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Optimization of ultrasound assisted aqueous oil extraction from (*Zea mays* L.) germ using response surface methodology

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Abstract
Extraction of oil from *Zea mays* L. germ was investigated using a ultrasound assisted aqueous extraction A Box-Bhenken design for experiments were employed to study and optimize the effect of Incubation temperature, ultrasonication time and water to seed ratio on oil recovery. A quadratic polynomial model was generated to predict oil recovery. Among the process parameters studied, interaction of Incubation temperature and ultrasonication time had the most significant effect on the recovery followed by quadratic of Incubation temperature and interaction of ultrasonication time and water to seed ratio. Model validation shows no significant ($p > 0.05$) between practical and predicted values. The optimum extraction condition for oil recovery within the experimental range of the variables researched was at 40°C incubation temperature, 60 min of ultrasonication time and water to seed ratio of 4:1. At this condition, the recovery of oil was observed to be 67±0.27%. The oil obtained was compared with the solvent extracted oil for physico chemical quality parameters. Gas-chromatography of the maize germ oil was carried out for the free fatty acid composition of the oil constituents. Both techniques yielded oil with no significant difference ($P > 0.05$) in the percentage composition of free fatty acid and iodine value, saponification value, and peroxide value but iodine values and linoleic acid was found to be slightly highest in UAE extracted oil. From present study it can be observed that UAE method can be used as alternative to SE, to prevent high solvent consumption and prevent the release the VOCs to atmosphere.

Keywords: RSM, maize germ oil, ultrasound, solvent

1. Introduction
Maize germ constitutes 5-14% of the weight of kernel and is a good source of key nutrients especially 18-41% of oil (Johnston *et al.* 2005). Extraction of oil from oil seeds commonly occurs using solvent in the Soxhlet apparatus. Since excessive use of organic solvents is not desirable and is associated with environmental hazards and also causes environmental issues, especially the increasing concern about volatile organic compounds (VOCs) caused by solvent emissions, (Rosenthal, Pyle, & Niranjan, 1996) [9] thus the use of an alternative methods can be useful in all aspects. Therefore, so far, various methods and solutions have been presented to enhance the extraction efficiency and reduce the solvent, energy and time consumption of various oil seeds such as grape seed cannabis (Garcia & Luque, 2004) [7]. In this void,
some of the needs triggering technology innovation in the oil extraction sector such as cost savings, environmental and safety concerns, and nutrition issues seem to be achievable by successful development of aqueous processes. Over the last few decades, several studies have been carried out on aqueous processing of oilseed and the concept appears potentially attractive compared to the conventional hexane-based process, the comparatively low oil yield and relatively high content of oil in the residue have discouraged its commercial application. The problem of low extraction efficiency of aqueous processes was overcome by the use of hydrolytic enzymes which disrupt the cell wall and help to release oil and increase the yield. But the use of enzyme was very uneconomical and high time consumption makes it a complex process. But the advancement in the food processing sector a new method is investigated which is used for the extraction of bio-actives and oil.

Ultrasound-assisted extraction can also provide the opportunity for enhanced extraction of heat-sensitive bioactive components at lower processing temperatures and is a more effective technique than conventional extraction. The mechanism of ultrasound in liquids relies on the mechanical effect caused by the implosion of cavitational bubbles (Vilkhu, Mawson, Simons, & Bates, 2008) [11]. The mechanism of separation by ultrasound is concerned to cavitation phenomenon during which tiny bubbles in the liquid mass are formed. Bubble burst in the presence of solid particles is asymmetric and therefore leads to a stream of liquid with high-speed to the particle and destroys particle surface and strong shear forces are created, while both high pressures and temperatures generated as a consequence of the bursting bubbles, cause rapid plant tissue disruption allowing cellular material release and improved mass transfer as well. In recent years the use of ultrasonic waves in the food industry also applicable on industrial scale has found many applications (Khan & Chemat, 2011) [4] extraction of oil from grape seeds (Da Porto, Porretto, & Decorti, 2013) [3]. According researches conducted about comparing the conventional and ultrasound extraction of pomegranate seeds oil, the use of ultrasonic waves with specified frequency can be a proper alternative to oil extraction by solvent in Soxhlet apparatus (Da Porto *et al*., 2013) [3]. The ultrasound assisted aqueous extraction was investigated for rice bran oil which gives low free fatty acid value and less color imparting than solvent extraction (Khoei & Chekin, 2015) [5].

