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Identification process parameters for refining of wild walnut (*Juglans regia* L.) Heijuga oil of Manipur, India using response surface methodology (RSM)

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Abstract
In this study, Response Surface Methodology (RSM) was used for optimising the refining process of crude wild walnut (*Juglans regia* L.), Heijuga oil (CWWO) of Manipur, India. Refining of crude wild walnut oil (CWWO) was conducted at different levels of refining parameters, phosphoric acid, caustic soda and bleaching earth. And also, different levels of refining temperature using design of experiment (DOE) method, response surface methodology (RSM) with Central Composite Design (CCD), Design Expert 11. Rotary evaporator unit is used to carry out the experiment of refining processes. Chemical properties are essentials in judging the suitability of oils in food preparation. The chemical properties are also responsible for storability of oils besides quality of end products. The important chemical properties judging the suitability of wild walnut oil are determined. The quality parameter for refined wild walnut oil (RWWO) monitored in this experiment was free fatty acid (FFA). The quadratic model was found appropriate to get the optimum results. The response Free Fatty Acid (FFA) was significantly affected by the selected process parameters and found as 0.7 (mg/kg) with phosphoric acid (%), bleaching earth (%), caustic soda (%) 1.25, 1.5, 2.55 respectively at temperature 85^0C for 30 minutes refining time.

Keywords: wild walnut, heijuga, crude wild walnut oil, refined wild walnut oil, refining

Introduction
Wild walnut (*Juglans regia* L.) Heijuga a member of Juglandaceae family, grown spontaneously in the forest of Manipur and Himalayan ranges, India, underutilised. Wild walnut flowering Period is April-June and fruiting period is July-Sept. Grows at an altitude up to 1200 m. Oil yielding crop plants are very important for economic growth of the agricultural sector. It was studied that oil content of Wild walnut (*Juglans regia* L.) Heijuga an average of 63.01 %. The challenge of feeding ever growing human population cannot be met with the increase of food production alone. Some crops have been so neglected that genetic erosion has become so severe. Neglected crops can overcome the constraints to the wider production and use. Many species have the potential to contribute to food security, nutrition, dietary and culinary diversification, health and income generation. They also provide environmental services. *Heijuga* of Manipur, will contributes as a rich source of oil bearing crop.
Refining process is a necessary step for the production of edible oils and fats products. The objective of this process is to remove the impurities and other components, which will affect the quality of finished product. The qualities of the finished product that need to be monitored are flavour, shelf-life stability and colour of the products (Leong, 1992) [8]. The objectionable substance or impurities in oil maybe biogenic i.e synthesised by plant themselves but they can be impurities taken up by the plants from their environment.
The oil is usually treated with phosphoric acid (to at least 0.05 %), which chelates the ca and mg. Due to the variable content of phospholipids in crude oils, analysis of phosphorus is necessary.
Caustic soda (sodium hydroxide) and loose fatty acids are converted into insoluble soaps, which may be easily separated via centrifugation. Consequently, the primary objective is the removal of free fatty acids. Also, caustic neutralization improves appreciably the oil colour partly via reacting with polar compounds (gossypol, sterols, hydroxy fatty acids, and so on) and partly with the aid of solubilisation.
In bleaching step oil (around 100ºc) is slurred with acid-activated bleaching earth (1-2%), typically calcium montmorillonite or herbal hydrated aluminium silicate (bentonite).

Under these situations adsorption of colour bodies, hint metals and oxidation products as well as residual soaps and phospholipids last after washing neutralized oils takes region.
Response Surface Method (RSM) technique is discovered to be is the most suitable design technique to be applied in this research study. According to Dey *et al*., (2001) [7] and Prado *et al*., (2004) [10], the chosen method for optimisation of the response factor was efficient, relatively simple with time and material saving by using response surface methodology. It is found out that, the RSM technique has been successfully applied in the field of quality experimental work (Muralidhar *et al*., 2001; Amin and Anggoro, 2003; Varnalis *et al*., 2004; Jose *et al.*, 2004) [9, 12].
In this study, the objectives are; to hit a target (to find the exact dosage of phosphoric acid, bleaching earth and caustic soda to be added to crude wild walnut oil (CWWO) fed) and to maximize a response (to optimize degumming and bleaching process).

