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ORIGINAL PAPER

Optimization of the Aqueous Enzymatic Extraction of Wheat Germ Oil Using Response Surface Methodology

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Abstract A process of aqueous enzymatic extraction of wheat germ was carried out by a multi-enzyme preparation consisting of cellulase, pentosanase, neutrase and fungal amylase (CPNF, 2:1:2:1 w/w/w/w). Hydro-thermal heating (at 112 °C for 60 min) was more effective than oven-drying regarding emulsified oil yield. Wheat germ was ground with a rate of 10,000 rpm for 90 s. The adding level (w/w) of multi-enzyme preparation of CPNF was 1.6%. Response surface methodology was used to obtain the desired data in the process optimization. The optimal set of variables was water to wheat germ ratio (v/wt, mL/g) of 3.46, pH of 5.24, temperature of 48.49 °C and time of 6 h. The emulsified oil yield was 86.74% at the optimal levels of the tested factors. Compared with organic solvent extracted oil, the content of free fatty acid of AEE extracted oil was higher and the color was slightly darker, while the peroxide value was lower and the oxidative stability was higher owing to high content of α-tocopherol. This technique for recovering oil from fresh wheat germ with enzymes is a significant improvement in both oil yield and quality over the traditional organic solvent process.

Keywords Aqueous enzymatic extraction · Wheat germ oil · Response surface methodology

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Introduction

Wheat germ is characterized by a high content of linoleic acid which is well known as an essential fatty acid [1–3]. Also of major importance, wheat germ is one of the vegetable materials with the highest vitamin E content [4]. The value of vitamin E lies in its antioxidant action by acting against the formation of free radicals [5]. In addition, the oil has been reported to improve human physical fitness, which is attributed to the long-chain *n*-alkanols (particularly octacosanol) [6]. Wheat germ oil has been used as a fertility agent, an antioxidant, and an additive in natural food and cosmetic products [7].

According to some researchers, conventional oil extraction methods are limited for wheat germ. Mechanical pressing methods applied to wheat germs produces high quality oil but only in low oil yields (50%) [8]. An organic solvent approach recovers 90% of the wheat germ oil, however, it needs refining steps including degumming, neutralization, bleaching, and deodorization and it has recently become a focus of concerns with respect to the organic solvent's safety and environmental pollution [7]. Extracting wheat germ oil with supercritical fluid CO_2 (SC) has been recognized by some workers, this generates high-quality oil. However, the SC technology is still not widespread because of its production costs and because of the difficulty using it in a continuous cycle on an industrial scale [9, 10].

In oil seeds of plants, the oil is localized in organelles called lipid bodies or spherosomes, which are surrounded by a biological membrane. Based on the mechanical rupturing, the aqueous enzymatic extraction method utilizes enzymes (i.e. cellulase, hemicellulase, pentosanase, amylase, glucanase and protease) to decompose cell walls and the lipid bodies. Aqueous enzymatic extraction is a new

