Advance Journal of Food Science and Technology 9(5): 367-371, 2015
DOI: 10.19026/ajfst.9.1917
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: March 9, 2015 Accepted: March 24, 2015 Published: August 20, 2015

Research Article

Optimization of Extraction Conditions on the Yield of Rana Chensinensis Egg Oil by Statistical Experimental Designs

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Abstract: Rana chensinensis egg oil is extracted from Rana chensinensis. The important variables (extraction temperature, extraction time and the ratio of oviductus ranae egg oil and extraction solvent) were optimized by Box-Behnken central composite design under response surface methodology. The statistical analysis showed that the optimum extraction conditions (extraction temperature 60.62°C, extraction time 3.16 h and the ratio of oviductus ranae egg oil and extraction solvent 8.91.) led to a maximum yield (19.3%). The Rana chensinensis egg oil was refined. The developed method has a potential to be used for efficient extraction conditions on the yield of Rana chensinensis egg oil.

Keywords: Model, optimization, rana chensinensis egg oil, statistical experimental designs

INTRODUCTION

Rana chensinensis egg oil is the fallopian tube of Rana chensinensis, which is widely known of its effects of nourishing the kidney and strengthening the essence, protecting and strengthening health and prolonging life, improving sight and hearing in china. Rana chensinensis egg oil contains a variety of amino acids and more than 30 kinds of microelement, various unsaturated fatty acids, vitamin D, vitamin E, estrogen, progesterone, fatty acids and other bioactive substance. Vitamin A, vitamin D, vitamin E, vitamin C is very useful for humans' growth and development, delay senility and so on. Therefore, Rana chensinensis egg oil is very expensive in international market. So, it was called "soft gold". With the development of the social economy and the improvement of the people's living standard, nutritional, healthy and drug effective value of Rana chensinensis egg oil was acknowledged by more and more people, especially its drug effective value (Lu *et al*., 2002). So, it is important to effectively obtain the Rana chensinensis egg oil from the fallopian tube of Rana chensinensis. The extraction was an easy and effective way to obtained Rana chensinensis egg oil. Therefore, an optimized extraction condition was essential for the research of Rana chensinensis egg oil.

Statistical experimental designs are becoming widespread in several sciences such as analytical chemistry, engineering environmental chemistry and bioprocess, because the methods carefully explore the experimental space while studying various variables using a small number of observations and disclose the relationship between the variable and response (Tran *et al*., 2010; Musyoka *et al*., 2012). However, it is lacking in literature so far about extraction conditions on the yield of Rana chensinensis egg oil by statistical experimental designs.

The goal of this present study was to develop a method for Rana chensinensis egg oil, to optimize extraction conditions on the yield of Rana chensinensis egg oil and to model extraction conditions on the yield of Rana chensinensis egg oil by statistical experimental designs and statistical analysis. At last, he Rana chensinensis egg oil was refined to obtain.

MATERIALS AND METHODS

Experiment materials: Rana chensinensis egg was purchased from market. Ethyl acetate, petroleum ether was purchased from Sinopharm Chemical Reagent Co. Ltd. China.

Experiment instrument: HH-6 Electro Thermostatic Water Bath; 101-1Electric Blast Drying Oven; FA-2004 electronic analytical balance; JW-2 pulverizer.

Experiment methods:

Extraction yield of Rana chensinensis egg oil: The fallopian tube of Rana chensinensis was obtained from Rana chensinensis and washed by normal saline. The fallopian tube of Rana chensinensis was homogenated by pulp refiner. Ammonium sulfate was added into the eluant at 4°C, 24h. The eluant was filtered and the filter liquor was collected. Wet Rana chensinensis egg was dried in Blast Drying Oven at 80°C, 24h (Water content Rana chensinensis egg should was less then 8% in order

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to enhance the extraction and quality of Rana chensinensis egg oil). 15 g of dried Rana chensinensis egg was put in Soxhlet extractor and reflux. Then, Rana chensinensis egg oil was obtained by reduced pressure distillation. The extraction yield was defined by weight of Rana chensinensis egg oil.

Box-Behnken central composite design under response surface methodology: The Box-Behnken central composite design under response surface methodology was employed in order to illustrate the nature of the response surface in the experimental region and elucidate the optimal conditions of the most significant independent variables. According to the CCD for two variables, 15 experimental runs (3 runs at centre point) were fitted to the following second order polynomial model:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^{\,2} + \sum \beta_{ij} X_i X_j$$

where, Y was the predicted response, β0 was the intercept term, βi was the liner term, βii was the square term, βij was the interaction term and X was independent variables. The developed regression model was evaluated by analyzing the regression coefficients, analysis of variance (ANOVA), P and F values. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2. The statistical software package statistical 8.0 (statsoft Inc, USA) was used to identify the experimental design as well as to generate a regression model and to predict the optimal levels of the selected variables which were obtained by solving the regression equation and analyzing the response surface contour.

