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Response Surface Optimization for The Extraction of Oil from Dog Rose Seed (*Rosa Canina*)

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Abstract

Solvent extraction of dog rose seed was conducted to establish best operation conditions for future study. Extraction variables were temperature, solvent type and L/S ratio. The best extraction conditions at laboratory scale were investigated applying Box-Behnken design (BBD) using response surface methodology (RSM). The highest extract yield was obtained (6.93 %) by isopropanol at the optimum conditions of 3 L/S ratio and 50-55 ^{0}C temperature range. Results of experiments confirmed the results of prediction.

Keywords*: dog rose seed, extraction of oil, response surface optimization, solvent ratio, solvent type*

1. INTRODUCTION

Dog hip, dog rose (*Rosa Canina L.*) is a widespread plant in the world. This wild plant possesses huge valuable ingredients. These include vitamins, carotenoids, phenols, carbohydrates, flavonoids etc. (Amin et al., 2018; Cui., at al., 2014; Demir et al., 2014; Inés at el., 2017; Nadpal et al., 2016). Dog rose is a source of the bioactive substrates and shows anti–inflammatory, antioxidant activities in pharmaceutical industry (Deliorman et al., 2007; Ioana et al., 2013). It can be used as a marmalade, tea, dried fruit, jam in food industry (Ana et al., 2013; Cihat et al., 2010). Dog rose seed is a waste product of the pharmaceutical and food industries. Dog rose (*Rosa Canina L.*) seed was processed to obtain oil with richer source of polyunsaturated fatty acids, as a linoleic acid (35-55%) and linolenic acid (17-27%) which may be used in cosmetics and other purposes (Jahongir et al., 2019; Kazaz et al., 2009; Musa, 2002). Essential fatty acids as linoleic (omega-6) and linolenic (omega-3) play a key role for developing of human body as well as to prevent certain diseases. These essential fatty

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acids are used for skin care, restriction of formation of eczemas, water loss and healing impacts on dermatoses (Aleksandra et al., 2014; Proksch et al., 2008).

Solvent extraction, mechanical extraction, supercritical fluid extraction and other extraction methods were employed for the recovery of dog rose seed oil. Traditional solvent extraction, has been executed to obtain oil from matrixes. However, due to the application of organic solvents and higher temperature in process, the oil quality may reduce.

Cold pressing is also utilized for extraction of rose hip seed (*Rosa affinis rubiginosa*) oil. The quality of produced oil is high enough but oil recovery percentage is relatively low comparing to other methods. Although, obtained oil amount is low (30-40%), it can be increased by the enzymatic ways (Concha et al., 2004; Concha et al., 2006). However, low oilcontent of the seed in pressing makes it economically infeasible. Solvent extraction is more applicable for seeds with low oil content (Szentmihályi et al., 2002).

Moreover, to find the greener solvent species, the best operational conditions and lower temperature ranges are necessary for oils with higher percentage of polyunsaturated fatty acids as well as solvents should be economically and environmentally viable.

Generally, optimization studies are implemented by classical methods and response-surface methodology (RSM). Systemic approach would consume much more time to local the optimum operation conditions and fail to understand the interactions between various operating parameters studied. RSM is an effective mathematical and statistical technique for optimization. Applying by RSM can be defined influence of parameters on response and interaction of them as well (Myers and Montgomery, 1995).

Dog rose seed was waste of the industries of Uzbekistan. Even though many studies have been implemented in the world, local verities of dog rose seed was not investigated at all. Additionally, studies on lower extraction temperature ranges have not been performed in laboratory scales.

Above mentioned issues lead to determination of preferable solvent, temperature ranges and L/S ratios. The goal of our study is to determine optimum extraction conditions of dog rose seed by applying response-surface methodology (RSM) in lab scale.

2. MATERIALS AND METHODS

2.1. Materials

Dog rose seed (*Rosa Canina*) was bought from market. Initial content of the oil and moisture of dog rose seed was determined by following AOCS Official Method Af 2,3-54 (AOAC, 1998) and was 8.9 %, 6.57% respectively. Milling of dog rose seed were carried out by applying lab scale miller (volume 500 g, RRH-350, China). Solvent extraction was fulfilled at0.315~1 mm particle sizes, 100 g of seeds was milled by following time and seed particle sizefractions were determined by applying standard sieves (Table 1). The highest percentage of fraction was 0.315~1 mm and the procedure were repeated to obtain necessary mass for experiments. Due to the imagination of solvent properties and solvent prices Table 2 was demonstrated.