To our knowledge, there are no published reports regarding ultrasound assisted aqueous extraction of maize germ oil. This research is aimed at studying and optimising the effect of these variables and their interaction on percentage oil recovery. Moreover this research is also aimed at comparing the physico-chemical characteristics and free fatty acids distribution of extracted oil with the solvent extracted oil.

2. Material and Methods

Distilled water was used as the solvent for ultrasound assisted aqueous extraction. N-Hexane was used as the organic solvent. Maize kernels of variety PMH I was procured from Punjab Agricultural University, Ludhiana Farms, and maize germ was obtained using wet milling techniques and germ was dried to a moisture content of 5% (wb) and stored at 0ºC to control any microbial effect. The pH of the solutions was controlled by adding 0.5 N NaOH or 0.5 N Hcl. The maize germ then grinded by steel grinder (Make: Tandem 10W) to increase the surface area.

Acid value, FFA, Saponification value, iodine value and peroxide value was determined by standard methods (AOAC, 2000) [1]. All the chemicals used were of analytical grade.

2.1 Soxhlet extraction

Grounded 10g of maize germ and solvent n-hexane 100ml were put into the soxhlet apparatus. The solvent was boiled and was refluxed for about 12h (Stanisavljevic I., Lakicevic, S., Velickovic, D., Lazic, M., Veljkovic, 2007) [10]. The extracted oil was obtained by removing hexane in oven at 85ºC to constant mass (Luque-Garcia & Luque De Castro, 2004) [7]. The oil content was found 2.56g of oil/10g of maize germ which was taken as the 100% in measuring the rate of oil recovery by ultrasound assisted aqueous oil extraction.

2.2 Ultrasound assisted aqueous extraction

About 20g grinded maize germ was heated at 104ºC in 250 ml flask for 15 min, and then it was allowed to cool down. It was the transferred to a 250ml beaker which was adjusted at a fixed pH of 4 (Moreau, Johnston, Powell, & Hicks, 2004) [8]. Now the different levels of independent variables viz., incubation temperature (40-60ºC), ultra sonication time (40-60 min) and water to seed ratio (4-6 w/w) was adjusted to the complete description design of experiment was obtained from response surface methodology (Box-Bhenken) using a trial version software Design-expert 10.0.1trial (State-Ease, Inc., Minneapolis MN, USA). Ultrasound assisted aqueous extraction was carried out in an Ultrasonic cleaning bath (Microclean-109, 250W, 40kHz Arihant Ultrasonicator Ltd., Mumbai (capacity 9l) having internal dimensions (30×25×12.5 cm). An in- water pipe was added to the opposite of out-water pipe in the bath, and the flux ratio between in-water and out-water was regulated to maintain the 25ºC temperature (Khoei & Chekin, 2015) [5] during the ultra-sonication time period. Then samples were transferred to the incubator with constant shaking at 250strokes/min for a fixed time of 6h for all design experiments. The supernatant layer of oil was then removed by centrifugation at 8000 rpm for 15 min by micropipette. The oil recovery was then calculated.

$$\text{Percentage oil recovery} = \frac{W_{OE}}{W_S} \times 100$$

Where, W_{OE} = weight of oil extracted (g); W_S = weight of oil by soxhlet extraction (g)