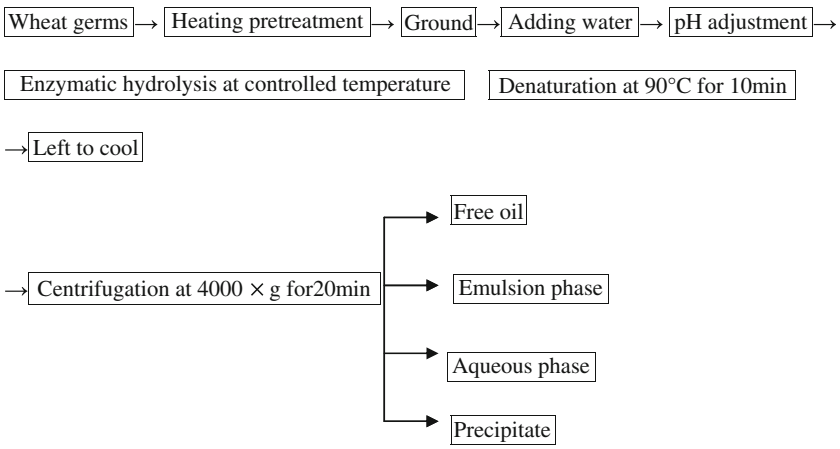

Fig. 1 The flow chart for enzymatic aqueous extraction of wheat germ oil

processing of oil extraction and is attracting a lot of attention from researchers, due to its mild conditions, low energy costs, good quality of the oil product and comprehensive utilization of oil seeds. Some researchers conducted their research on this novel oil extraction method with coconut, maize germ, soybeans, rapeseeds, peanuts, sesame and sunflower seeds [11–13]. Aqueous enzymatic extraction processing involves paying attention to parameters including crude material rupturing degree, particle size, pre-treatment, enzyme types, enzyme adding dose, substrate concentration or water/seed ratio, reaction time, reaction pH, and other factors [11–21]. Compared with the traditional method, the development of the aqueous enzymatic method leads to little difference between fatty acid compositions, high oil yield rate, low peroxide value, low iodine value, low unsaponifiable matter content, and other properties [14, 15, 19–21]. However, no studies have reported on the application of aqueous enzymatic extraction to wheat germ.

Therefore, the aim of this present research was to assess the effects of different commercial enzymes (i.e. cellulase, pentosanase, neutrase and fungal amylase) on the extraction of wheat germ oil and to investigate the processing parameters (i.e. adding concentrations of multi-enzyme preparation, ratio of water to raw material (v/wt, mL/g), time of reaction, pH of reaction, temperature of system). This was followed by an optimization of the aqueous enzymatic extraction process by means of response surface methodology (RSM). And the quality of AEE extracted wheat germ oil was compared with organic solvent extracted oil.

Materials and Methods

Materials

Wheat germ and organic-solvent-extracted wheat germ oil were provided by the Hebei Yongsheng Food Co. Ltd. Wheat germ oil was extracted by liquefied butane from the same batch as the wheat germ sample. Fungal amylase 2 500 SG, neutrase 5.0 BG, pentosanase 2 500FXU, Cellulase Celluclast BG were purchased from the Novozymes (China) investment Co. Ltd.

Pre-treatment and Aqueous Enzymatic Extraction of Oil from Wheat Germ

The flow chart for enzymatic aqueous extraction of wheat germ oil is shown in Fig. 1. Appropriate pre-treatment methods (Table 1) were selected for subsequent hydrolysis process parameters. One was a hydrothermal pre-treatment, i.e. 100 g of a ground sample was put into a pressure cooker equipped with a pressure regulator for 60 min at 112 °C [14].The other was oven-drying pre-treatment, i.e. 100 g of a ground sample was put into an electric far-infrared oven and baked for 60 min at 112 °C.

Wheat germ was ground at a rate of 10000 rpm in a DFY-400 grinder purchased from Wenling Dade Chinese Herb Machine Co. Ltd., controlled the rupturing degree by the grinding time (Fig. 2) because it is difficult to sieve the viscous wheat germs. The sample was dispersed in distilled water and its diameter determination was carried directly after 90 s rupturing treatment by laser light scattering (Malvern Mastersizer 2000 equipped with a 100 mm lens, Malvern Instruments Ltd., Malvern–Worcestershire, UK with HydroMU sample dispersion unit).

The water to wheat germ ratio (v/wt, mL/g) and pH of the slurry were adjusted to different levels respectively. The enzyme(s), either singly or in combination (Table 2), were then added to the slurry to a final proportion of the wheat germ weight (w/w) to commence the hydrolysis at the desired temperature for a specified time period with an agitation rate at 200 rpm. Following the incubation, the suspension was centrifuged at 4,000*g* for 20 min. The layers of free oil and emulsion phrase were collected separately, while the precipitate was freeze dried. And this

Table 1 Effects of pre-treatment on emulsified oil yield

Pre-treatment method	Emulsified oil yield (%)
Hydro-thermal pre-treatment	84.82[a]
Oven-drying pre-treatment	78.37[b]

Means with different letters in the same column are significantly different at the 5% level