Table 1. Granulatic analyses of dog rose seed

	Particle size, mm (mean±SD)		*Time (s)		
			10	**15**	**20**
1.	0.125~0.315 mm	a (%)	5.97±0.2	9.92±0.35	9.8±0.06
2.	0.315~0.5 mm	b (%)	18.74±3.2	23.47±1.1	28.47±0.27
3.	0.5~1 mm	c (%)	20.8±0.7	26.01±0.23	28.58±1.4
4.	1~3 mm	d (%)	36.25±0.08	31.93±3.12	27.99±0.26
5.	higher then 3 mm	e (%)	15.78±1.12	5.96±0.39	2.9±0.74

*****milling time

Table 2. Price and characteristics of solvents

Solvent types	Density 20 ^{0}C (kg/m^3)	Viscosity (Pa*s)	Boiling point (^{0}C)	Price (USD/kg)
Chloroform	1148	0.57	61.2	7.45
Isopropanol	786	2.39	82.5	8.44
Petroleum benzine	700-730	0.48	80-120	3.48

Oil yields were calculated using equation (1)

$$Q\ (\%) = (M_1/M_2)*100\% \qquad (1)$$

Where, Q (%) is dog rose seed oil yield, M_1=amount of extracted oil (g), M_2=amount of dog seed used in the process.

The experiments were processed at laboratory scales and 60 g of seed was weighted by digital balance (Scout Pro SPS602F, max weight 600 g) and charged into 500 ml of flask by following plan of experiments (Table 3). Particle sizes proportion was 37.7 % for 0.315~0,5 mm and 62.3 % for 0.5~1 mm for each experiments respectively. Experiments were conducted in water bath coupled with temperature control and due to the difference of temperature of outside and inside of the flask average temperature were accepted in calculations. Overall extraction period was 2 hours and the flask was shacked 3 minutes during the 10 minutes intervals for each experiment. Subsequently, solid and liquid phase was separated by filter paper using rotary evaporator. All planned experiments were duplicated.

2.2. Design of experiments of dog rose seed

Coded and uncoded levels and factors were demonstrated in Table 3. These are (A)-L/S, (B)-temperatures and (C)-solvent types.

Table 3. Levels and factors of extraction

Levels	Factors		
	A	B	C
	* L/S	temperature	solvents
Units	ml/g	^{0}C	type
-1	1.5	30-35	Chloroform
0	3	40-45	Petroleum benzine
1	5	50-55	Isopropanol

*L- liquid, S-seed.

RSM was used for three factors and three levels with BBD design and 15 experimental runs were performed by two replications as described in Table 4.

The second order polynomial model was used as described in equation (2).

$$Y=\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+\beta_{12} x_1 x_2+\beta_{13} x_1 x_3+\beta_{23} x_2 x_3+\beta_{11} x_1^2+\beta_{22} x_2^2+\beta_{33} x_3^2 \quad (2)$$

Where, Y is response; β_0 is a constant; β_1, β_2 and β_3 are coefficients; β_{12}, β_{13} and β_{23} are the interaction coefficients; β_{11}, β_{22} and β_{33} are the quadratic coefficients.

Minitab 19 software and ANOVA was used to analyze the responses and statistical analysis. Analyses were executed by Box-Behnken design (BBD) for the two continues factors such as L/S, temperature and categorical factor as a type of solvent. Used categorical factor for the three levels have been represented as -1, 0 and 1 (chloroform, petroleum benzine and isopropanol in order). Experiments were conducted in a randomized order.

