Response surface methodology to optimize enzyme-assisted aqueous extraction of maize germ oil

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Revised: 31 May 2016 / Accepted: 28 July 2016 / Published online: 25 August 2016
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Abstract The present study was aimed at optimizing the enzyme assisted aqueous extraction (EAAE) process for extraction of maize germ oil. The different EAAE process parameter viz., pH of the slurry, seed to water ratio, the temperature of incubation and time of hydrolysis were optimized for improving oil recovery and oil quality. The combined effect of independent variables on recovery of oil, time taken for oil extraction and various quality parameters were studied using response surface methodology. The designed experimental runs were conducted to obtain the optimal conditions as 5.85 pH of slurry, 1:6.92 seed to water ratio, 45.12 °C temperature of incubation and 1 h time of hydrolysis. Oil extracted under these conditions was light yellowish in color and had a pleasant nutty taste, with maximum oil recovery of 70 %. The extraction variables viz., pH of slurry, seed to water ratio, the temperature of incubation had a significant effect on recovery of oil and various quality characteristics, however the time of hydrolysis had a non significant effect.

Keywords EAAE extraction method · Enzyme · Maize germ · Maize germ oil · Response surface methodology · Solvent extraction method

Introduction

Maize (*Zea mays*) is one of the most important cereal crops in the world agricultural economy and is grown in many countries in each of the continents of the world. In India, maize is the third important cereal crop after rice and wheat in terms of area. Worldwide production of maize is 854 Mt and in India 21.7 Mt (Anonymous 2012). In Punjab, the maize production was estimated at 502 Tht in (Anonymous 2013a). The PMH 1 variety of maize which is short duration kharif crop has oil content 3.34–3.4 %, developed by Punjab Agricultural University, Ludhiana. Maize is an abundant food and feed crop. Diversified use of this crop adds higher value to its cultivation and production; it is one such crop which yields several useful products (Rajendran et al. 2012). The Maize Kernel is a complex mixture of starch, protein, oil, water, fiber, vitamins and pigments all wrapped in a package. The Maize wet milling process increases the nutritional and economic value of this package by separating it into homogenous fractions, each having its specific identity and end use. This is valuable by-product being rich in oil. It has a pleasant nutty taste when fresh but can quickly become rancid, if not treated within a short period of time after milling. Its main value is in the oil, which can be extracted (Anonymous 2014).

Maize oil extraction also opens up new avenues of entrepreneurship to maize growers and processors. Maize oil is becoming popular among edible oils owing to its unique health related benefits (Rajendran et al. 2012). The high PUFA content meets the essential fatty acid requirements in human nutrition. In maize oil, the total percentage of PUFA constituted by linoleic acid (18:2) alone is about 60 % and MUFA (oleic acid; 18:1) is about 24 %. Among saturated fatty acids (SFA), palmitic acid (16:0) is almost 13 % and stearic acid (18:0) is 1 %. The percentage of

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PUFA is high in maize among cereals. Maize oil is a rich source of linoleic acid, which is one of two essential acids necessary for the integrity of the skin, cell membranes, and the immune system (Mestrovic 2015). It is highly effective in lowering serum cholesterol, primarily low-density-lipoprotein cholesterol. Omega-6 fatty acid is rich in maize oil (Rajendran et al. 2012).

Oil extraction from maize germ is conventionally done by solvent extraction (Matill et al. 1964). In 2001, the US Environmental Protection Agency issued stricter guidelines for hexane emissions by vegetable oil extraction facilities (EPA 2001), providing new incentives to develop alternative methods of edible oil extraction. A number of aqueous (Rhee et al. 1972; Shi et al. 1998), aqueous enzymatic (Bocevska et al. 1993; Karlovic et al. 1994; Latif 2009) and enzyme-assisted solvent extraction (Owusu-Ansah 1997) methods have been developed, but the current consensus is that hexane extraction is still much less expensive than any of these alternative approaches. Enzyme assisted aqueous oil extraction has emerged as an eco-friendly process for oil extraction (Mcglone et al. 1986; Bocevska et al. 1993) and this method could reduce the breakage for the functional component of oil (Qian et al. 2011). The addition of specific enzymes during oil extraction enhances the oil recovery by breaking the cell wall and lipid bodies (Rosenthal et al. 1996; Singh et al. 1999).

Optimization is done in order to improve the efficiency of the system in terms of performance, product yield and cost. The numerical optimization has been done using response surface methodology (Lee et al. 2015; Mishra et al. 2015). In this study, we show that appropriate choice of the enzyme(s) and optimization of extraction conditions make it possible to improve the oil recovery from maize germ of a PMH 1 variety using enzyme-assisted aqueous oil extraction method. The objective of present study was to optimize process parameter for the extraction of germ oil and to study the effect of enzyme assisted aqueous extraction on the quality of maize germ oil.