2.3 Experimental design and statistical analysis

A three-variable, three-level Box–Bhenken design (BBD) (Box & Wilson, 1951) [2] was applied to optimizing the extraction condition in order to obtain the high oil recovery from ultrasound assisted aqueous extraction of maize germ oil. The three independent variables set were incubation temperature (min, X_1), ultra sonication time (ºC, X_2), and water to seed ratio (g/g, X_3), and each variable set at the three levels. A total of seventeen experiments were designed (Table 1). Values in triplicate were taken for each experiment and the average oil recovery (%) was taken as the response, Y. Regression analysis was performed for the experiment data and was fitted into the empirical second order polynomial model, as shown in the following equation:

$$Yi=\beta_o+\beta_1X_1+\beta_2X_2+\beta_3X_2+\beta_{12}X_1X_2+\beta_{23}X_2X_3+\beta_{31}X_3X_1+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_2^2 \quad (1)$$

Where, Yi is predicted response, β_0 is offset term, β_1, β_2 and β_3 are the regression coefficients for linear effect terms, β_{11}, β_{22} and β_{33} are quadratic effects and β_{12}, β_{23} and $\beta_{31}$are interaction effects. In this model, X_1, X_2 and X_3 represent Incubation temperature ºC, Ultrasonic time (min) and water to seed ratio (w/w) respectively. The significant terms (p<0.05) in the model were found by analysis of variance (ANOVA) based on p-value. The three-dimensional response surface plot was generated for the graphical interpretation of the interaction effect of independent variables on the response. Numerical optimization was carried out to predict the exact optimum level of independent variables leading to the desirable response goal. The model adequacy was determined using model analysis, lack of fit test, coefficient of determination (R^2) and adj R^2. Furthermore, experimental data were compared with predicted values (method validation) in order to verify the adequacy of final reduced model.

2.4 Gas chromatography analysis

Thermo Scientific TSQ 8000 Gas Chromatograph with the TRACE 1300 GC (Thermo Scientific Trace Finder Software, a common platform for routine GC quantification).a silica column (30mm × 0.25mm id, film thickness of 0.25µm) was used for separation. Fatty acid composition of oils extracted by UAE and SE were determined using GC after derivation of fatty acid methyl esters. FAME was prepared according to the method of (van wijngaarden 1967) [15] via saponification of 0.5M NaOH-MeOH solution and methylation with 14% BF_3-MeOH (sigma, India). Injector temperature and detector temperature were set at 250ºC and 220ºC, respectively. The amount of sample injected was 1.0 µL. Nitrogen at a constant flow of 1.0 ml/min was used as a carrier gas with a split ratio of 25:1. The column temperature initially was 40ºC which was maintained for 1 min, from 40ºC to 195ºC at 25ºC/min, from 195ºC to 205ºC at 4ºC/min, from 205ºC to 230ºC at 8ºC/min and held for 2 min and then to a final temperature of 235ºC at 2.5ºC/min. Fatty methyl esters were identified by comparing with standard fatty acid methyl esters. Then they are quantified in percentages of total methyl ester peak areas.

3. Results and Discussions

3.1 Response surface optimization of ultrasound assisted aqueous extraction

In order to determine the optimum process parameters, i.e. Incubation temperature, ultrasonication time and water to seed ratio on oil recovery, the experiments were designed according to a Box-Bhenken design. The experimental set-up and corresponding experimental responses are shown in Table 1. The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique and are presented in Table 2. Interaction of Incubation temperature and ultrasonication time had the most significant effect on the recovery followed by quadratic of Incubation temperature and interaction of ultrasonication time and water to seed ratio. The Model F-value of 4.34 implies the model is significant. There is only a 3.30% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case AB, BC, A2 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 0.61 implies the Lack of Fit is not significant relative to the pure error. There is a 64.07% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good for the model to fit. The "Pred R-Squared" of 0.0694 is not as close to the "Adj R-Squared" of 0.6524 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. our ratio of 6.124 indicates an adequate signal. This model can be used to navigate the design space. the predicted second-order polynomial model was:

$$\text{Oil recovery} = +66.52-1.25X_1-0.32X_2+0.21X_3-2.48X_1X_2+1.45X_1X_3-1.97X_2X_3-2.22X_1^2-0.92\ X_2 2-1.41X_3 2 \quad (2)$$