Table 4. Experimental design and results of dog rose seed extraction

Runs	Independent parameters						Yield (%)	Predicted yield (%)
	Coded			Uncoded				
	A	B	C	Ratio L/S	Temperature T	Solvent types		
1	0	1	-1	3	50-55	Chloroform	5.71	5.903
2	1	-1	0	5	30-35	Petroleum benzine	6.72	6.72
3	0	1	1	3	50-55	Isopropanol	6.93	6.736
4	0	0	0	3	40-45	Petroleum benzine	5.8	5.896
5	0	-1	-1	3	30-35	Chloroform	5.275	5.46
6	-1	1	0	1.5	50-55	Petroleum benzine	4.86	4.86
7	-1	0	-1	1.5	40-45	Chloroform	5.14	4.94
8	1	0	-1	5	40-45	Chloroform	5.3	5.106
9	1	0	1	5	40-45	Isopropanol	4.85	5.04
10	0	0	0	3	40-45	Petroleum benzine	6	5.896
11	-1	0	1	1.5	40-45	Isopropanol	3.49	3.683
12	1	1	0	5	50-55	Petroleum benzine	6.92	6.92
13	0	0	0	3	40-45	Petroleum benzine	5.89	5.896
14	-1	-1	0	1.5	30-35	Petroleum benzine	4.74	4.74
15	0	-1	1	3	30-35	Isopropanol	3.5	3.306

3. RESULTS AND DISCUSSION

3.1. Effect of process variables and optimization

Analysis of variance (ANOVA) was performed to evaluate influence of parameters as A, B and C (Table. 5).

Table 5. Analysis of variance of dog rose seed extraction

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	11	15.3114	1.39195	13.11	0.028
Linear	4	9.1648	2.29121	21.58	0.015
A	1	2,5063	2.50632	23.61	0.017
B	1	3.2401	3.24012	30.52	0.012
C	2	3.4184	1.70919	16.10	0.025
Square	2	0.8808	0.44039	4.15	0.137
A*A	1	0.5129	0.51290	4.83	0.115
B*B	1	0.3021	0.30210	2.85	0.190
2-Way Interaction	5	4.9688	0.99376	9.36	0.047
A*B	1	0.0016	0,00160	0.02	0.910
A*C	2	1.1538	0.57690	5.43	0.101
B*C	2	3.8134	1.90669	17.96	0.021
Error	3	0.3184	0.10615		
Lack-of-fit	1	0.2984	0.29838	29.74	0.032
Pure error	2	0.0201	0.01003		
Total	14	15.6299			

Significancy ($p<0.05$).

In model summary R^2-value is 97.54 % and adjusted-R^2 value is 88.52 %. The results of analysis of variance (ANOVA) in Table 5 showed that A, B and C as well as B*C effects on oil yield significantly ($p<0.05$).

Regression equation in uncoded units represent below: At C levels -1, 0 and 1 equations are (3), (4) and (5) respectively.

$$R=5.4+0.08A+0.217B-0.373A*A+0.286B*B+0.02\ A*B \quad (3)$$

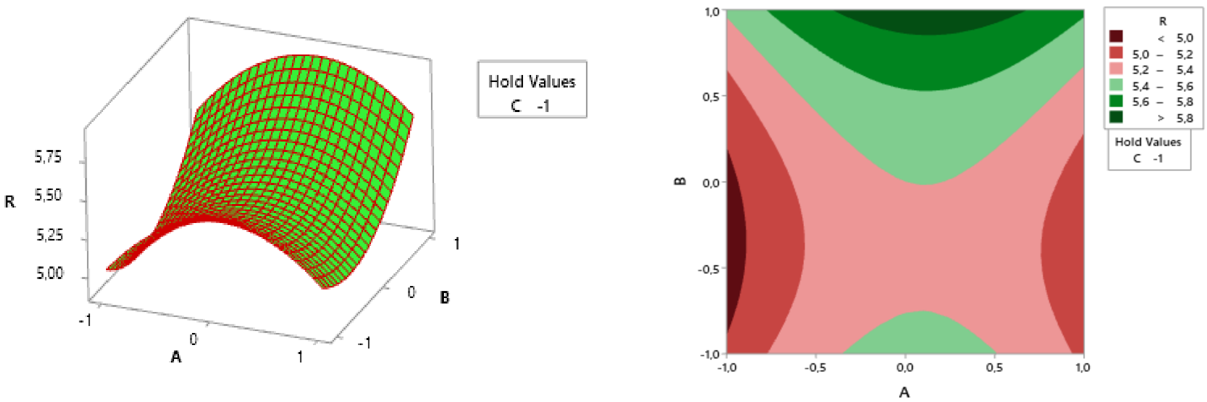
$$R=5.897+1.01\ A+0.08B-0.373A*A+0.286B*B+0.02\ A*B \quad (4)$$

$$R=4.73+0.68A-1.715B-0.373A*A+0.286B*B+0.02\ A*B \quad (5)$$

Where, C is solvent types, A is L/S, B is temperature.

