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Optimization of cashew nut (*Anacardium occidentale*) oil extraction yield by the enzymatic method

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Abstract

This study aimed to extract as much cashew oil as possible by enzymatic means, an environmentally friendly method. To achieve this, cashew almond paste was reacted with the enzyme alcalase 2.4 L, taking into account the matrix provided by a Box-Behnken design. The factors highlighted were those of enzyme concentration, incubation temperature, incubation time, pH ratio of the medium, and substrate/water. These factors were optimized to obtain the maximum cashew oil yield. The results showed that the alcalase 2.4 L enzyme optimized the cashew oil yield. The optimum yield (38.20 %) was achieved with an enzyme concentration of 2.5 %, a temperature of 60 °C, an incubation time of 8 h, a pH of 8 and a substrate/water ratio of 1:5. Thus, enzymatic extraction using alcalase 2.4 L can be considered an effective extraction method for obtaining oil from cashew nuts.

Keywords: *Anacardium occidentale*; Cashew oil; Enzyme extraction; Optimization

1 Introduction

Cashew trees (*Anacardium occidentale*) grow in the tropics and subtropics, particularly in Brazil, India, Africa, and Southeast Asian countries including Vietnam, and have spread to parts of tropical South and Central America [1]. Vietnam has become a leading country in the processing and export of cashew kernels. Vietnam cashew kernels are exported mainly to the United States, China, European countries, Australia, and New Zealand [2]. In the world, the cashew industry occupies third place in the production of edible nuts in 2000 [3]. India, Brazil, Tanzania, and Nigeria were the four major regions of cashew processing [4]. India, Brazil, Tanzania and Nigeria were once the four main cashew production and processing countries [4]. In recent years, Côte d'Ivoire has become the world's leading exporter of raw cashew nuts, with production of more than one million tons in 2022 [5].

Cashew nut contains a large amount of oil (47.0 %), proteins (21.0 %), moisture (5.9 %), carbohydrates (22.0 %), vitamins B6 D, E, and K, and minerals (potassium, calcium, phosphorus, magnesium and sodium [6]. The oil is abundant in unsaturated fatty acids, which bring many health benefits to consumers [7]. Besides, cashew nuts also provide the body with many essential vitamins, for instance, pyridoxine (Vitamin B-6), vitamin E, and squalene. Vitamin E and squalene are potential antioxidants that support effects on cardiovascular health; squalene is also an important steroid and precursor that has a role as an anticancer agent [8]. Phenolic compounds are important sources of bioactive compounds in the human diet [9]. Frequent nut consumption is associated with a lower risk of cardiovascular disease and diabetes [10]. Unprocessed cashew nut oil is neutral and good for human health because it is rich in unsaturated fatty acids [11]. According to Hongmei [12] world vegetable oil consumption has increased by 2.3 % per annum during the past two decades, with China and Brazil as the highest consumers at 30 and 24 kg/capita. Consumption is predicted to continue to rise by 0.9 % in the coming years. Thus, the gap between demand and the increase in oilseed production also requires a critical examination of extraction and processing technologies.

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In general, solvent extraction is one of the most widely used methods in the industry due to its high oil yield. However, many highly toxic and flammable organic solvents come from non-renewable sources [13]. Pressure extraction, the classic method, does not use solvents, but this technique is less efficient in terms of extraction yield. Aremu and Akinwumin [14] observed a yield of 26 % with mechanical extraction versus 40 % with hexane solvent. Thus, given the global concerns about the damage caused by organic solvents to the environment and the low yield of mechanical extraction, the development of alternative methods of extracting good quality oil needs to be evaluated. Aqueous enzymatic extraction is, therefore, a clean technology that presents itself as a promising alternative to the technique using organic solvents for the extraction of vegetable oils, taking into account the principles of green chemistry [15].

Aqueous enzymatic extraction uses enzymes that hydrolyze the cell wall and oleosome membranes Ribeiro et al. (2016) releasing the oil into the aqueous medium. The use of 2.4 L alcalase for the aqueous enzymatic extraction of oil and protein hydrolysates resulted in a relative recovery of 92 % of the total oil contained in peanut seeds. This enzyme can therefore be adapted for the extraction of cashew oil. In the present work, the objective was to optimize the parameters that can affect the performance of enzyme-assisted aqueous extraction of cashew seed oil.