RESULTS AND DISCUSSION

The choice of extraction solvent: From the Table 1, we can see that when ethyl acetate was extraction solvent, the extraction yield was less the other two kind of petroleum ether. The two kinds of petroleum ether's Extraction yield are closed. The best extraction solvent is petroleum ether (60-90). So, petroleum ether (60-90) is used by petroleum ether in the latter experiment.

Optimization of variable by Box-Behnken central composite design under response surface methodology: From the Table 2 to 4, we can see that the F value and P value were used to check the significance of each coefficient, which also indicated the interaction strength between independent variables. The lager the magnitude of the F value and the smaller the P value, the more significant was the corresponding coefficient (Elibol, 2004). It was observed that the linear and the square effect of enzyme concentration and mole ratio were significant at the level of $p<0.05$. Furthermore, the interactive term between enzyme concentration and mole ratio shown in the ANOVA analysis from Table 4 were not significant ($p>0.05$). Linear and quadratic effects of parameters were significant, meaning that they could act as limiting variable and little variation in their concentration would alter the conversion to a considerable extent. The value of determinations coefficient R^2 was 0.9988. It meant that 99.88% variability of response could explain, indicating a good agreement between experimental and predicted values. The "lack of fit" measured the failure of the model to represent data in the experimental domain at points which were not included in the regression. The non-significant "lack of fit" also indicated that the model was a good fit.

Table 1: The choice of extraction solvent

Extraction solvent	Extraction yield
Ethyl acetate	8.5
Petroleum ether (30-60)	12
Petroleum ether (60-90)	13

Table 2: Variables and levels of the reaction

Level	Extraction temperature (°C)	The ratio of egg oil and extraction solvent	Extraction time (h)
-1	45	6	2
0	60	9	4
1	75	12	6

Table 3: Experimental design and results with response surface methodology

Runs	Reaction temperature	Substrate molar ratio	Reaction time	Y (%)
1	-1	-1	0	10.0
2	1	-1	0	16.0
3	-1	1	0	12.0
4	1	1	0	15.0
5	-1	0	-1	15.0
6	1	0	-1	17.0
7	-1	0	1	13.5
8	1	0	1	14.0
9	0	-1	-1	16.0
10	0	1	-1	15.5
11	0	-1	1	15.0
12	0	1	1	16.0
13	0	0	0	18.5
14	0	0	0	19.0
15	0	0	0	19.5

The regression equation coefficients were calculated and the data were fitted to a second-order polynomial equation. The regression equation obtained for conversion was as follows:

$$Y\ (\text{Extraction yield}) = 19.00+1.44*A+0.19*B-0.63*C-0.75*A*B-0.37*A*C+0.38*B*C-3.25*A^2-2.50*B^2-0.88*C^2$$

where, Extraction yield (Y) was a function of A (extraction temperature), B (the ratio of oviductus ranae egg oil and extraction solvent) and C (extraction time). Figure 1 showed the fitted curve of predicted and actual value. It demonstrated that regression equation was very close to the actual value.

Table 4: Results of the ANVOA analysis of regression equation

Source	Sum of square	Degree of freedom	Mean square	F	p>F
Mold	81.92083	9	9.102315	5.825481	0.0333
A	16.53125	1	16.53125	10.58000	0.0226
B	0.281250	1	0.281250	0.180000	0.6890
C	3.125000	1	3.125000	2.000000	0.2164
AB	2.250000	1	2.250000	1.440000	0.2839
AC	0.562500	1	0.562500	0.360000	0.5747
BC	0.562500	1	0.562500	0.360000	0.5747
A2	39.00000	1	39.00000	24.96000	0.0041
B2	23.07692	1	23.07692	14.76923	0.0121
C2	2.826923	1	2.826923	1.809231	0.2364
Lack of fit	7.812500	5	1.562500		
Pure error	7.312500	3	2.4375	9.750000	0.0944
	0.500000	2	0.2500		
	89.73333	14			