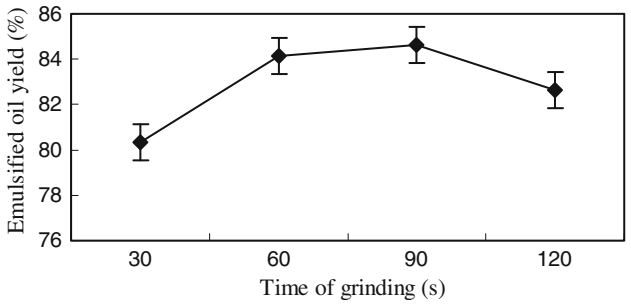

Fig. 2 Effects of time of grinding on emulsified oil yield following extraction with a 1.6% concentration (w/w) of a combination of cellulase, pentosanase, neutrase and fungal amylase (2:1:2:1 w/w/w/w) and a ratio of four to one of water to wheat germ at 50 °C and pH 6 for 6 h with gentle agitation

meal was pulverized and analyzed for residual oil and moisture content determinations.

The emulsified oil yield was calculated according to the relationship expressed in Eq. 1. Because little free oil was obtained, a suitable enzyme or combination was selected based on the emulsified oil yield. The enzyme combination consisting of cellulase–pentosanase–neutrase–fungal amylase at a ratio of 2:1:1:2 (w/w/w/w), gave the highest oil recovery (Table 2) and was subsequently applied in the optimization process.

Emulsified Oil Yield of Wheat Germ

The yield of emulsified oil was expressed using Eq. 1.

$$\text{Emulsified oil yield, \%} = \frac{[\text{total oil in wheat germ}] - [\text{residual oil in meal}]}{[\text{total oil in wheat germ}]} \times 100\% \quad (1)$$

Experimental Design, Evaluation, and Statistical Analysis

Response surface methodology (RSM) was used to predict the optimum conditions for enzymatic hydrolysis treatment to obtain the maximum emulsified oil yield. A cubic order response surface was fitted using four factors (i.e. water-to-wheat germ ratio, incubation pH, temperature and time) and five levels (Table 3) using central composite design (CCD) with five replicates at the center point [22, 23]. The

Table 2 Effects of single enzyme and multi-enzyme on emulsified oil yield

Enzyme	Emulsified oil yield (%)
Control (without enzyme)	31.09 ± 1.23
Cellulase	55.04 ± 0.83
Pentosanase	47.74 ± 0.85
Neutrase	68.27 ± 1.08
Fungal amylase	57.81 ± 0.87
Cellulase + pentosanase (2:1)	76.95 ± 0.71
Cellulase + pentosanase (1:1)	71.17 ± 0.98[b]
Cellulase + pentosanase (1:2)	71.52 ± 0.94[b]
Cellulase + pentosanase + neutrase + fungal amylase (2:1:2:1)	84.94 ± 0.56
Cellulase + pentosanase + neutrase + fungal amylase (2:1:1:1)	80.93 ± 0.99[c]
Cellulase + pentosanase + neutrase + fungal amylase (2:1:1:2)	80.00 ± 1.05[c]

Emulsified oil yield following extraction with a 1.6% concentration (w/w) of a single enzyme or a combination of cellulase, pentosanase, neutrase or fungal amylase and a 4 of water-to-wheat germ at 50 °C and pH 6 for 6 h with gentle agitation

All values represent the mean of triplicate determination ± standard deviation. Means followed by the same superscript letters are not significantly different ($P > 0.05$)

Table 3 Coded and actual variable levels employed in the central composite design

Variables	Levels				
	−1.68179	−1	0	1	1.68179
A: Water-to-wheat germ ratio	2.32	3	4	5	5.68
B: Incubation pH	4.07	4.75	5.75	6.75	7.43
C: Incubation temperature (°C)	41.59	45	50	55	58.41
D: Incubation time (h)	3.32	4	5	6	6.68

CCD contained an embedded full and small factorial matrix where the distance from the start point (i.e. low and high values) is designated as alpha. A small factorial matrix, the rotatable ($k < 6$) type and the value of alpha 1.68179 were chosen.

The actual and coded levels of the independent variables used in the experimental design were shown in Table 3. The cubic model for predicting the optimal point is expressed according to Eq. 2.