2 Material and methods

2.1 Material

Cashew nuts were harvested at Kongoti in the Bouaké region (Côte d'Ivoire). The processing of the cashew nuts was inspired by the process described by [17]. After collection, the cashew nuts were sorted manually to remove physical impurities (stems, misshapen nuts, stones, metal parts). They were then sun-dried for four days. After drying, the nuts were placed in jute bags and sent to be shelled at I2T (Société Ivoirienne de Technologie Tropicale, Abidjan, Côte d'Ivoire). The cashew nuts were cleaned, cooked in a steamer (FISDES CIV-0005948, Côte d'Ivoire) at 115 °C for 45 min, then left to dry at room temperature for 48 h before being shelled using a manual shelling machine (FISDES CIV-0005958, Côte d'Ivoire). Afterward, the almonds were baked in an oven at a temperature of 70 °C for 8h; atlast, the dried almonds were ground using a grinder (Platinum MG 139, India). Afterward, the kernels were baked in an oven at a temperature of 70 °C for 8h. All reagents and chemicals were of analytical grade and purchased from the local market. The enzyme (alcalase 2.4 L: *Bacillus licheniformis protease*, ≥2.4 U/g) was provided by Novozymes (Denmark).

2.2 Methods

2.2.1 Solvent extraction

The crushed sample of 5g was packed in a thimble and placed in the Buchi extraction system (Buchi B- 811 Labortechnik Switzerland) and the oil was extracted using n-hexane for 6 hr. After extraction, the hexane was distilled under a vacuum in a rotary evaporator (Eyela, N-N Series; Tokyo, Japan) at 50 °C. The oil obtained was stored under refrigeration (4 °C) until used for further analysis. The yield was calculated according to equation 1 (Eq 1)

$$Yield\ (\%) = \frac{\text{Weight of oil obtained (g)}}{\text{Cashew almond of the sample (g)}} \times 100 \ldots\ldots\ldots\ldots\ldots \textbf{Eq 1}$$

2.2.2 Aqueous enzymatic oil extraction from Cashew Nut

Several factors affect the yield and quality of the oil during enzyme-assisted aqueous oil extraction. Enzyme concentration, seed/water ratio, mixture pH, incubation temperature, and incubation time have been identified as independent variables (factors) in enzyme-assisted aqueous extraction. The enzyme-assisted extraction process used in this study is a slightly modified version of an earlier method reported by [18]. Cashew nuts 5 g were ground to a thick paste and dispersed in distilled water at various ratios (1:5; 1:6 and 1:7 w/v) to obtain a slurry and homogenized in a 100 ml falcon. The mixture was boiled for 5 minutes and allowed to cool to room temperature (25 °C). The pH was then adjusted to the enzyme's optimum level with a solution of NaOH and HCl at 0.1 mol/L each. The alcalase 2.4 L. (protease from *Bacillus licheniformis*, ≥2.4 U/g) enzyme (1 % v/w) was then added to the mixture. The mixture was hydrolyzed for a while at 60 °C in a water bath (Memmert WTB24, India) for a hydrolysis time of 8h with constant stirring at 200 rpm. At the end of the treatment, the enzyme was deactivated at 100 °C for 5 minutes. The suspension was then centrifuged at 8,000 g for 30 minutes to allow phase separation. The oil was recovered as the top layer after centrifugation. The top layer of oil was removed using a Pasteur pipette. The extraction yield has been determined based on the initial cashew kernel quantity used for the extraction, and the obtained oil quantity, which can be observed from Figure 1.