Materials and methods

Extraction of crude wild walnut oil (CWWO)

Wild walnut (*Heijuga*) was procured from the local market of Imphal, Manipur, India. For extraction of CWWO, the wild walnut is cracked and the walnut kernel was separated manually and is shown in Fig. 1, Fig. 2 and Fig. 3. CWWO (Fig. 4) was extracted using soxhlet apparatus, solvent extraction.
Extraction of oil was carried out by the method summarized by Asha Srinivasan, *et al*. (2008) [4]. In order to get maximum yield of oil, the seeds were extracted for period of time 2 hours and other parameters *viz.* amount of wild walnut seeds (10g) and quantity of solvent (150ml) are kept constant. 10g of seeds were crushed and extracted with ether at 55^0 C for 2 hours in Soxhlet apparatus. The extract was concentrated under reduced under hot air oven. The extracted oil obtained is CWWO and stored in dark place at room temperature. Process flow chart for extraction of crude wild walnut oil (CWWO) as given in Fig. 6.
The ranges of parameters identified based on the maximum acceptable and allowable values used by the refiners are set as tabulated in Table 1.
Design of Experiment (DOE) was applied to arrange the number of experiments run for the study. With the help of DOE method, 30 set of experiment were arranged as in Table 2. All the 30 set of experiments with quality checking experiments each, has been determined through Central Composite Design (CCD) technique via Response Surface Method (RSM) using Design Expert 11 environment.

Fig 1: Wild walnut, *Heijuga* of Manipur, India

Fig 2: Broken Wild walnut, *Heijuga* of Manipur, India

Fig 3: Wild walnut, *Heijuga* kernel of Manipur, India

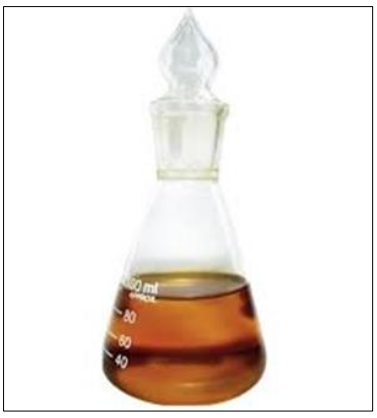

Fig 4: Crude Wild walnut Oil

Fig 5: Defatted wild walnut kernel

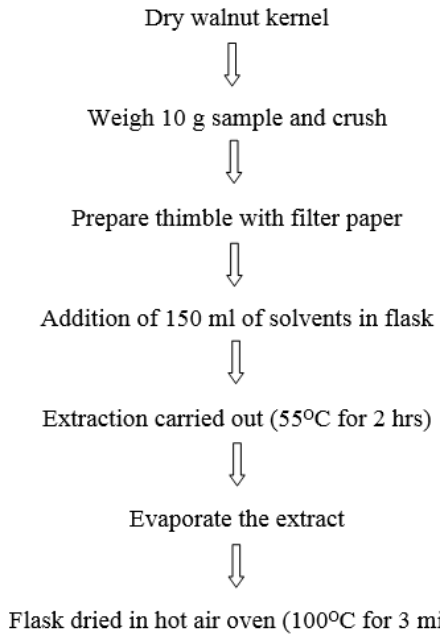

Fig 6: Flow chart for extraction of crude wild walnut oil (CWWO)

Table 1: Ranges of parameters for refining crude wild walnut oil (CWWO)

Parameter	Range	
	High	Low
Dosage of phosphoric acid (wt %)	1.5	1
Dosage of Bleaching Earth (wt %)	2	1
Dosage of caustic soda (wt %)	2.5	2
Temperature (^{0}C)	115	70
Pressure (Torr)	50	
Time (minutes)	30	

Table 2: Arrangement of experiment sequence by DOE (Design of Experiment), Design Expert 11