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x_i^2 + \sum \beta_{ijk} x_i x_j x_k + \sum \beta_{iij} x_i^2 x_j + \sum \beta_{iii} x_i^3 \quad (2)$$

where Y is the response variable (emulsified oil yield), β_0 is the constant term, β_i is the coefficient of the linear term, β_{ij} is the coefficient of the quadratic cross product term, β_{ii} is the coefficient of the quadratic single term, β_{ijk} is the

Table 4 Experimental design and results of the central composite design (CCD)

Trial no.	A	B	C	D	Emulsified oil yield (%)
1	1	−1	−1	1	78.18
2	0	0	1.68179	0	80.44
3	0	1.68179	0	0	77.26
4	0	0	0	0	85.56
5	−1	1	−1	1	80.70
6	0	0	0	0	85.59
7	−1	−1	−1	−1	77.17
8	−1	1	1	1	78.38
9	0	0	0	0	85.55
10	0	0	0	0	85.58
11	0	0	0	0	85.53
12	1	1	1	−1	80.18
13	0	0	0	1.68179	85.89
14	0	0	−1.68179	0	82.29
15	1.68179	0	0	0	76.34
16	1	−1	1	1	79.84
17	−1.68179	0	0	0	79.37
18	0	0	0	−1.68179	81.93
19	1	1	−1	−1	78.72
20	0	−1.68	0	0	80.14
21	−1	−1	1	−1	75.53

Values represent the means of two experiments

coefficient of the cubic three cross product terms, β_{iij} is the coefficient of the cubic two cross product terms, β_{iii} is the coefficient of the cubic single term. The x_i, x_j and x_k terms represent the independent variables (i.e. water-to-wheat germ ratio (v/wt, mL/g), incubation pH, temperature and time) as coded values.

Data from the CCD shown in Table 4 were used for determining the regression coefficients of the third-order multiple regression models. All experiments were done in duplicate, and mean values of the data are reported. Design-Expert package version 7.1.3 (Stat-Ease Minneapolis, MN, USA) was employed for regression analysis of the data obtained and for estimation of the coefficients of the regression equation. The statistical significance of the model was evaluated by the application of the Fisher test. The 3-dimensional graphical representation of the system behavior, called the response surface, was used to describe the individual and the cumulative effects of the variables as well as the mutual interactions between the variables and the dependent variables (Table 5).

Analytical Methods

The oil contents of wheat germ and meal obtained from the process were determined by the Soxhlet extraction method. The solvent used for Soxhlet extraction was hexane [24].

Official methods of the American Oil Chemists' Society (AOCS) [25] were used to determine the FFA contents (AOCS Ca 5a-40), peroxide values (PV) (AOCS Cd 8-53), oxidative stabilities by the active oxygen method (AOM) (AOCS Cd 12-57), colors (AOCS Cc 13b-45) and tocopherol contents (AOCS Ce 8-89). All analyses were performed in duplicate and mean values of the data are reported Statistical analysis was done using SPSS ver.13 of Windows (SPSS Institute, Cary, NC).

Results and Discussion

Effects of Pre-treatment on Emulsified Oil Yield

The effects of pre-treatment on emulsified oil yield are shown in Table 1. A thermal treatment of wheat germ was first applied to deactivate lipase, but also to gelatinize starch prior to the reaction with α-amylase. Hydro-thermal heating was more effective than oven-drying regarding the emulsified oil yield, because with hydro-thermal heating it was possible to loosen its structure to help liberate the germ oil, thereby bringing about a higher emulsified oil yield. Also, the aqueous enzymatic extraction process of corn germ used by Karlovic et al. [26] included an essential "hydrothermal pre-treatment" step. Bocevska et al. [14] stated that by applying the hydrothermal pre-treatment of

Table 5 Analysis of variance for response surface cubic model

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*p*-value Prob > *F*	Significance
Model	236.5938	16	14.78711	25,942.3	<0.0001	**
A–A	4.59045	1	4.59045	8,053.421	<0.0001	**
B–B	4.1472	1	4.1472	7,275.789	<0.0001	**
C–C	1.71125	1	1.71125	3,002.193	<0.0001	**
D–D	7.8408	1	7.8408	13,755.79	<0.0001	**
AB	0.795021	1	0.795021	1,394.773	<0.0001	**
AC	6.2658	1	6.2658	10,992.63	<0.0001	**
AD	10.30809	1	10.30809	18,084.36	<0.0001	**
BC	0.0968	1	0.0968	169.8246	0.0002	**
BD	7.892757	1	7.892757	13,846.94	<0.0001	**
CD	0.0288	1	0.0288	50.52632	0.0021	**
A^2	84.85407	1	84.85407	148,866.8	<0.0001	**
B^2	67.26721	1	67.26721	118,012.6	<0.0001	**
C^2	25.16401	1	25.16401	44,147.39	<0.0001	**
D^2	3.89872	1	3.89872	6,839.86	<0.0001	**
ABD	0.145874	1	0.145874	2,55.9197	<0.0001	**
A^2C	0.656222	1	0.656222	1,151.266	<0.0001	**
Pure error	0.00228	4	0.00057			
Cor total	236.5961	20				