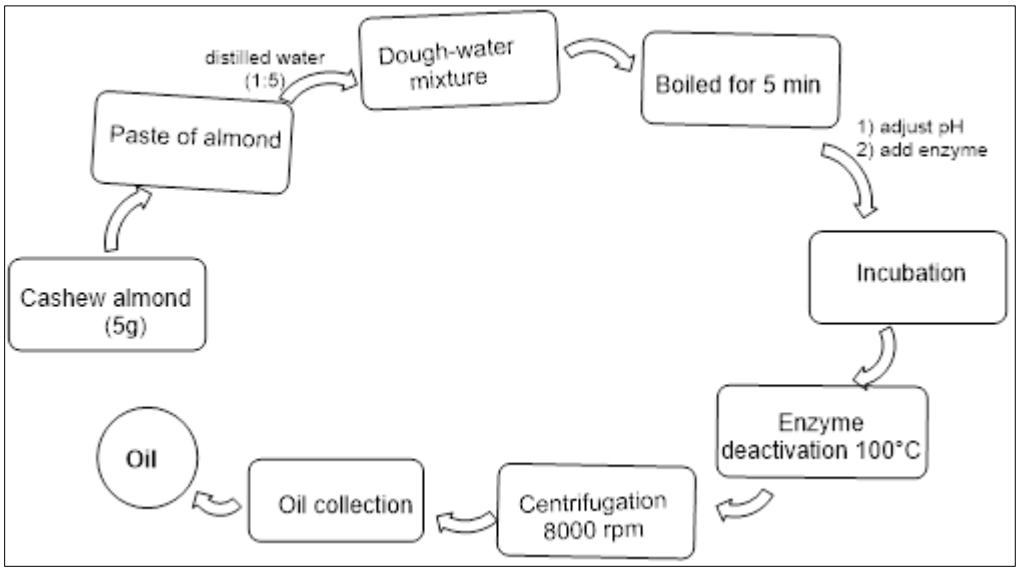

Figure 1 Cashew kernel oil extraction diagram

The yield of enzymatic extraction was determined by equation 1 (Eq 1), and that of the yield recovery by equation 2 (Eq 2).

$$Yield\ recovery = \frac{Weight\ of\ oil\ obtained\ by\ enzyme}{Weight\ of\ oil\ obtained\ by\ solvent} \times 100 \ldots\ldots\ldots\ldots\ldots \textbf{Eq 2}$$

2.3 Optimization of enzyme-assisted aqueous extraction by alcalase 2.4L

Response surface methodology was applied to identify optimum levels of four key independent variables and three levels including enzyme concentration (1-2; 5-4 %), hydrolysis temperature (55-75-60 °C), hydrolysis time (4; 6; 8 h) and pH (8-7.5-9.5) for the selected enzyme in Table 1. After a series of preliminary mono-factor tests, a Box–Behnken design (BBD) was used to survey the effects of independent variables at three levels on the dependent variable (oil recovering rate). A total of 27 randomized experiments including 24 factorial and 3 zero-point tests were designed. Experiments were conducted in random order to minimize the effects of unexpected variability in observed responses due to external factors. The experimental plan was designed and the results obtained were analyzed using Minitab version 18 software.

Table 1 Independent variables and their levels used in Box-Behnken design

				Factors Levels	
Independent variables			(-1)	(0)	(+1)
Amount of enzyme (X_1)	%	X_1	1	2.5	4
Temperature (X_2)	°C	X_2	55	57.5	60
Incubation time (X_3)	h	X_3	4	6	8
pH (X_4)		X_4	8	8.75	9.5

2.4 Statistical analysis

The significant difference in the yield of cashew nut oil was calculated using an analysis of variance (ANOVA). Analysis of variance was performed using the standard statistical software Minitab 18.0. A probability value of $p < 0.05$ was considered statistically significant. All the experimental data for the analysis were assessed in triplicate, and the actual values of each run-on BBD (Box–Behnken design) were conveyed as the mean values, while the other data were presented as the mean values ± standard deviation.

3 Results and discussion

Table 2 shows the matrix design of the response surface plane of the three-level (-1 to 1), four-factors. This study showed that factors such as enzyme concentration (%), temperature hydrolysis (°C), incubation time (h) and pH of the mixture influenced the performance of enzyme-assisted aqueous extraction.

Table 2 Experimental data and the observed response values with different combinations of different factors for aqueous enzymatic oil extraction by alcalase 2.4 L.