Run Order	Factor 1A: Phosphoric Acid (%)	Factor 2B: Bleaching Earth (%)	Factor 3C: Temperature (^{0}C)	Factor 4D: Caustic Soda (%)
1	1	1	100	2
2	1.25	1.5	85	2.25
3	1.75	1.5	85	2.25
4	1	2	70	2.5
5	1	2	100	2.5
6	1	1	100	2.5
7	1.25	1.5	85	1.75
8	1	1	70	2.5
9	1	1	70	2
10	1.25	1.5	85	2.25
11	1.25	1.5	115	2.25
12	1.5	1	70	2
13	1.25	1.5	85	2.75
14	1.25	1.5	85	2.25
15	0.75	1.5	85	2.25
16	1.5	1	70	2.5
17	1.5	2	70	2
18	1.5	1	100	2.5
19	1	2	100	2
20	1.25	2.5	85	2.25
21	1	2	70	2
22	1.5	2	100	2
23	1.5	2	70	2.5
24	1.25	1.5	55	2.25
25	1.25	0.5	85	2.25
26	1.5	1	100	2
27	1.5	2	100	2.5
28	1.25	1.5	85	2.25
29	1.25	1.5	85	2.25
30	1.25	1.5	85	2.25

Refining Method

In this study, rotary vacuum evaporator unit is used to carry out the experiment of refining processes. The CWWO sample used for degumming and bleaching processes is 100g for each run. The CWWO sample, bleaching earth, caustic soda and phosphoric acid are put into a conical flask before it is attached to rotary evaporator unit. Heating element used in this experiment is distilled water. The distilled water is initially heated up and vacuum is set to 50 Torr (0.0679 kg/cm2) before attaching the conical flask containing the CWWO sample, certain dosages of bleaching earth, caustic soda and phosphoric acid.

Free Fatty Acid (%)

FFA is the amount of fatty acid occurring naturally or produced during storage or processing that exists in edible oils as a distinct chemical unit in an uncombined state. The quantity of FFA present is a good measure of the quality of the oil. FFA levels in crude oils can be adversely affected by bleaching earth conditions and acidity of the adsorbent (bleaching earth) as well as nature properties of the phosphoric acid used. Small changes in FFA levels can have a great impact on oil loss. High FFA not only infers lower extractable oil but also corresponds to the higher content of emulsifiers (Abdul Azis, 2000) [2].

The free fatty acid in oil is estimated by titrating it against KOH in presence of phenolphthalein indicator. And was calculated by using the given relationship (A.O.A.C. (1990) [1] in equation (1).

$$\text{FFA \%} = \frac{A \times N \times 28.2}{W} \quad \text{…….. (1)}$$

Where,
A = Titration value
N = Normality of KOH
W = weight of oil

Results and Discussion

Effect of bleaching earth (%), phosphoric acid (%), caustic soda (%) dosages and operational temperatures on FFA (mg/kg) of refined wild walnut oil (RWWO) analysed by RSM

Table 3 and Fig. 7 shows the effects on FFA(mg/kg) content on RWWO by varying the bleaching earth dosage and caustic soda. From the graph, when phosphoric acid is added at 1.25 wt % but with no bleaching earth the increment of FFA content is highest about 0.7 %. However, even though bleaching earth are added during the phosphoric acid and bleaching process there is still some rise of the FFA content in degummed oil.

Traces of phosphoric acid remaining in the oil after phosphoric acid treatment are said to be responsible for such situation to occur (Rossi *et al*. 2003) [11].

From ANOVA table 4 the Model F-value of 8.09 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case D, AC, C^2, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The Lack of Fit F-value of 2.16 implies the Lack of Fit is not significant relative to the pure error. There is a 20.52% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good - we want the model to fit.

Fit Statistics for FFA (mg/kg) of refined wild walnut oil (RWWO)

From table 4 the Predicted R^2 of 0.4216 is not as close to the Adjusted R^2 of 0.7739 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with the model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 11.224 indicates an adequate signal. This model can be used to navigate the design space.