** Significant at the 5% level

corn germ, it was possible to inactivate native enzymes present in the germ and to loosen its structure. Soto et al. [27] claimed that pre-heating would open cellular channels, reduce the oil viscosity, and enhance the oil fluidity through the cell wall that has not been degraded. Hernandez et al. [28] asserted that a thermal treatment of rice bran is first applied to deactivate lipase, but also to gelatinize starch previous to reaction with α-amylase.

Effects of Grinding Time on Emulsified Oil Yield

The critical step in the aqueous extraction process which affects the oil yield is the grinding operation which determines the oil-seed particle size. Time of grinding influenced the extraction yield of the emulsified oil (Fig. 2). This may be due to the fact that grinding for a long time means creating a large surface area to help the enzymes and substrate react more easily resulting in a high extraction yield of the emulsified oil. The values after 90 s of rupturing treatment of volume–surface average diameter D [2, 3] and weight mean diameter D [3, 4] of wheat germ samples are 102.869 and 295.949 μm, respectively. However, as the time of grinding became longer, the emulsification of enzymatic reaction solutions became stronger to some degree, which contributed to declining of extraction yield because it was difficult to separate the suspensions [19]. The time of 90 s gave the highest yield of oil and this time was employed in subsequent experiments.

Effects of Single Enzymes and Cooperative Multi-Enzymes on Emulsified Oil Yield

Enzymes are used to facilitate release of oil from oil bodies enmeshed in protein and cellulosic or hemicellulosic networks [29]. Table 2 also shows the result of using various commercial preparations of enzymes. Among single enzymes used, neutrase gave a significantly ($P < 0.05$) higher oil yield (68.28), which conformed to the early observation that hemicellulases and cellulases were less effective than proteases in oil extraction from some seeds such as *Jatropha curcas* L. seeds [30, 31]. Cellulase, pentosanase, neutrase and fungal amylase increased the oil yield by 23.95, 16.65, 37.18 and 26.72%, respectively, compared with the control, and the differences between the four enzymes treatment and the control with respect to the oil yield were significantly, therefore the four enzymes were all tested in subsequent experiments.

The enzymes were combined (Table 2) to evaluate their cooperative effects on extracting the oil. We desired the highest possible oil yield, so we chose the combination of cellulase, pentosanase, neutrase and fungal amylase (2:1:2:1 w/w/w/w) for the subsequent experiments. A good recovery of emulsified oil indicated that the wheat germ

cell wall was more effectively degraded by the enzyme combination, leading to the release of most of the oil and other materials enmeshed within the cells into the aqueous medium. Different materials require different types of enzymes. Cell-wall degrading enzymes can be used to extract oil by decomposing the structural cell wall components of the oil-seed. In addition, carbohydrases, proteolytic enzymes were also found to improve yields of oil and protein by hydrolyzing the structural fibrous protein in which fat globules are embedded. Thus, cellulase and protease are suitable for maize germ oil extraction [14–16], while cellulase, glucanase, pectinase and protease are beneficial to rapeseed oil extraction [18].

Effects of Concentrations of Multi-Enzymes on Emulsified Oil Yield

As is shown in Fig. 3, even a low concentration (0.8%) of CPNF (2:1:2:1, w/w/w/w) had a marked effect on the extractability of oil, which was only slightly improved by higher concentrations of enzyme. At higher enzyme concentrations, there were more active sites available on the enzymes to interact with substrates, resulting in a greater emulsified oil yield. However, the enzyme concentration should be a compromise between the improvement of oil recovery and the cost of the enzyme. In this study, a 1.6% concentration (w/w) of CPNF (2:1:2:1, w/w/w/w) was adopted.