3.1.1. Effect of process variables

As indicated in figure 1 significant effect of ratio of L/S was not observed comparing to temperatures when chloroform has been used as a solvent. Ratio of L/S has a little impact on oil extraction efficiency and at the ratio of 3 of L/S, the highest oil yield was obtained at highest temperature diapasons (50-55 ^{0}C). Chloroform based extraction model was described in formula (3).

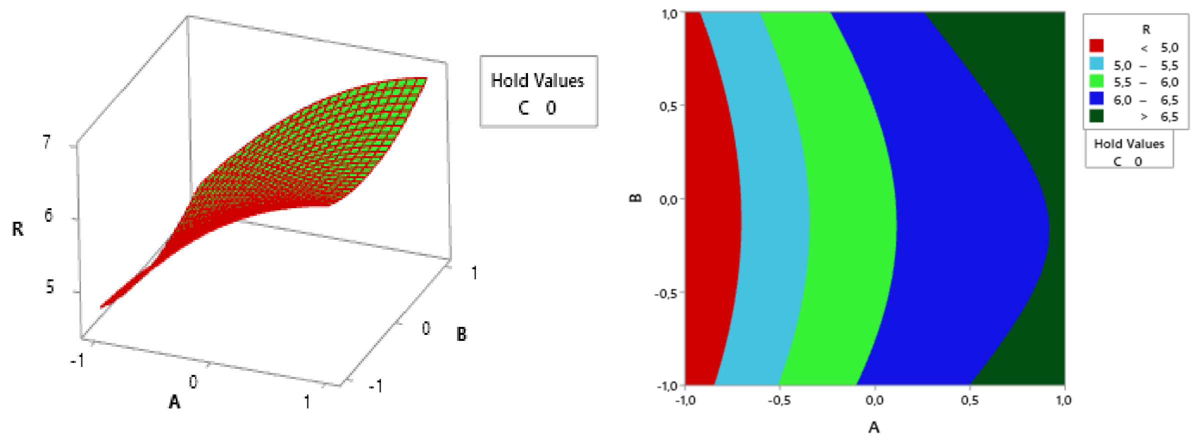


a) b)

Figure 1. Surface (a) and contour plot (b) of extraction of dog rose seed (chloroform)

Model of the extraction by petroleum benzine demonstrated in formula (4). In figure 2 (a) and (b) response curves revealed that the highest oil recovery was observed at the temperature diapasons of 50-55 (^{0}C) and L/S ratio 5 (ml/g). Additionally, it can be seen from overall picture of the curves that no significant effect of the temperature on oil yield was observed. Incontrast to that L/S ratio influenced on oil yield significantly. Higher oil yield can be obtained(about 7%) when petroleum benzine was used for dog rose seed extraction at particle sizes between 0.315~1 mm and shaking method was applied in laboratory scale. Juhaimi et al.,

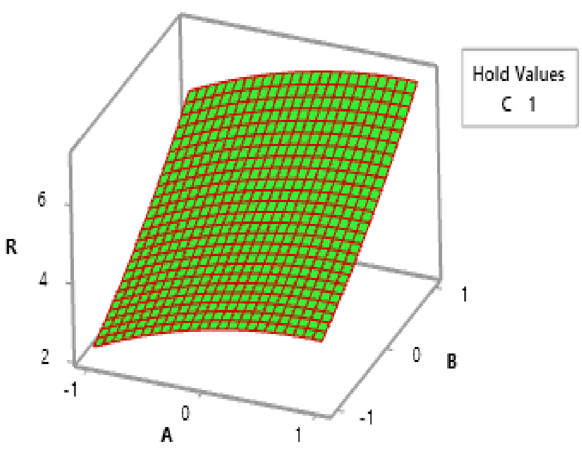
(2019) demonstrated that safflower seed oil yield was lower in hot extraction (39.53%) than cold extraction by petroleum benzine (39.96%). However, this is not notable high yield differences between methods of extraction. Essential oil extraction was performed by hexane and petroleum benzine from pomegranate seeds and there was no significant difference in oil yields between solvent types (Hajar et al., 2008). Somnuk et al., (2017) compared solvent types when spent coffee grounds was extracted and the highest oil yeild was recovered by hexane (14,7%).