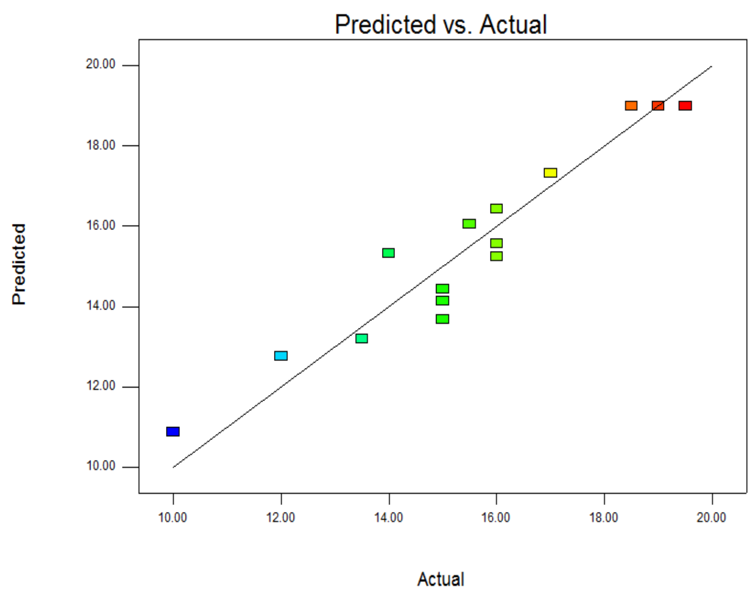

Fig. 1: The fitted curve of predicted and actual value

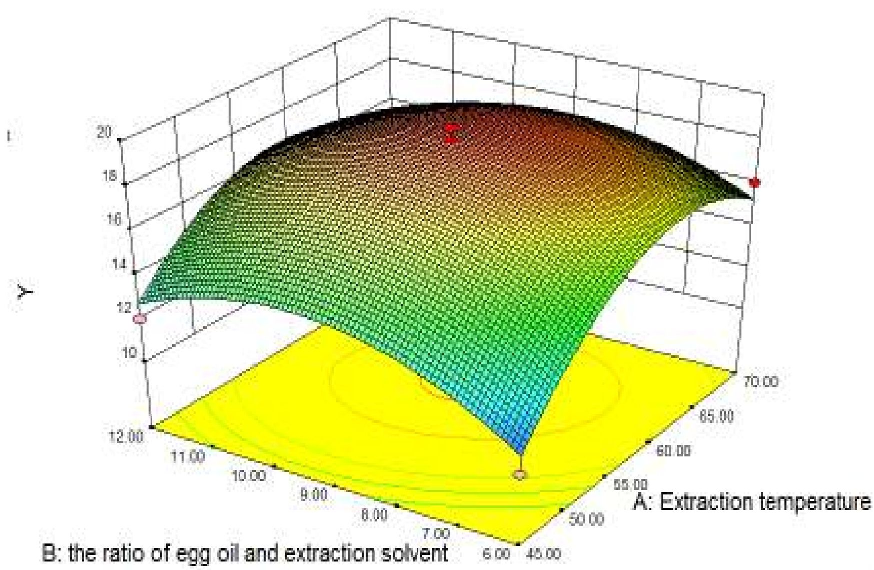

Fig. 2: 3D response surface shows the effect of extraction temperature and the ratio of Rana chensinensis egg oil and extraction solvent on the yield

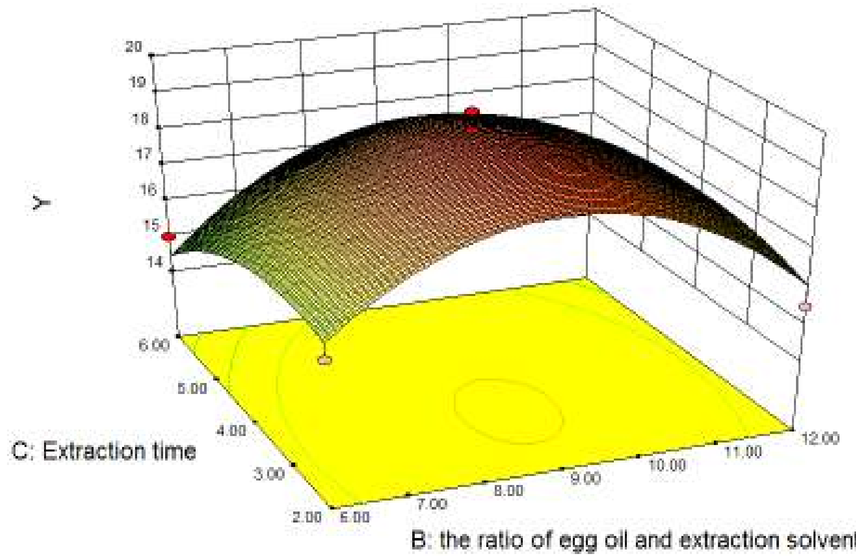

Fig. 3: 3D response surface shows the effect of extraction time and the ratio of Rana chensinensis egg oil and extraction solvent on the yield