The Central Composite Design and Response Surface Methodology

The Model F-value of 25942.30 implied that the model was significant. There was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob $>$ F" less than 0.0500 indicated that the model terms were significant. In this case A, B, C, D, AB, AC, AD, BC, BD, CD, A^2, B^2, C^2, D^2, ABD, A^2C were significant model terms. Values greater than 0.1000 indicated that the model terms were not significant. If there were many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

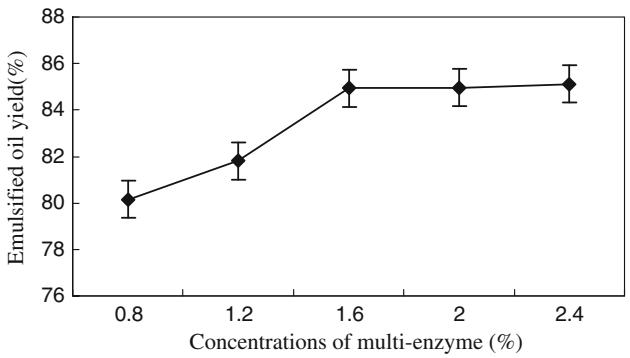

Fig. 3 Effects of concentrations of multi-enzyme on emulsified oil yield following extraction with different concentrations (w/w) of a combination of cellulase, pentosanase, neutrase and fungal amylase (2:1:2:1 w/w/w/w) with a four of water-to-wheat germ at 50 °C and pH 6 for 6 h with gentle agitation

Similarly, the application of RSM yielded the following cubic order regression equation as shown in equation 3, which described the relationships between response variables (i.e. emulsified oil yield) and independent variables (i.e. water-to-wheat germ ratio (v/wt, mL/g), incubation pH, incubation temperature and incubation time) in coded units.

$$\begin{aligned}\text{Emulsified oil yield } (\%) = {} & 85.56 - 0.90 \\ & \times A - 0.86 \times B - 0.55 \times C + 1.18 \times D \\ & + 0.49 \times A \times B + 0.88 \times A \\ & \times C - 1.76 \times A \times D - 0.11 \times B \times C - 1.54 \\ & \times B \times D - 0.060 \times C \times D - 2.72 \times A^2 - 2.43 \\ & \times B^2 - 1.48 \times C^2 - 0.58 \times D^2 - 0.24 \times A \times B \times D \\ & + 0.45 \times A^2 \times C \end{aligned} \quad (3)$$

The Cubic Model

Figure 4 shows the response surfaces generated by the proposed models. These figures express the interactions between the two independent variables, in which the other two variables were both maintained at the central point.

The water-to-wheat germ ratio (v/wt, mL/g) significantly affected the extraction yield of the emulsified oil, showing a quadratic trend (Fig. 4a). This may be due to the fact that thick suspensions prevent the effective penetration of the enzymes, while the chance of an interaction between the enzyme and substrate molecules is low in very dilute suspensions. The trend is consistent with the research about aqueous enzymatic extraction of corn germ and rapeseed oil [14–16, 18]. The ratio of 1:4.25 (v/wt, mL/g) gave the highest yield of oil.

In Fig. 4a and b, a quadratic trend can be seen between the pH and the extraction yield of the emulsified oil. According to Whitaker [32], the pH affects enzymes in several ways. First, catalytic activity is related to the ionic state of the active site, changes in hydrogen ion concentration can affect the ionization of active site groups. In addition, substrates may also be affected. If a substrate contains an ionizable group, a change in pH may alter its capacity to bind to an active site. Second, changes in ionizable groups may change the tertiary structure of the enzymes. Drastic changes in pH often lead to denaturation. Although, a few enzymes tolerate large changes in pH, most enzymes are active only within a narrow pH range.