Materials and methods

Materials

The Maize kernels of variety PMH 1 were procured from Punjab Agricultural University farm, Ludhiana, Punjab, India. Cleaning and grading was done to remove undesirable materials and to obtain uniform size kernels. Healthy kernels of initial moisture content 15 % (wb) have been selected after cleaning and grading by selecting uniform

size of kernel for this study. The combination of enzymes cellulase and protease was used, enzyme were procured from Himedia Pvt. Ltd., Ludhiana, Punjab, India.

Extraction of germ

One kg of cleaned maize was steeped at 55 °C for 18 h in steeping solution of 0.2 % sulphur dioxide (SO₂) and 0.55 % lactic acid for germ separation (Eckhoff et al. 1993). The sample was first ground in the grinder for 15 s. The germ skimming process allowed the germ component and another kernel component to be separated by density differences. The skimmed germ was dried in hot air tray dryer for 18 h up to 5 % (wb) moisture content and then stored in a refrigerator at a temperature of 4–5 °C for further process. The maize germ oil was extracted using enzyme assisted aqueous extraction (EAAE) method (Moreau et al. 2004).

Enzyme assisted aqueous extraction (EAAE) method

About 100 g ground germ was mixed with distilled water, at different seed to water ratios (1:5, 1:7.5 and 1:10). The mixture was cooked and allowed to cool down to room temperature (Abdulkarim et al. 2005). The pH was adjusted at 4, 6 and 8 for the enzyme mixture with 0.5 N NaOH and 0.5 N HCl. Enzyme concentrations were predicated on the basis of studies as 0.50 % seed weight basis (Moreau et al. 2004). The mixture was stirred or hydrolysed for different periods of time 1.0, 2.5 and 4.0 h and incubated at temperature of 40, 50 and 60 °C for 20 h. After incubation, the mixture was centrifuged at 5000 rpm in hot centrifuge machine for 25 min (Fig. 1). The oil phase floating at the top was collected using a pipette and transferred to a beaker. Residue of the slurry was used for estimation of proximate composition (Latif 2009; Olaniyan 2007). The oil was placed in a hot air oven at a temperature of 70 °C for 30 min to remove the residual water present in the oil.

The recovery of oil, time required for oil extraction and various quality characteristics of oil extracted viz., unsaponifiable matter, saponification value, total phenolics, acid number and peroxide value, was determined using standard methods as described below.

Unsaponifiable matter

The unsaponifiable matter is defined as all the substance present in the products which, after saponification of the oil, latter by potassium hydroxide and extraction by diethyl ether, are not volatile under the specified operating conditions (AOCS 1997). It is expressed using the following equation,

Process flow chart for aqueous enzymatic oil extraction

Fig. 1 Flow diagram for maize germ oil extraction

$$\text{Unsaponifiable matter (\%)} = \frac{a}{m} \times 100 \quad (1)$$

a = weight of residue after oven drying (g), m = weight of the sample (g).

Saponification value

The saponification value is defined as the weight of potassium hydroxide expressed in milligrams required to saponify 1 g of fat or oil (AOCS 1997). It is expressed using the following equation,

$$\text{Saponification value (mgKOH/g)} = \frac{(t_1 - t_2) \times 28.1}{W} \quad (2)$$

t_1 = blank value, t_2 = sample value, W = weight of sample (g).

Total phenolics content

Total phenolic content was determined using the modified Folin-Ciocalteu procedure (Chaovanalikit and Wrolstad 2004; Tuan 2011). A 0.5 mL of the oil extract was mixed with 0.5 mL of Folin-Ciocalteu reagent (Loba Chemie Pvt Ltd, Mumbai, India) and 7.5 mL of deionized water. The mixture was held at room temp for 10 min before adding 1.5 mL of 20 % sodium carbonate (w/v). The mixture was measured for total phenolics by the absorbance at 755 nm in a spectrophotometer. Results were expressed as mg of Gallic acid equivalent (GAE) per kg of fresh weight.

Acid number

Acid number may be defined as milligram of Potassium Hydroxide required for neutralizing the free fatty acid present in 1 g of oil (FSSAI 2012).

$$\text{Acid number (mg KOH/g)} = \frac{5.6 \times (V_1 - V_2)}{W_1} \quad (3)$$

$$\text{Free Fatty Acids} = \frac{2.82 \times (V_1 - V_2)}{W_1} \quad (4)$$

V_1 = Volume in ml of potassium hydroxide solution required for the sample, V_2 = Volume in ml of potassium hydroxide solution required for the blank, W_1 = Weight of sample in g.

Peroxide value

This is an indication of the extent of oxidation suffered by oil (Khan et al. 2011; FSSAI 2012).

$$\begin{aligned} \text{Peroxide value} & \left(\text{milliequiv.} \frac{\text{Peroxide}}{\text{kg}} \text{ sample} \right) \\ &= \frac{(S - B) \times N \times 1000}{\text{sample weight (g)}} \end{aligned} \quad (5)$$

S = mL of 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$ required by sample, B = mL of 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$ required by blank, N = Normality of $\text{Na}_2\text{S}_2\text{O}_3$ solution.