Table 3: Effect of phosphoric acid (%), bleaching earth (%), caustic soda (%) and operational temperature on FFA (mg/kg) of refined (RWWO)

Run	Factor 1 A: Phosphoric Acid (%)	Factor 2 B: Bleaching Earth (%)	Factor 3 C:Temperature (^OC)	Factor 4 D: Caustic Soda (%)	Response FFA (mg/kg)
1	1.00	1.00	100	2.00	2.10
2	1.25	1.50	85	2.25	1.10
3	1.75	1.50	85	2.25	1.20
4	1.00	2.00	70	2.50	0.70
5	1.00	2.00	100	2.50	1.00
6	1.00	1.00	100	2.50	1.00
7	1.25	1.50	85	1.75	2.50
8	1.00	1.00	70	2.50	0.70
9	1.00	1.00	70	2.00	1.50
10	1.25	1.50	85	2.25	0.75
11	1.25	1.50	115	2.25	1.50
12	1.50	1.00	70	2.00	2.70
13	1.25	1.50	85	2.75	1.20
14	1.25	1.50	85	2.25	1.20
15	0.75	1.50	85	2.25	1.20
16	1.50	1.00	70	2.50	1.20
17	1.50	2.00	70	2.00	2.30
18	1.50	1.00	100	2.50	1.10

19	1.00	2.00	100	2.00	2.30
20	1.25	2.50	85	2.25	10
21	1.00	2.00	70	2.00	2.20
22	1.50	2.00	100	2.00	2.10
23	1.50	2.00	70	2.50	1.70
24	1.25	1.50	55	2.25	1.50
25	1.25	0.50	85	2.25	1.10
26	1.50	1.00	100	2.00	1.90
27	1.50	2.00	100	2.50	0.75
28	1.25	1.50	85	2.25	0.70
29	1.25	1.50	85	2.25	0.75
30	1.25	1.50	85	2.25	0.75

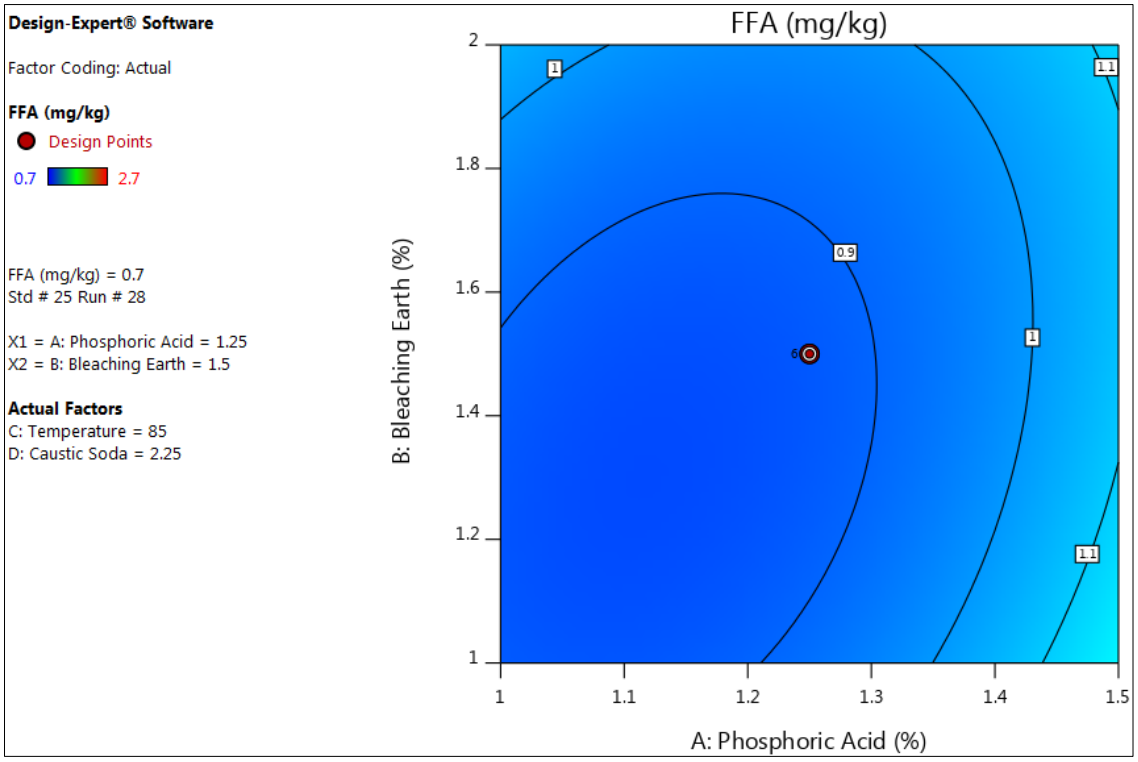

Fig 7: Optimal range of FFA (mg/kg) of refined wild walnut oil (RWWO) by varying phosphoric acid(%), bleaching earth(%), caustic soda(%) and refining temperature via RSM (Design Expert 11 Environment)