To determine the optimal level of oil recovery from UAE, the three dimentional surface plots were constructed according to equation (2). The response surface 3-D graphs in Fig 1(a) shows the effect of incubation temperature and ultrasonic time on the oil recovery at fixed 5:1 water to seed ratio. At a definite incubation temperature the oil recovery increses with the increase in temperature and reaches nearly peak but with further increase in the incubation temperature decreases the oil recovery, incubation temperature shows a quadratic effect on the oil recovery. The ultrasonication time has a linear effect on the recovery of oil it increases with the increase in the ultrasonication temperature and reaches its peak at 60 min. However the interaction between the incubation temperature and water to seed ratio is shown in Fig. 1(b) at the fixed ultrasonication time of 50 min. With increase in Incubation temperature recovery first increases then level off on the further increase in the incubation temperature, both incubation temperature and water to seed ratio shows quadratic effect on oil reovery. Fig. 1(c) shows the effect of the ultrasonic time and the water ratio on the oil at fixed incubationb temperature of 50ºC. it was observed that he oil recovery increased with the ultrasonication time and the water to seed ratio upto a certain level then it remains constant. The optimum condition obtained by response surface methodology using numerical analysis was as follows: Incubation temperature 40.13ºC, ultrasonication time 60 min and water to seed ratio 4:1. To compare the predicted result (68.55%) with the practical value (67±0.27%). X^2 goodness of fit test was used to examine the validity of (Mooney and Swift, 1999) [14] the model at (n=3). The test shows that there is no significant(p>0.05) difference between the predicted and practical values since the X^2 value (0.035) is much smaller than the cut-off value of X^2 for 95% confidence level. This indicates that the generated model is valid at 95% confidence level. The strong correlation between the practical and the predicted results confirmed that the response model was adequate to reflect the predicted optimization.

3.2 Free fatty acid composition of maize germ oil.

The free fatty acid analysis was carried out with GC for maize germ oil extraction obtained from solvent extraction (SE) and ultrasound assisted aqueous extraction (UAE). As shown in Table 3, all the oils were rich in unsaturated fatty acids roughly (84% of linoleic and oleic acid), maize germ oil is low in saturated fatty acids. The variance analysis was carried out between data of fatty acid composition oil extracted by SE and UAE. The results showed that there was no remarkable difference between (p>0.05) in the oil compositions. results of our findings were found to be compatible results obtained by (Zhang *et al*., 2008) [12] for ultrasound assisted extraction of oil from flaxseed.

Table 1: Box-bhenken design and observed responses

Run	Independent variable			Response[a] (Y%)
	X_1 Incubation temperature. ºC	X_2 Ultrasonication time, min	X_3 Water to seed ratio,(g/g)	
1	50 (0)	50 (0)	5 (0)	66.95
2	50 (0)	60 (+1)	6 (+1)	62.97
3	60 (+1)	40 (-1)	5 (0)	64.7
4	60 (+1)	60 (+1)	5 (0)	58.55

5	40 (-1)	40 (-1)	5 (0)	63.27
6	60 (+1)	50 (0)	4 (-1)	61.09
7	50 (0)	40 (-1)	6 (+1)	67.01
8	50 (0)	50 (0)	5 (0)	67.54
9	50 (0)	50 (0)	5 (0)	65.32
10	50 (0)	50 (0)	5 (0)	64.23
11	40 (-1)	50 (0)	4 (-1)	65.46
12	50 (0)	50 (0)	5 (0)	68.55
13	40 (-1)	50 (0)	6 (+1)	61.78
14	50 (0)	40 (-1)	4 (-1)	61.46
15	40 (-1)	60 (+1)	5 (0)	67.02
16	60 (+1)	50 (0)	6 (+1)	63.22
17	50 (0)	60 (+1)	4 (-1)	65.32