Table 1 Experimental factors for EAAE oil extraction method, their coded and uncoded values

Factor	Name	Units	Coded factors		
			−1	0	+1
A	Ratio (seed to water)	%	1: 5.0	1: 7.5	1: 10.0
B	Time	h	1.0	2.5	4.0
C	ph		4	6	8
D	Temperature	°C	40	50	60

Experimental design and data analysis

The response surface methodology (RSM) was performed using a commercial statistical package, Design-Expert version 9.0.2.0' (Trial version StatEase) to identify optimum levels of four independent variables viz., pH of the slurry, seed to water ratio, the temperature of incubation (°C) and time of hydrolysis (h). The dependent variables were oil yield, residual oil, time of extraction, unsaponifiable matter, saponification value, total phenolics, acid number and peroxide value. The coded and uncoded independent variables used in the RSM design are listed in Table 1. The experiments were designed according to the Box-Behnken design, as presented in Table 2. The order of experiments was fully randomized.

The data obtained for the responses were fitted into second order polynomial multiple regression equations as a function of independent variable.

$$y_K = \beta_0 + \sum_{i=1}^4 \beta_{Ki}x_i + \sum_{i=1}^4 \beta_{Ki}x_i^2 + \sum_{i=1}^4 \sum_{j=1}^4 \beta_{ij}x_i x_j \quad (6)$$

The predicted response (y_K) was therefore correlated with the set of regression coefficients (β): the intercept (β_0), linear (β_{Ki}), interaction (β_{ii}) and quadratic (β_{ij}) coefficients. The significance of terms in the model was found by analysis of variance (ANOVA). The adequacy of developed model was tested by performing lack of fit test and coefficient of determination ' r^2 ' analysis was carried out. Optimization was carried out individually for all the responses. The numerical optimization technique was applied for optimization of multiple responses. All the independent variables were kept within range, while the responses were either maximized or minimized according to the requirement of the process. Desirability function method was applied for generating optimum conditions having some specific desirability values. The experiments were carried out in triplicates.

Results and discussion

The maximum recovery of germ using wet milling of maize was about 6.5 % and the germ recovery was affected by time and temperature of soaking. A similar result for maize germ recovery was reported by Singh and Eckhoff (1996). The oil content of extracted maize germ was determined using solvent extraction method (Abdulkadir and Abubakar 2011) was 25.58 %. The effect of various process parameters viz., pH of the slurry, seed to water ratio, temperature of incubation (°C) and time of hydrolysis (h) were studied on yield of germ oil using EAAE oil extraction method. The response surface methodology (RSM) was used to optimize various process parameters for EAAE oil extraction method. The multiple regression analysis was tested to fit the high order polynomial equation to the experimental combinations and their response functions were presented in Table 3. The quadratic model equation was fitted to experimental data and the regression coefficient was obtained. However correlation of oil yield and the residual oil was found to be significant ($p \leq 0.01$); whereas lack of fit was non-significant ($p \geq 0.05$).

Enzyme assisted aqueous extraction (EAAE)

In EAAE process, the enzymes improve oil recovery by degrading the seed cell wall, resulting in rupturing the polysaccharide–protein colloid system (Latif 2009). The percent oil yield from maize germ varied from 36.71 to 88.58 %. Similar results for the EAAE extraction of maize germ oil was reported by Moreau et al. (2004). Oil recovery was the highest when pH of slurry was 6 and seed to water ratio, temperature of incubation and time of hydrolysis were 1:7.5, 50 °C and 2.5h, respectively. Whereas, it was the lowest when pH of slurry was beyond 6 with seed to water ratio of 1:5, temperature of incubation of more than 50 °C and time of hydrolysis beyond 2.5 h. The residual oil in slurry ranged from 13.17 to 72.75 % of oil content of extracted maize germ.

The suitability of a second order polynomial to predict oil yield ($R^2 \geq 0.95$, $p \leq 0.01$) and residual oil ($R^2 \geq 0.95$, $p \leq 0.01$) as a function of operational parameters was fitted (Table 3). The correlation between experimental versus actual results was found to be perfectly good for oil yield and residual oil in the slurry (Table 3). The response surface contour graphs were generated by different interactions of two variables while keeping the other two variables constant.

Table 2 Experimental design and responses for EAAE maize germ oil extraction method