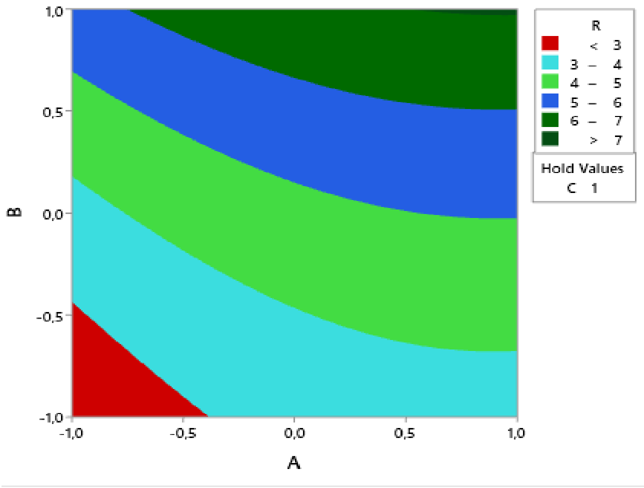


a) b)

Figure 2. Surface (a) and contour plot (b) of dog rose seed extraction (petroleum benzine)

Isopropanol (IPA) is an alternative solvent and considered as a "green solvent" (Calvo-Flores et al., 2018), it is less flammable and lower toxic than the other solvents (Proctor et al., 1996; Tir et al., 2012) as well as IPA would not contaminate the product. It is relatively easy to recycle, quality of the extract is higher compared to others. However, demerits of the alcohols are low selectivity, additional substance in oil such as phospholipids, sugars, pigments, waxes etc. (Toda et al., 2016). Isopropanol was used for extraction of rubber seed and extraction was conducted during 3 hours, ratio of the seed and solvent was 0.05 g/mL (Chiazor et al., 2020), sunflower oil was obtained from enzyme-treated collets by ethanol and isopropanol and recovery was 98-99 % (Luciana M. R., et al. 2020.), 79 % of oil was extracted from rapeseed flakes by isopropanol applying 20 min of ultrasound effect and then 2 h of extraction was processed (Perrier et al., 2017), as well as soy bean oil (Sawada et al., 2014; Seth et al., 2010), cotton oil (Harris et al., 1950) was extracted by isopropanol.





a) b)

Figure.3 Surface (a) and contour plot (b) of dog rose seed extraction (isopropanol)

Model of the extraction of dog rose seed by using isopropanol is described in formula (5). According to surface (a) and contour (b) plot curves, the highest oil yield was around 7 % at a ratio of L/S 3, temperature was 50-55 ^{0}C. Moreover, at the process temperature 30-35 ^{0}C and 3 L/S ratio the lowest oil yield was observed.

ANOVA Table 5 revealed that effect of the solvent types and interaction temperatures with solvent species is significantly different. Additionally, when we compare the types of the solvents in term of the quality, IPA is the best among the three solvents. However, extracted oil may include additional compounds such as sugar, phospholipids, pigments etc. Higher oil yield can be recovered utilizing petroleum benzine for the extraction of dog rose seed but toxicity and loss of the solvent is high enough. Moreover, petroleum benzine is cheapest solvent among others (isopropanol and chloroform).

3.1.2. Optimum conditions

Optimum conditions were determined for the goal of maximum oil recovery. Optimumprocess conditions are temperature between 50-55 (^{0}C), 4.87 of L/S (ml/g) ratio and solvent type is isopropanol. Under optimal conditions, experimental responses in agreement with predicted.

4. CONCLUSION

Our studies show (laboratory scale) that it is important to optimize solvent type, temperature and L/S ratio as the process variables. Interaction between temperature and solvent type is significantly. Experimental results confirmed the predicted results at the optimum conditions, and these are temperature 50-55 (^{0}C), 4.87 of L/S (ml/g) ratio and solvent type is isopropanol. Although isopropanol is greener solvent for extraction of dog rose seed, the price of the solvent (tab 2) is high enough and also selectivity is low. Petroleum benzine is a relatively cheaper solvent (tab 2) and able to extract higher oil yield. However, it is toxic, flammable and losing of solvent is high enough. Taking into account application of solvent types dependon users' goals.

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