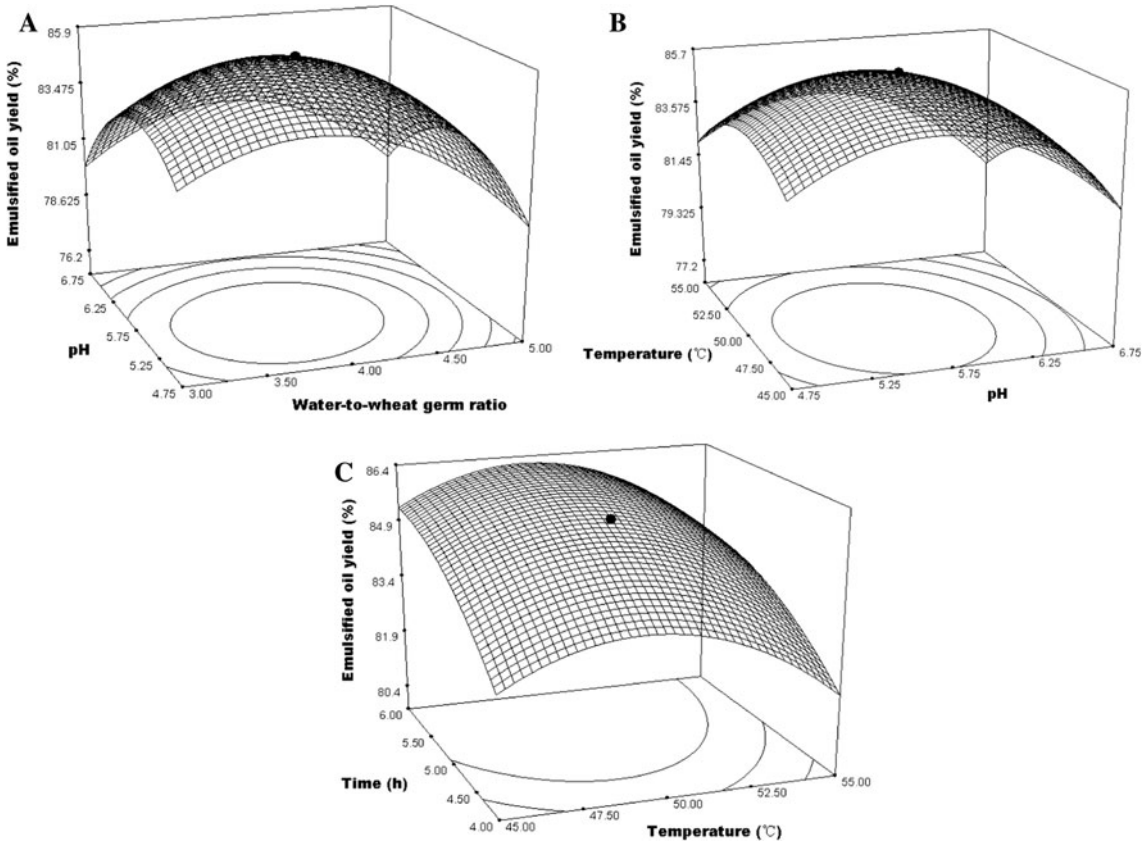

Fig. 4 Response surface plot of the combined effects of two independent variables, when the other two variables were both at center points: **a** water-to-wheat germ ratio × pH, **b** pH × temperature, **c** temperature × time

Therefore, an optimum extraction pH varies quite sensitively with the oil-bearing material. The optimum pH of multi-enzyme preparation is approximately 6. Optimum pH conditions for oil extraction found in other studies were 4.0 for corn germ [15] and 10.0 for rapeseed [18].

All chemical reactions are affected by temperature. The higher the temperature, the higher the reaction rate. The reaction velocity increases because more molecules have sufficient energy to enter into the transition state. In Fig. 4b and c, a quadratic trend can be seen between the temperature and the extraction yield of the emulsified oil. The rates of enzyme-catalyzed reactions also increase with increasing temperature. However, enzymes are proteins that become denatured at high temperatures. Each enzyme has an optimum temperature at which it operates at maximal efficiency. If the temperature is raised beyond the optimal temperature, the activity of many enzymes declines [32]. Therefore the temperature has a considerable effect on yields. The optimum temperature of multi-enzyme preparation is close to 50 °C. Various other temperatures have been employed in aqueous extraction process with other oil seeds: 50–65 °C for corn germ [15] and 60° C for rapeseed [18].