Experiment no.	Ratio (seed to water)	Time (h)	pH	Temperature (°C)	Oil yield (%)	Residual oil (ml)	Time of extraction (h)	Unsaponifiable matter (%)	Saponification value (mgKOH/g)	Total phenolics (mgGAE/kg)	Acid number (mgKOH/g)	Peroxide value (meq/kg)
1	1:7.5	2.5	4	40	39.42	15.67	22.5	2.12	196.54	78.965	3.342	2
2	1:10	2.5	8	50	32.66	18.61	22.5	2.01	211.65	79.08	3.66	7
3	1:7.5	2.5	8	60	33.78	18.28	22.5	2.26	211.65	79.07	3.26	8
4	1:5	1	6	50	50.67	12.43	21	1.57	169.31	79.08	3.09	4
5	1:7.5	2.5	8	40	33.78	18.26	22.5	2.33	183.43	78.99	3.26	6
6	1:5	2.5	4	50	42.79	15.68	22.5	2.24	195.43	79.06	3.18	4
7	1:5	2.5	6	40	58.56	10.16	22.5	1.56	169.32	78.98	3.09	3
8	1:5	4	6	50	67.57	7.57	24	1.76	197.43	79.08	3.18	4
9	1:7.5	2.5	6	50	74.33	5.63	22.5	1.72	211.65	79.07	3.26	5
10	1:7.5	1	6	40	73.13	5.98	21	1.81	209.87	79.00	3.18	3
11	1:7.5	1	8	50	36.03	17.64	21	2.23	155.21	79.08	3.34	6
12	1:7.5	2.5	4	60	39.42	16.67	22.5	2.41	197.87	79.02	3.34	7
13	1:10	1	6	50	61.94	9.18	21	1.65	225.21	79.08	3.42	5
14	1:10	2.5	4	50	47.30	14.31	22.5	2.18	224.65	79.06	3.50	6
15	1:7.5	2.5	6	50	73.20	5.76	22.5	1.86	192.43	79.08	3.42	4
16	1:7.5	4	6	60	69.82	6.64	24	1.88	225.76	79.05	3.26	7
17	1:10	4	6	50	74.33	5.53	24	1.79	221.65	79.08	3.51	5
18	1:7.5	2.5	6	50	78.83	4.37	22.5	1.71	211.65	79.08	3.34	3
19	1:7.5	4	8	50	37.16	17.25	24	2.45	197.54	79.08	3.34	4
20	1:10	2.5	6	40	77.63	4.68	22.5	1.68	296.31	78.98	3.58	5
21	1:7.5	2.5	6	50	78.83	3.371	22.5	1.71	197.54	79.06	3.34	4
22	1:10	2.5	6	60	67.57	7.47	22.5	1.71	219.87	79.05	3.51	8
23	1:7.5	4	6	40	78.83	4.24	24	1.82	197.54	78.99	3.26	3
24	1:7.5	1	4	50	47.30	13.28	21	2.25	197.54	79.08	3.34	2
25	1:7.5	1	6	60	65.32	8.19	21	1.80	183.54	79.07	3.17	6
26	1:7.5	4	4	50	42.79	15.49	24	2.21	198.65	79.08	3.26	5
27	1:5	2.5	8	50	30.40	17.62	22.5	2.46	197.54	79.08	3.17	7
28	1:7.5	2.5	6	50	76.58	4.88	22.5	1.79	193.43	79.07	3.34	4
29	1:5	2.5	6	60	69.82	6.73	22.5	1.87	253.98	79.01	3.09	7

Table 3 Condensed analysis of variance for response functions in coded level of variables

