RSA).

INTRODUCTION

Areca (*Areca catechu* L.) is one of the traditional herbal medicines used in Hainan and widely distributed in East Africa, Southeast Asia and Pacific islands. Its flower contain essential oils, arecoline[1], phenolics[2], amino acids[3], is a favorite healthcare products for its nutritional content[4]. Essential oils compounds are the main body of the aroma.

The water vapor distillation, which has been one of the most frequently used methods for the isolation of volatiles, has already shown its better reliability for the extraction of volatile compounds of various spices, such as rose, clove and rosemary[5].

The technology of areca flower essential oils extraction is analysed with the response surface methodology (RSM), in order to optimize the technical parameters process. It will obtain more suitable essential conditions for extraction of areca flower for development and utilization, aiming at providing the scientific experiment basis for areca flower and its odoriferous substances extraction[6].

MATERIALS AND METHODS

Materials

Fresh areca flower was obtained from the Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, Hainan Province, China. The samples were comminuted by a pulverizer using high speed.

Experimental design

On the basis of single-factor experimentation, soaking time, distillation time and solid to liquid ratio were determined. A three-factor and three-level Box-Behnken design (BBD) consisting of seventeen experimental runs was used, including three replicates at the center point[7]. Soaking time (*A*, h), distillation time (*B*, h) and solid to liquid ratio (*C*, volume per mass) were chosen for independent variables, the dependent variable was extraction yield of areca flower (*Y*, %). Other factors in the extraction were kept constant: the amount of areca flower (200 g). The three variables and their levels in coded and natural values are shown in TABLE 1. The whole design consisted of 17 experimental points generated by Box Behnken design are presented in TABLE 2.

TABLE 1 : Independent variables and their coded and actual values used for optimization

Independent variables	Symbol		Code levels		
	Coded	Uncoded	-1	0	1
Soaking time/h	X_1	x_1	1	3	5
Extraction time/h	X_2	x_2	1	3	5
Solid to liquid ratio (volume per mass)	X_3	x_3	1	3	5

Essential oils compounds extraction

The water vapor distillation conditions, such as extraction temperature, amount of areca flower, water and solvent, have been optimized in our laboratory. The essential oils extraction compounds were extracted by diethyl ether. 200g of areca flowers was laced in a 1000ml round-bottom flask with purified water. The samples in flasks were heated to boiling. Collected the distillate, and extraction with diethyl ether. After cooling to ambient temperature for some hours, the diethyl ether extract was dried over anhydrous sodium sulfate. The extract was carefully concentrated at 40°C with a rotary evaporator at atmospheric pressure, and then dichloromethane was used to metered volume to 10mL.

In the present work, the extraction yield (*Y*, %) was the ratio of extract mass and test sample mass (weight).

$$Y=(W_1/W_0)\times 100\%. \quad (1)$$

Where Y is the ratio of extraction mass in test sample areca flowers mass, W_1 is the extraction mass from each run of the extraction, W_0 is the test sample mass.

TABLE 2 : Experimental designs and the results of Box-Behnken design

Run Number	Coded Factor Values			Uncoded Factor Values			Response Values
	X_1	X_2	X_3	x_1	x_2	x_3	Y
1	0	0	0	3	3	3	0.0149
2	0	1	1	3	5	5	0.0167
3	1	0	-1	5	3	1	0.0149
4	0	-1	1	3	1	5	0.0051
5	-1	1	0	1	5	3	0.0140
6	1	-1	0	5	1	3	0.0061
7	1	0	1	5	3	5	0.0152
8	0	0	0	3	3	3	0.0151
9	-1	-1	0	1	1	3	0.0050
10	0	1	-1	3	5	1	0.0161
11	0	0	0	3	3	3	0.0189
12	0	0	0	3	3	3	0.0161
13	0	0	0	3	3	3	0.0122
14	0	-1	-1	3	1	1	0.0041
15	-1	0	1	1	3	5	0.0081
16	1	1	0	5	5	3	0.0195
17	-1	0	-1	1	3	1	0.0131

Data analysis

Analysis of each set of experimental design and calculation of predicted responses were carried out using Design Expert software (version8.0).

A second-order polynomial equation was used to fit the experimental data of the studied variables. The generalized second-order polynomial model used in the response surface analysis is shown in Eq. (2):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{k} \beta_{ij} X_i X_j \quad (2)$$

Where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and Xi, and Xj are the independent variables. The significances of all terms in the polynomial were judged statistically by computing the F value at a probability (P) of 0.05 and 0.1.