[a] average value of triplicate experiments

Table 2: Analysis of variance for experiment results of oil recovery

Parameter[a]	Estimated cofficients	Standard error	Df[b]	Sum of squares	F value	Prob > F
Intercept				Model		
β_o	66.51	0.70	1	98.01	4.33	0.03
β_1	-1.24	0.56	1	12.42	4.94	0.06
β_2	-0.32	0.56	1	0.83	0.33	0.58
β_3	0.20	0.56	1	0.34	0.13	0.72
β_{12}	-2.47	0.79	1	24.50	9.75	0.01
β_{13}	1.45	0.79	1	8.43	3.36	0.10
β_{23}	-1.97	0.79	1	15.60	6.21	0.04
β_{11}	-2.21	0.77	1	20.70	8.24	0.02
β_{22}	-0.91	0.77	1	3.52	1.40	0.27
β_{33}	-1.41	0.77	1	8.40	3.34	0.11
Lack of fit			3	5.54	0.61	
Pure error			4	12.03		
R^2	0.84		Adj R^2	0.65		
C.V.%	2.46		PRESS	107.56		

[a] cofficient reference to general model; [b] degree of freedom

Table 3: Fatty acid composition (% of total fatty acids) of maize germ oil extracted by SE and UAE

Fatty Acid	SE (%)	UAE (%)
C14:0	1.47	1.09
C16:0	12.45	12.97
C18:0	3.54	3.37
C18:2 n-6	51.32	52.68
C18:2 n-9	31.68	30.32
C18;3 n-3	1.06	1.07

Table 4: Physico-chemical properties of extracted maize germ oil

Physico-chemical properties	Solvent extracted (SE)*	UAE*	Control (Aqueous extraction)*
Iodine value (wijs)	132 [b] ±1.2 [b]	128[a] ±1.7 [b]	129[a]±1.3 [b]
Saponification value (mgKOH/g oil)	194[a] ±2.1 [b]	192[a] ±1.8 [b]	191[a]±1.5 [b]
Peroxide value (meq/kg)	3.6[a] ±0.4 [b]	3.5[a] ±0.5 [b]	3.8[a]±0.4 [b]
Refractive index	1.50 [a] ±0.03 [b]	1.49[a]±0.02[b]	1.51[a]±0.02[b]
Acid value	3.63 [a] ±0.03 [b]	3.15[a]± 0.01 [b]	6.63[a] ±0.11 [b]
Free fatty acid value	1.82 [a] ±0.04 [b]	1.58[a]± 0.02 [b]	3.33 [a] ±0.084 [b]
Density (kg/m³)	884.73 [a] ±1.10[b]	883.55 [a]±0.92 [b]	885.46[a]±1.98[b]

[a]average value; [b]standard deviation

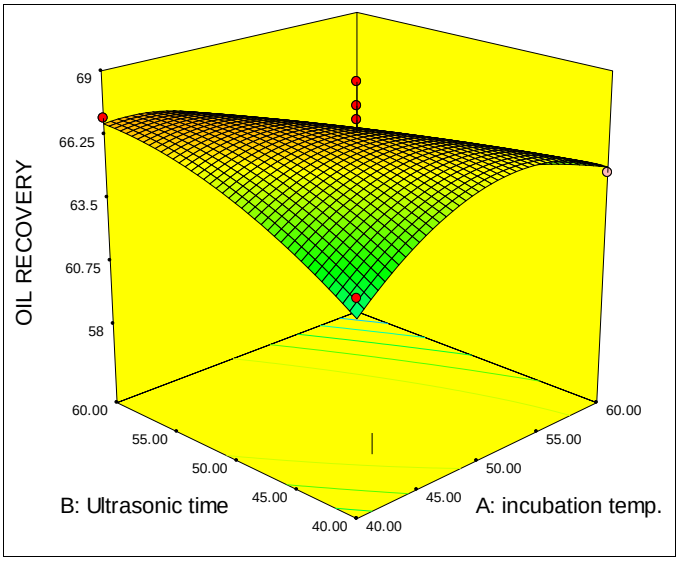

Fig 1(a)