Source of variation	Oil yield			Residual oil			Time required			Unsaponifiable value			Saponification value			Total phenolics			Acid number			Peroxide value		
	CP	F-	value	CP	F-	value	CP	F-	value	CP	F-	value	CP	F-	value	CP	F-	value	CP	F-	value	CP	F-	value
Constant	21.95			4.80			22.50			1.76			201.34			79.07			3.34			4.00		
A	0.88	3.85 ^c		-0.87	3.47 ^c		-7.95E-016			-0.04	2.39 ^{NS}		18.03	34.80 ^a		4.58E-003	1.94 ^{NS}		0.20			133.80 ^a		16.73 ^a
B	0.86	3.74 ^c		-0.83	3.18 ^c		1.50			0.05	4.37 ^c		8.16	7.13 ^b		-1.33E-003	0.16 ^{NS}		0.02			1.43 ^{NS}		1.37 ^{NS}
C	-1.20	7.23 ^b		1.38	8.77 ^b		6.12E-033			0.03	1.30 ^{NS}		-4.47	2.14 ^{NS}		0.01	10.20 ^a		6.79E-003			0.16 ^{NS}		49.17 ^a
D	-0.37	0.70 ^{NS}		0.42	0.80 ^{NS}		1.02E-031			0.05	4.49 ^c		3.31	1.17 ^{NS}		0.03	80.98 ^a		-6.79E-003			0.16 ^{NS}		150.59 ^a
AB	-0.33	0.18 ^{NS}		0.30	0.14 ^{NS}		2.68E-015			-0.01	0.07 ^{NS}		-7.92	2.24 ^{NS}		2.25E-003	0.16 ^{NS}		9.80E-016			0.00 ^{NS}		0.00 ^{NS}
AC	-0.52	0.45 ^{NS}		0.59	0.53 ^{NS}		1.15E-031			-0.09	5.14 ^b		-3.78	0.51 ^{NS}		-1.50E-003	0.069 ^{NS}		0.04			1.91 ^{NS}		4.10 ^c
AD	-1.53	3.92 ^c		1.56	3.72 ^c		-4.54E-047			-0.07	2.83 ^{NS}		-40.28	57.90 ^a		9.00E-003	2.50 ^{NS}		-0.02			0.48 ^{NS}		1.02 ^{NS}
BC	0.41	0.27 ^{NS}		-0.65	0.65 ^{NS}		-3.77E-033			0.06	2.50 ^{NS}		10.30	3.79 ^c		1.50E-003	0.07 ^{NS}		0.02			0.48 ^{NS}		25.61 ^a
BD	-0.087	0.01 ^{NS}		0.049	3.61 ^{NS}		-6.78E-032			0.02	0.21 ^{NS}		13.64	6.64 ^b		-3.25E-003	0.33 ^{NS}		5.84E-018			0.00 ^{NS}		1.02 ^{NS}
CD	-1.37E-015	0.00 ^{NS}		-0.24	0.092 ^{NS}		4.08E-031			-0.09	4.51 ^c		6.72	1.61 ^{NS}		5.00E-003	0.77 ^{NS}		7.11E-018			0.00 ^{NS}		9.22 ^a
A ²	-1.68	7.67 ^b		1.86	8.65 ^b		8.07E-016			-0.07	4.74 ^b		16.39	15.56 ^a		-3.47E-003	0.60 ^{NS}		0.02			1.05 ^{NS}		24.41 ^a
B ²	-1.01	2.78 ^{NS}		1.04	2.72 ^{NS}		8.99E-016			0.02	0.44 ^{NS}		-8.99	4.68 ^b		0.01	7.38 ^b		-0.06			6.21 ^b		4.61 ^b
C ²	-9.53	245.88 ^a		10.46	272.73 ^a		-3.66E-016			0.52	245.37 ^a		-10.44	6.30 ^b		-2.85E-003	0.41 ^{NS}		0.02			1.05 ^{NS}		18.46 ^a
D ²	-0.94	2.37 ^{NS}		0.99	2.43 ^{NS}		-3.66E-016			0.03	0.56 ^{NS}		11.81	8.07 ^b		-0.06	169.57 ^a		-0.06			6.21 ^b		24.41 ^a
Lack of fit	-	5.76 ^{NS}		-	3.38 ^{NS}		-			-	1.77 ^{NS}		-	1.30 ^{NS}		-	1.39 ^{NS}		-			1.07 ^{NS}		0.28 ^{NS}
r ²	0.9550			0.9552			1.0000			0.9560			0.9195			0.9549			0.9172			0.9588		

CP coefficient of polynomial, NS not significant ($p \geq 0.10$), ND not detected^a $p \leq 0.01$ ^b $0.01 < p \leq 0.05$ ^c $0.05 < p \leq 0.10$

Fig. 2 Response surfaces plot for effect of extraction parameters on recovery of germ oil

Effect of process parameters on oil recovery

The three-dimensional response surface plot for the effect of independent variables (pH of the slurry, seed to water ratio, temperature of incubation and time of hydrolysis) on germ oil yield of maize are presented in Fig. 2. It was observed that oil yield increased with increasing seed to water ratio because the increase in seed to water ratio results in effective action of enzymatic formulation for oil extraction and more release of oil from oil cells of oil globules occur (Latif 2009). When the seed to water ratio was decreased below 1:7.5, the oil yield decreased dramatically which may be due to the difficulty of keeping the mixture in suspension during treatment. However, at the higher seed to water ratio (1:10), the viscosity of the mixture increased. This made it difficult to maintain mixture homogeneity and decreased the oil recovery. In the present study, a seed to water ratio of 1:7.5 was found to be adequate for the extraction process.

It was observed from the same figure that hydrolysis time has appreciable effect on oil extraction yield up to 2.5 h because when the time of hydrolysis was more, enzymes got more time for degradation of oilseeds cell wall, but after 2.5 h the oil yield was not enhanced may be due to the increase in levels of reducing sugar when enzyme was used (Latif 2009). The effect of hydrolysis time on the oil yield with an optimum enzyme concentration and pH 5–6 at 50 °C were observed and at these parameters the oil yield was maximum due to higher efficiency of enzymes required for oil extraction.

The enzymes activities were strongly dependent upon the pH value. The pH range of 4–6 was found suitable for the present study. With the increase in pH beyond 6, the oil yield decreased because the action of the enzyme was

found effective in the pH range of 5–6. The higher pH can result in higher viscosity due to cells degradation, this makes it more difficult to separate solid–liquid phase by centrifuging which results in a decrease in the oil yield. It may also result in deterioration of oil quality through saponification (Rhee et al. 1973).