RESULTS AND DISCUSSION

Fitting the models

Mean values of all responses by water vapor distillation are presented in TABLE 2. The following regression equation was obtained.

$$Y = 0.015 + 0.001938*X_1 + 0.005775*X_2 - 0.004125*X_3 + 0.001125*X_1*X_2 + 0.00125*X_1*X_3 - 0.000075*X_2*X_3 - 0.000975*X_1*X_1 - 0.0033*X_2*X_2 - 0.001675*X_3*X_3. \quad (3)$$

Where Y, extraction yield of areca flower (%), X_1, soaking time(h); X_2, extraction time(h); X_3, ratio of liquid to solid. The quadratic model indicated that the linear variables X_1, X_2, X_1X_2, X_1X_3 demonstrated positive effects on the extraction yield, whereas the linear variables X_3, the quadratic variables X_1^2, X_2^2, X_3^2, and the two variable interactions X_2X_3 showed negative effects.

Analysis of variance (ANOVA) was performed to investigate the adequacy of the suggested models and identify the significant factors (TABLE 3). The fitness of the quadratic polynomial model was inspected by the regression coefficient R^2. *F*-value and p-value can check the significances of experimental variables. Moreover, the regression model can well fit the response values when generated small *P* value and high R^2 values. R^2 was calculated to b 0.9222 for the extraction efficiency. This implies that the sample variation of 92.22% could be attributed to the independent variables and the model did not explain only 7.78% of the total variations (TABLE 3). The model's *p*-value of 0.0039 was less than 0.05 implying that the model is significant.

TABLE 3 : Analysis of variance for BBD

Source	*SS*	*DF*	*MS*	*F*	*Pr>F*
Model	0. 00037645	9	0.00004183	9.21528051	0.0039
X_1	0.00003003	1	0.00003003	6.61637422	0.0369
X_2	0.00026681	1	0.00026681	58.78149343	0.0001
X_3	0.00000136	1	0.00000136	0.29990558	0.6010
X_1X_2	0.00000506	1	0.00000506	1.11535133	0.3260
X_1X_3	0.00000625	1	0.00000625	1.37697695	0.2790
X_2X_3	0.00000002	1	0.00000002	0.00495712	0.9458
X_1^2	0.00000400	1	0.00000400	0.88184502	0.3790
X_2^2	0.00004585	1	0.00004585	10.10208265	0.0155
X_3^2	0.00001181	1	0.00001181	2.60263137	0.1507
Residual	0.00003177	7	0.00000454		
(Lack of Fit)	0.00000657	3	0.00000219	0.34775132	0.7940
(Pure Error)	0.00002520	4	0.00000630		
Total	0. 00040822	16			

Optimization of water vapor distillation and verification of models

In common, the efficiency of the extraction is influenced by multiple parameters such as temperature, time and solvent polarity, among others, and their effects may be either interactive or independent[8]. Selection of appropriate conditions is crucial for the extraction of volatile compounds. The three factors and lower, middle and upper design points for RSM in coded and uncoded values are shown in TABLE 1.

According to the experimental design, 17 experimental results are shown in TABLE 2. By ANOVA (analysis of variation) on the results, the different significances of all variation sources were obtained (TABLE 3). The linear variables X_2 was statistically very significant at $P < 0.01$; the linear variables X_1 had significant influences ($P < 0.05$) on the extraction yield; and the quadratic variables X_2^2 had significant influences ($P < 0.05$) on the extraction yield of aniseed; whereas the linear variables X_3, the quadratic variables X_1^2 and X_3^2, the two-variable interactions X_1X_2, X_1X_3 and X_2X_3 had no significant influence ($P > 0.05$) on the extraction yield.

To compare the predicated results with the practical values, verification experiments were performed using the deduced optimal conditions. The mean value of extraction yields obtained from experiments was 0.0201%. Under the same conditions, the model predicated that the maximum result was 0.0196%, it was not significantly different between predicated values and experimental values within 95% confidence interval, confirming the validity and adequacy of the predicted models.

CONCLUSIONS

The water vapor distillation showed the best extraction rates for all kinds of volatiles. An extraction method of areca flower was developed with the application of the water vapor distillation. On the basis of the preliminary investigation, optimal extraction parameters for the water vapor distillation were: the soaking time 4.8h, extraction time 5h, and the ratio of solid to liquid 1:2.7. These conditions not only could increase the extraction yield, but also greatly shortened the total experiment time. Thus, the method of the water vapor distillation has a promising application for the extraction of areca flower.

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