A linear increase in the hydrolysis time and the extraction yield of the emulsified oil was observed, as seen in Fig. 4c. An increase in incubation time up to 6 h did not provide any significantly higher oil yield compared with 5.5 h, this may be due to the depletion of the substrates and/or product inhibition of enzymes [32]. Therefore, the time required to reach a desired extraction level depends on the oilseed as well as the process variables mentioned above.

Optimization

According to the model, the predicted result for the maximal emulsified oil yield was 87.60% under the following critical values: water to wheat germ ratio (v/wt, mL/g) =3.46, pH = 5.24, temperature = 48.49 °C and time = 6 h. The validation tests were repeated three times under the optimal conditions in order to determine the adequacy of the cubic model. A mean value of the 86.74% was found, which was only 0.86% lower than the predicted value. Therefore, there was a good fit between the predicted result and the experimental responses, which

Table 6 Quality of wheat germ oil

	FFA (%)	PV (mequiv/kg)	Color (Red, 1In.)	AOM (h)	Tocopherol content (ppm)		
					α	β	Total
Organic solvent extracted oil	1.67[a]	40.54[a]	6.5[a]	7.9[a]	1,717[a]	754[a]	2,471[a]
AEE extracted oil	5.41[b]	20.13[b]	8.4[b]	>48[b]	2,104[b]	737[a]	2,841[b]

Means with different letters in the same column are significantly different at the 5% level

suggested that the optimized model was proper and effective for explaining the actual enzymatic aqueous process.

Quality of Wheat Germ Oil

A wheat germ oil quality comparison between aqueous enzymatic extraction and organic solvent extraction is shown in Table 6. Compared with organic-solvent-extracted oil, the content of free fatty acid of AEE extracted oil was higher, while the peroxide value was lower. The multi-enzyme combination is helpful for the release of free fatty acid, which is consistent with a peanut oil quality comparison between aqueous enzymatic extraction and organic solvent extraction [19]. In addition, the hydrothermal treatment had a substantial effect on the FFA content of the corn germ oil extracted by AEE, because the high processing temperature may lead to oil hydrolysis [14, 33]. The starting peroxide values of the AEE extracted oil and organic solvent extracted oil were 40.54 and 20.13 mequiv/kg, respectively. The AOM (active oxygen method) is expressed in hours and is the length of time needed for the PV to reach 100 mequiv/kg. Apparently, the AEE extracted oil was stable, which attributed to the antioxidant activity of α-tocopherol with high content [5, 34]. The color of AEE extracted oil was slightly darker than that of organic solvent extracted oil, owing to the lack of a bleaching procedure. Therefore, the quality of AEE extracted wheat germ oil was high.

Conclusion

The multi-enzyme preparation optimal combination consisted of cellulase, pentosanase, neutrase and fungal amylase (CPNF, 2:1:2:1 w/w/w/w). Hydro-thermal heating (at 112 °C for 60 min) was more effective than oven-drying regarding the emulsified oil yield. Wheat germ was ground with a rate of 10,000 rpm for 90 s. The optimal adding level of multi-enzyme preparation of CPNF was 1.6% (w/w). The aqueous enzymatic extraction of wheat germ oil was optimized by means of response surface methodology. The optimal set of variables was water to wheat germ ratio (v/wt, mL/g) of 3.46, pH of 5.24, temperature of 48.49 °C and time of 6 h. The emulsified oil yield was 86.74% at the optimal levels of the tested factors. Compared with organic solvent extracted oil, the quality of AEE extracted wheat germ oil was high. Therefore, the optimized model was applicable and effective for explaining the actual enzymatic aqueous process. This technique for recovering oil from fresh wheat germ with enzymes is a significant improvement in both oil yield and quality over the traditional organic solvent process.

Apart from these strengths, demulsification may become a bottleneck in the development of an aqueous enzymatic extraction technology. Generally, heating [35], freezing-thawing [12, 19, 36], phase inversion [37, 38] and a phospholipase demulsification [39] have been used in an attempt to break the emulsion by some researchers. The demulsification mechanism and method of freezing-thawing are being applied to wheat germ by our research team. Thus, effective demulsification and potential comprehensive utilization of protein hydrolysates should facilitate this eco-friendly process becoming a practical technique for wheat germ processing in the future.

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