The oil yield increased with the increase in temperature of incubation up to 50 °C because the enzyme activity strongly depends on the optimal temperature range. For cellulase and protease enzyme mixture the optimal temperature range of 45–50 °C was effective for higher oil yield. But beyond 50 °C oil yield decreased, because at high temperatures it was difficult to maintain homogeneity during extraction and also it resulted in protein denaturation (Rosenthal et al. 1996). The effect of process parameters on residual oil in slurry is presented in Fig. 3. It can be seen from the figure that at pH 4 and 8 the residual oil is maximum because oil yield is minimum at these pH value. Similarly, the residual oil was determined maximum at seed to water ratio of 1:5 and 1:10 and at time of hydrolysis 1–2.5 h. But temperature of incubation does not affect the residual oil in slurry, may be due to oil extraction was done near the optimum enzyme activity temperature range.

The coefficient of determination value of recovery of germ oil was 0.9550, it shows that the model predicting increased oil yield is significant at 5 % level of significance. The high model F value (245.8) and the low p value less than 0.05 indicates that the model terms are significant. To test the fit of the model, the regression equation and determination of coefficient (r^2) are evaluated. The value of r^2 (0.95) is in reasonable agreement with the adjusted r^2 of (0.90); hence there is a close agreement between the experimental results and theoretical values predicted by the proposed models. Therefore this model can be used to

Fig. 3 Response surface plot for effect of extraction parameters on residual oil in slurry

navigate the design space. The significant model terms were A, C, A^2 and C^2 were (Table 3).

Whereas for residual oil in slurry, the value of r^2 (0.9552) was in reasonable agreement with the adjusted r^2 of (0.9099); hence there was a close agreement between the experimental results and theoretical values predicted by the proposed models. Therefore this model can be used to navigate the design space. The significant model terms were A, B and C (Table 3). The regression equation of the model showing the effect of independent parameters on the oil yield and residual oil in slurry are given below:

$$\begin{aligned} \text{Oil yield(\%)} = & +76.35 + 3.47A \\ & + 3.01B - 4.60C - 1.30D - 1.13AB \\ & - 0.56AC - 5.33AD + 1.41BC \\ & - 0.30BD - 2.825E - 0.16CD - 6.27A^2 \\ & - 3.32B^2 - 33.57C^2 - 3.04D^2 \end{aligned} \quad (7)$$

$$\begin{aligned} \text{Residual oil in slurry (ml)} = & +4.80 - 0.87A \\ & - 0.83B + 1.38C + 0.42D + 0.30AB \\ & + 0.59AC + 1.56AD - 0.65BC \\ & + 0.049BD - 0.24CD + 1.86A^2 + 1.04B^2 \\ & + 10.46C^2 + 0.99D^2 \end{aligned} \quad (8)$$

where, A = Seed to water ratio; B = Time of hydrolysis; C = pH; D = Temperature of hydrolysis.

Effect of process variables on extraction time

The effect of different process parameters on time required for oil extraction are shown in Table 2 and Fig. 4. It can be observed from the figure that the time required for oil extraction decreased as the time of hydrolysis decreased. The additional incubation time of 20 h was constant so it has no effect on oil extraction time. The seed to water ratio, pH of slurry, temperature of incubation and time of

Fig. 4 Response surface plot for effect of extraction parameters on time of oil extraction

hydrolysis had the least effect on oil extraction time. The time of oil extraction increased with the increase in seed to water ratio, it may be due to the increase in cooking time. It also increased with the increase in pH of the slurry because higher pH caused higher viscosity of slurry, which resulted in the increase in centrifugation time for oil extraction (Latif 2009; Abdulkarim et al. 2005). The extraction time decreased with the increase in temperature of incubation due to more release of oil from oil globules at a higher temperature (Rhee et al. 1972). In this case, there were no significant model terms and the lack of fit was not detected (Table 3). The multiple regression analysis results are shown in the following equations:

$$\begin{aligned} \text{Extraction Time(h)} = & +22.50 - 7.949E - 016A \\ & + 1.50B + 6.119E - 033C + 1.022E - 031D \\ & + 2.676E - 015AB + 1.152E - 031AC - 4.539E \\ & - 047AD - 3.773E - 033BC - 6.779E - 032BD \\ & + 4.088E - 031CD + 8.073E - 016A^2 + 8.993E \\ & - 016B^2 - 3.664E - 016C^2 - 3.664E - 016D^2 \end{aligned} \quad (9)$$

Effect of extraction variables on physicochemical characteristics of oil

The quadratic model equation was fitted for all the quality parameters of oil to experimental data and regression coefficient was obtained as discussed below.

Unsaponifiable matter

Tocopherols are a particularly important functional constituent of the unsaponifiable fraction of vegetable oils (Tuan 2011). These compounds displayed antioxidant