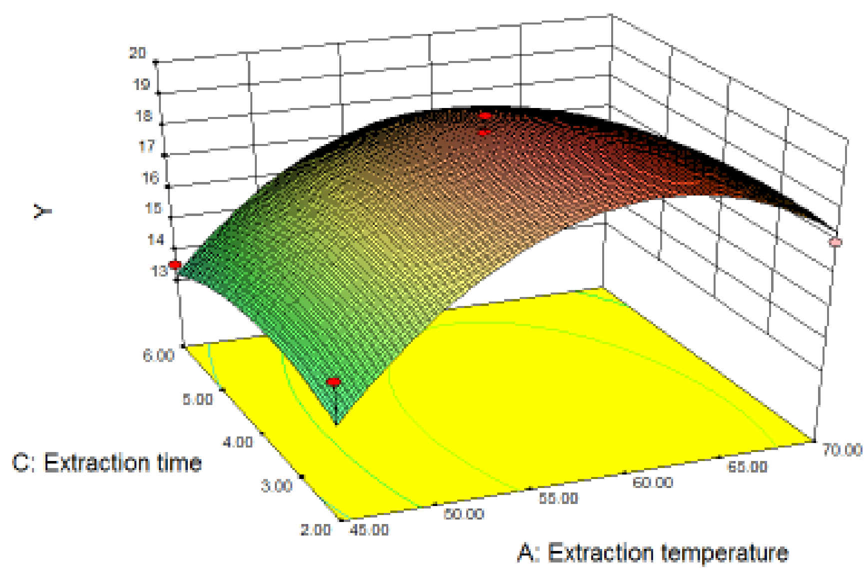

Fig. 4: 3D response surface shows the effect of extraction temperature and the ratio of Rana chensinensis egg oil and extraction solvent on the yield

The 3D response surface plot was the graphical representations of the regression equation to investigate the interaction among extraction temperature, the ratio of oviductus ranae egg oil and extraction solvent and extraction time to determine the optimum values of each factor for maximum conversion (Fig. 2 to 4). These results showed the Extraction yield was considerably affected by extraction temperature, the ratio of oviductus ranae egg oil and extraction solvent and extraction time. The maximum conversion of about 19.3% was predicted at extraction temperature 60.62°C, extraction time 3.16 h and the ratio of oviductus ranae egg oil and extraction solvent 8.91.

Optimum conditions and model verification: According to Box-Behnken central composite design response surface methodology, the optimum reaction with the maximum predicted conversion was suggested as 19.3% at (extraction temperature 60.62°C, extraction time 3.16 h and the ratio of Rana chensinensis egg oil and extraction solvent 8.91. The adequacy of the predicted model was examined by performing three additional independent experiments at the suggested optimum synthesis conditions. The predicted conversion was 19.3% and the actual experimental values were 19.1, 19.3 and 19.5%, respectively. The mean was 19.3%. A chi-square test (P value = 0.9, degrees of freedom = 2) indicated that the observed values were essentially the same as the predicted values and that the generated model adequately predicted the conversion (Ott and Longnecker, 2008; Lerin *et al*., 2012).

The refine of Rana chensinensis egg oil: The extracted Rana chensinensis egg oil was put into the flask, stired and heated by oil bath. The temperature slowly warm up to 120 degrees when the temperature was up to 110 degrees. Then, the temperature slowly cooled to 80 degrees when the bubbles disappeared. The refining agent (hydrogen peroxide solution (30%, pH = 9)) was put into the flask. The amount of refining agent was the volume of extracted Rana chensinensis egg's 5%. Then, the temperature slowly warmed up to 110 degrees when the many bubbles appeared. The temperature was kept until the bubbles disappeared. The solution was transferred to the separating funnel. The solution was washed by warm water until the pH was closed to normal. The activated clay was added into by weight of 4%. The decoloration was at 80-90 degrees, 20 min. The yellow, lucency and fishy Rana chensinensis egg oil was obtained by filtration.

CONCLUSION

Rana chensinensis egg oil was acknowledged by more and more people, especially its drug effective value So, it is important to effectively obtain the Rana chensinensis egg oil from the fallopian tube of Rana chensinensis. The best extraction solvent is petroleum ether (60-90) by screening three kinds of extraction solvent. Statistical experimental designs for optimization of extraction conditions on the yield of Rana chensinensis egg oil were developed. The optimum values were determined by Box-Behnken central composite design under response surface methodology by optimizing the extraction condition (extraction temperature, extraction time and the ratio of oviductus ranae egg oil and extraction solvent). The statistical analysis showed that the optimum conditions (extraction temperature 60.62°C, extraction time 3.16 h and the ratio of oviductus ranae egg oil and extraction solvent 8.91.) led to a maximum yield (19.3%). Validation experiments verified the availability and the accuracy of the model. The predicted values were in agreement with the experimental values. Obviously, extraction conditions on the yield of Rana chensinensis egg oil by statistical experimental designs and statistical analysis was proved to be a useful and powerful tool in developing optimal extraction conditions. At lastly, the Rana chensinensis egg oil was refined. The yellow, lucency and fishy Rana chensinensis egg oil was obtained.

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