Table 4: ANOVA for Response 1: Free Fatty Acid, FFA (mg/kg) of refined wild walnut oil (RWWO) Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.38	14	0.6697	8.09	0.0001	significant
A-Phosphoric Acid	0.2109	1	0.2109	2.55	0.1312	
B-Bleaching Earth	0.0176	1	0.0176	0.2127	0.6513	
C-Temperature	0.0234	1	0.0234	0.2832	0.6024	
D-Caustic Soda	5.56	1	5.56	67.16	< 0.0001	
AB	0.0564	1	0.0564	0.6815	0.4220	
AC	0.7014	1	0.7014	8.47	0.0107	
AD	0.0127	1	0.0127	0.1529	0.7013	
BC	0.0352	1	0.0352	0.4248	0.5244	
BD	0.0189	1	0.0189	0.2284	0.6396	
CD	0.0014	1	0.0014	0.0170	0.8980	
A^2	0.3376	1	0.3376	4.08	0.0617	
B^2	0.1479	1	0.1479	1.79	0.2012	
C^2	0.9483	1	0.9483	11.46	0.0041	
D^2	2.05	1	2.05	24.78	0.0002	
Residual	1.24	15	0.0828			
Lack of Fit	1.01	10	0.1008	2.16	0.2052	not significant
Pure Error	0.2338	5	0.0468			
Cor Total	10.62	29				

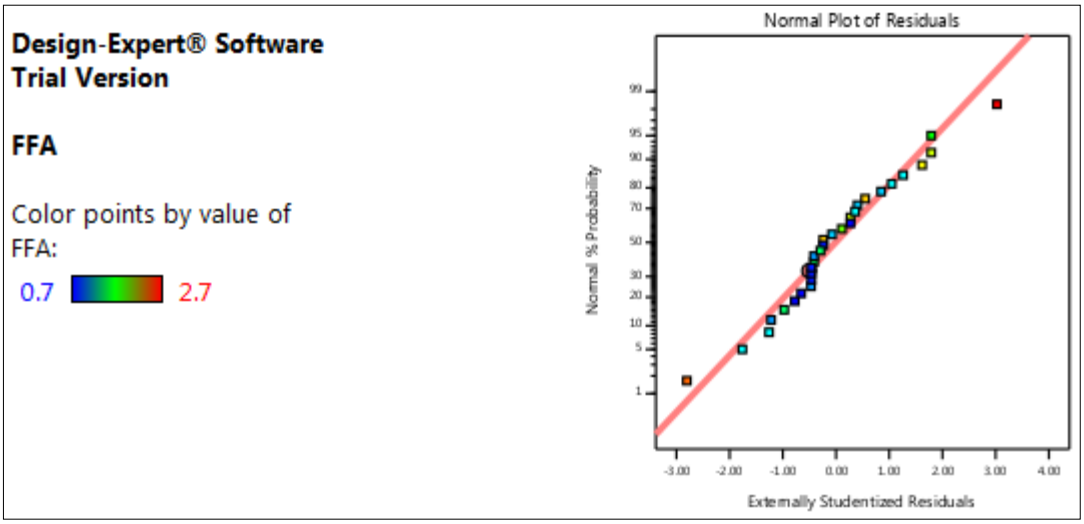

Fig 8: Fit Statistics for FFA (mg/kg) of refined wild walnut oil (RWWO)

Conclusion

The RSM was successfully applied to identify the effect of multiple variables, including the phosphoric acid (%), bleaching earth (%), caustic soda (%) dosages and operational temperature for the refining of wild walnut, *Heijuga* oil of Manipur, India. The quadratic model was found appropriate to get the optimum results. The response Free Fatty Acid (FFA) was significantly affected by the selected process parameters and found as 0.7 (mg/kg) with phosphoric acid (%), bleaching earth (%), caustic soda (%) 1.25, 1.5, 2.55 respectively at temperature 85^0C.

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