properties and were active as vitamin E, which made them particularly essential for human nutrition (Tuan 2011; Farhoosh et al. 2011). The unsaponifiable matter of EAEE extracted maize germ oil in the present study was found as $1.38 \pm 0.08 \%$, which was within limits and similar result reported by Abdulkadir and Abubakar (2011). It can be observed from the Fig. 5 that the value of unsaponifiable matter of EAEE extracted oil decreased with the increase in seed to water ratio, this may be due to less extraction of lipid substances associated with unsaponifiable matter viz., sterols, fat-soluble vitamins, hydrocarbons and pigments. It increased with the increase in time of hydrolysis may be because enzyme got more time to degrade oil seed cell walls for oil extraction and extract lipid substances associated with unsaponifiable matter. At slurry pH of 6 and incubation temperature of 40–50 °C, the oil had the least unsaponifiable matter may be due to the greater action of enzymes on grain cell wall leading to higher oil extraction and lesser lipid substance extraction associated with unsaponifiable matter (Latif 2009; Abdulkarim et al. 2005). The value of r^2 (0.956) was in reasonable agreement with the adjusted r^2 (0.912); hence there was also a close agreement between the experimental and predicted values determined by the proposed models. In this case AC, A^2 and C^2 were significant model terms (Table 3). The regression equation of the model showing the effect of independent parameters on the unsaponifiable matter in coded level is shown below:

$$\begin{aligned} \text{Unsaponifiable matter} = & +1.76 - 0.04A + 0.05B \\ & + 0.03C + 0.05D - 0.01AB - 0.09AC - 0.07AD \\ & + 0.07BC + 0.02BD - 0.09CD - 0.07A^2 \\ & + 0.02B^2 + 0.52C^2 + 0.03D^2 \end{aligned} \quad (10)$$

Fig. 5 Response surface plot for effect of extraction parameters on unsaponifiable matter of oil

Fig. 6 Response surface plot for effect of extraction parameters on saponification value of oil

Saponification value

Saponification is a process that produces soap, usually from fats and lye (Anonymous 2013b). Vegetable oils and animal fats are the main materials that are saponified. These greasy materials, triesters called triglycerides, are mixtures derived from diverse fatty acids. The saponification value of EAAE extracted maize germ oil in the present study was determined as 214.02 ± 10.58 mgKOH/g. A similar result for saponification value of maize germ extracted oil was determined by Abdulkadir and Abubakar (2011). It can be observed from Fig. 6 that the saponification value of EAAE extracted oil increased with the increase in time of hydrolysis and seed to water ratio, due to more extraction of fatty acids which contributed to the saponifiable matter in oil. The pH of slurry and temperature of incubation had least effect on the saponification value of oil (Latif 2009). The value of r^2 (0.9195) was in reasonable agreement with the adjusted r^2 (0.8390); hence there was a close agreement between the experimental results and predicted values by the proposed models. A, B, AD, BD, A^2 , B^2 , C^2 and D^2 were significant model terms (Table 3). The regression equation of the model showing the effect of independent parameters on the saponification value in coded level is given below:

$$\begin{aligned} \text{Saponification value} = & +201.34 + 18.03A \\ & + 8.16B - 4.47C + 3.31D - 7.92AB - 3.78AC \\ & - 40.28AD + 10.30BC + 13.64BD + 6.72CD \\ & + 16.39A^2 - 8.99B^2 - 10.44C^2 + 11.81D^2 \end{aligned} \quad (11)$$

Total phenolics

Phenolic compounds are important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into radicals (Deiana et al. 2002). The total phenolics content of EAAE extracted maize germ oil in the present study was determined as 79.08 ± 0.01 mg GAE/kg. A similar result for total phenolics content of maize germ oil was determined by Tuan (2011). It can be observed from the Fig. 7 that the total phenolics content of oil was the highest when the temperature of incubation or hydrolysis was in the range of 50–55 °C i.e. at the optimal temperature of enzyme activity. The other factors showed the negligible effect on the total phenolics content of extracted oil. The use of enzymatic preparations containing cell wall degrading enzymes during oil extraction results in the release of greater amounts of phenolics due to hydrolysis of seed cell wall, resulting in higher availability of such bioactive components into the oil (Ranalli et al. 2003; Qiu et al. 2003). A total phenolic content produces scavenging capacity and inhibition of linoleic acid peroxidation of the oils obtained by EAAE method (Tuan 2011). The value of r^2 (0.95) was in reasonable agreement with the adjusted r^2 of (0.90); hence there is a close agreement between the experimental results and predicted values by the proposed models. C, D, B^2 and D^2 are significant model terms (Table 3). The regression equation of the model showing the effect of independent parameters on the total phenolics content in coded level is given below:

Fig. 7 Response surface plot for effect of extraction parameters on total phenolics of oil

Fig. 8 Response surface plot for effect of extraction parameters on acid number of oil

$$\begin{aligned}
 \text{Total phenolics} = & +79.07 + 4.583E - 003A - 1.333E \\
 & - 003B + 0.01C + 0.03D + 2.250E - 003AB \\
 & - 1.500E - 003AC + 9.000E - 003AD + 1.500E \\
 & - 003BC - 3.250E - 003BD + 5.000E - 003CD \\
 & - 3.475E - 003A^2 + 0.01B^2 - 2.850E - 003C^2 - 0.06D^2
 \end{aligned}
 \quad (12)$$