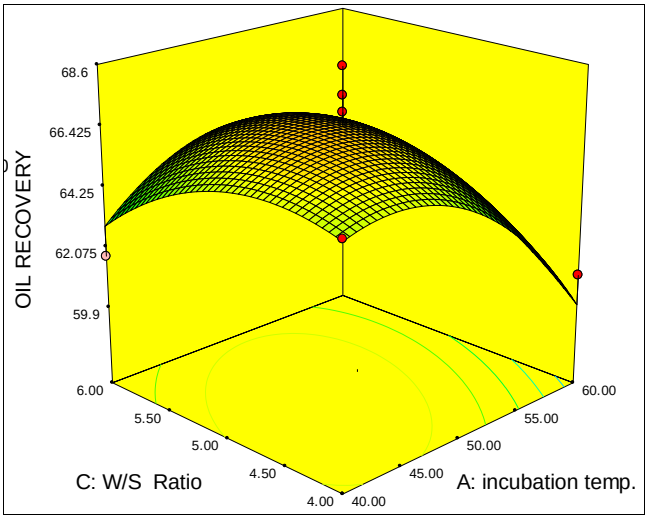

Fig 1(b)

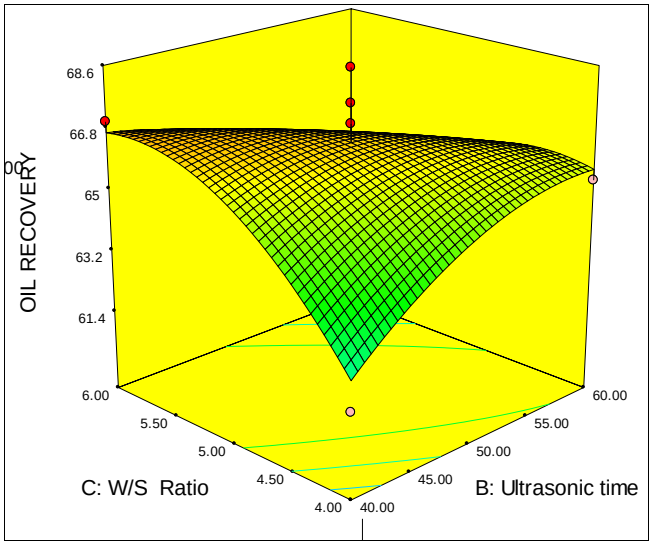

Fig 1(c)

3.3 Physico-chemical quality parameters

Extracted oil by both methods were subjected to examination of saponification value, peroxide value and iodine value. The analysis of variance were carried out and it showed that there is no remarkable difference between the saponification value, peroxide value. Iodine value of UAE extracted oil was sighltly higher that SE extracted oil although there was no significant difference (p>0.05) between them. The iodine value, was used to determine the amount of unsaturation in fatty acids, was measured through iodine compounds reacting with C=C bonds of unsaturated fatty acids (Li *et al*., 2013) [6]. The higher the iodine value, the more unsaturated fatty acids are present in the oil (Thomas, 2002) [13]. This high iodine value was further confirmed by the fatty acids composition of maize germ oil, whose unsaturated fatty acids (C18:1, C18:2, and C18:3) comprised approximately 84%.

4. Conclusion

In this study it was investigated that, the ultrasound-assisted aqueous extraction of oil from maize germ was performed with a three-variable, three-level Box–Behnken design (BBD) based on the RSM. The experiment results showed that the interaction of Incubation temperature and ultrasonication time was the major contributing factor to the oil extraction. It was revealed that the fatty acid composition and other quality parameters were not significantly affected by the method of oil extraction. Moreover the results indicated that ultrasound-assisted aqueous extraction of maize germ oil was an efficient extraction method at optimum conditions of 40ºC incubation temperature, 60 min ultrasonication time and 4:1 water to seed ratio giving the highest oil recovery of 67±0.27%. More importantly, ultrasound assisted aqueous extraction was an environmental friendly alternative to conventional solvent extraction methods.

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