Acid number

The acid value is considered as an indicator of the quality of the oil and the degree of its degradation during heating. An increase in the acid value leads to the development of unpleasant tastes and odors in oils (Li et al. 2014). The increase in acid value is attributed to the hydrolysis of TAG (triacylglycerol) and/or cleavage and oxidation of

fatty acid double bonds (Abdulkarim et al. 2007). The acid number of EAAE extracted maize germ oil in the present study was determined as 3.35 ± 0.05 mg KOH/g. A similar result for acid number of edible oil was determined by Abdulkarim et al. (2007). It can be observed from Fig. 8 that there is increase in acid number with increase in seed to water ratio because more water needs more heating to cook the slurry, more impact of high temperature on oil which degrades the quality of the oil. The increase in temperature up to 50 °C does not have an effect on acid number but beyond 55 °C there is increase in acid number of oil due to degradation of oil at a higher temperature (Concha et al. 2004). The pH of slurry and time of hydrolysis does not have any effect significant effect on the acid number of extracted maize germ oil (Abdulkarim et al. 2005). The r^2 value (0.9172) was in agreement with the adjusted r^2 (0.8344); A, B² and D² are

Fig. 9 Response surface plot for effect of extraction parameters on peroxide value of oil

significant model terms and the lack of fit was non-significant (Table 3). The regression equation of the model showing the effect of independent parameters on the acid number in coded level is given below:

$$\begin{aligned} \text{Acid number} = & +3.34 + 0.20A + 0.02B \\ & + 6.792E - 003C - 6.792E - 003D + 9.801E \\ & - 016AB + 0.04AC - 0.02AD + 0.02BC \\ & + 5.836E - 018BD + 7.112E \\ & - 018CD + 0.02A^2 - 0.06B^2 + 0.02C^2 - 0.06D^2 \end{aligned} \quad (13)$$

Peroxide value

The number of peroxides present in edible fats and oils is an index of their primary oxidative level. The lower the peroxide value, the better the fat or oil quality and its status of preservation (Tuan 2011). The peroxide value of EAAE extracted maize germ oil in the present study was determined as 3.51 ± 0.49 meq/kg. A similar result for peroxide value of maize oil was determined by Abdulkadir and Abubakar (2011). Figure 9 shows that when seed to water ratio increased the peroxide value of oil also increased, may be because slurry take more time to cook and evaporate the water for increasing the viscosity of the slurry. The slurry remains at higher temperature for more time thus, the oxidation of oil occur. The increase in time of extraction has no significant increase in peroxide value of oil, but with the increase in pH of the slurry, there was a slight increase in peroxide value of oil may be because at higher pH the enzyme activity decreased and the consistency of the slurry increased which increased the oxidation of the oil. And as the temperature of the incubation increased, the increase in oxidation of oil occurs thus the peroxide

value also increased (Latif 2009). The r^2 (0.9588) and adjusted r^2 (0.9176) were closely associated. A, C, D, BC, CD, A^2 , B^2 , C^2 and D^2 are significant model terms and the lack of fit was non-significant (Table 3). The regression equation of the model showing the effect of independent parameters on peroxide value in coded level is given below:

$$\begin{aligned} \text{Peroxide value} = & +4.00 + 0.58A + 0.17B + 1.00C \\ & + 1.75D - 3.042E - 3.013E - 016AB - 0.50AC \\ & - 0.25AD - 1.25BC + 0.25BD - 0.75CD \\ & + 0.96A^2 - 0.42B^2 + 0.83C^2 + 0.96D^2 \end{aligned} \quad (14)$$

Optimization of extraction variables

The optimization of process parameters was carried out using 'Design Expert DX9 9.0.2.0', simultaneously optimization of the multiple responses was carried out using numerical optimization technique of the Design Expert software. A solution having the maximum desirability value of 0.787 was selected as the optimum condition for extraction of germ oil from maize. The optimized results of process parameters according to the Design Expert were given the pH of the slurry, seed to water ratio, the temperature of incubation and time of hydrolysis as 5.85, 1:6.92, 45.12 °C and 1 h respectively. The predicted values for various responses were given by the result of the optimized process parameters. Thus, for the yield of oil, residual oil, extraction time, unsaponifiable matter, saponification value, total phenolics, acid number and peroxide value the predicted value at optimized process parameters were 19.76 ml, 7.15 ml, 21 h, 1.68 %, 204.02 mgKOH/g, 79.08 mg GAE/kg, 3.39 mg KOH/g and 4.41 meq/kg respectively.

Conclusion

The EAAE method for oil extraction from maize germ is considered as a good alternative for this purpose being environment-friendly and green technology; also the extracted oil was light yellowish in color and had a pleasant nutty taste. Twenty-nine experiments runs in triplicate were conducted to obtain the optimal conditions as 5.85 pH of the slurry, 1:6.92 seed to water ratio, 45.12 °C temperature of incubation and 1 h time of hydrolysis, with a highest oil yield of 70 %. The results showed that pH of slurry, seed to water ratio, temperature of incubation had a significant effect ($p < 0.05$) on oil yield and various quality parameters, but the time of hydrolysis had a non significant effect.

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