Run	X_1	X_2	X_3	X_4	Yield (%)	
					Measured	Predicted
1	-1 (1)	-1 (55)	0 (6)	0 (8.75)	32.00	32.36
2	1 (4)	-1 (55)	0 (6)	0 (8.75)	34.00	34.10
3	-1 (1)	1 (60)	0 (6)	0 (8.75)	35.32	35.41
4	1 (4)	1 (60)	0 (6)	0 (8.75)	35.68	35.50
5	0 (2.5)	0 (57.5)	-1 (4)	-1 (8)	33.54	33.66
6	0 (2.5)	0 (57.5)	1 (8)	-1 (8)	35.99	36.27
7	0 (2.5)	0 (57.5)	-1 (4)	1 (9,5)	35.27	35.20
8	0 (2.5)	0 (57.5)	1 (8)	1 (9,5)	37.55	37.61
9	-1 (1)	0 (57.5)	0 (6)	-1 (8)	34.44	34.37
10	1 (4)	0 (57.5)	0 (6)	-1 (8)	33.60	33.86
11	-1 (1)	0(57.5)	0 (6)	1 (9.5)	34.38	34.40
12	1 (4)	0(57.5)	0 (6)	1 (9.5)	36.40	36.74
13	0 (2.5)	-1 (55)	-1 (4)	0 (8.75)	32.96	33.36
14	0 (2.5)	1 (60)	-1 (4)	0 (8.75)	33.96	3451
15	0 (2.5)	-1 (55)	1(8)	0 (8.75)	35.05	34.78
16	0 (2.5)	1 (60)	1 (8)	0 (8.75)	38.20	38.08
17	-1(1)	0 (57.5)	-1 (4)	0 (8.75)	34.02	33.54
18	1(4)	0 (57.5)	-1 (4)	0 (8.75)	36.20	35.67
19	-1(1)	0 (57.5)	1 (8)	0 (8.75)	37.20	37.26
20	1(4)	0 (57.5)	1 (8)	0 (8.75)	36.94	36.95
21	0 (2.5)	-1 (55)	0 (6)	-1 (8)	32.72	32.37
22	0 (2.5)	1 (60)	0 (6)	-1 (8)	34.75	34.52
23	0 (2.5)	-1 (55)	0 (6)	1 (9.5)	34.00	33.75
24	0 (2.5)	1 (60)	0 (6)	1 (9.5)	36.16	36.04
25	0 (2.5)	0 (57.5)	0 (6)	0 (8.75)	34.90	35.06
26	0 (2.5)	0 (57.5)	0 (6)	0 (8.75)	34.96	35.06
27	0 (2.5)	0 (57.5)	0 (6)	0 (8.75)	35.32	35.06

X_1: enzyme concentration (%), X_2: incubation temperature (°C), X_3: incubation time (h) and X_4 pH of the mixture

Table 2 shows the experimental data and response values observed with the different enzyme combinations. These observed yields ranged from 32 to 38.20 %, with the maximum oil yield of 38.20 % observed at an enzyme concentration of 2.5 %, an incubation temperature of 60°C, a hydrolysis time of 8 h and a pH of 8.75 (assay 16). These results are

similar to those of Gibbins et al. [19] Umego et al. [20] who found that incubation temperature, enzyme amount and buffer pH influenced oil yield during enzyme-assisted aqueous extraction in their various works.

5.1. Analysis of model goodness of fit

The p-value indicating the significance of the coefficients was important for understanding the pattern of mutual interactions between the variables. The results of factor estimation and regression coefficient significance (Table 3) of the Box-Behnken second-stage model, representing the relationship between yield (Y) and the four factors studied, indicate all regression coefficients are significant ($p \leq 0.05$). Table 3 shows that aqueous enzymatic extraction of oil by alcalase 2.4 L was positively affected by all four factors ($p < 0.001$). Of all the factors, incubation time(X_3) most strongly affected aqueous enzyme extraction, followed by mixture pH (X_4) incubation temperature (X_2) and enzyme concentration (X_1). The positive effect of incubation time on extraction yield may be due to cell wall degradation during enzymatic treatment. Some authors like Jiang et al. [21], Passos et al. [22] confirmed in their work that a longer incubation time allows greater degradation of cell wall components, leading to greater oil release. Concerning temperature, this could be explained by the fact that 60 °C lies within the range of maximum activity of the alcalase 2.4 L enzyme. Indeed, according to Jiang et al. [21] the optimal temperature range for alcalase 2.4 L in the enzymatic extraction process is between 55 and 65 °C. The same observation was made by Abdulkarim et al. [23] who verified the influence of a temperature between 45 and 60 °C on the enzyme-assisted aqueous extraction of Moringa oleifera oil with the Celluclast 1.5 L enzyme, with the highest yield obtained at 60°C. Regarding the enzymatic activity of alcalase 2.4 L, it is strongly dependent on pH. The study of the influence of pH showed that a pH of 8.75 was found to be better for the extraction of cashew kernel oil. This increase in oil yield significantly ($P < 0.05$) at this pH could be explained by the fact that pH 8.75 would be in the optimal range for this enzyme. Indeed, according to Li et al. [24] the optimum pH of alcalase is in the interval (8-9.5) and therefore a pH beyond this interval would lead to a reduction in oil yield. The involvement of enzyme concentration in oil extraction is thought to be related to enzyme-induced degradation of plant material (plant seed membranes and cell walls that form a solid barrier against oil release from the cells), which increases oil release [25]. In our study, the quadratic coefficients (β21, β24) and interaction coefficients (β12, β23, β24, β34) were not significant ($p \geq 0.05$) (Table 3). Thus, the second-order equation of the general polynomial model expressing the cashew kernel oil yield as a function of the independent variables was generated (equations 3 and 4):

Yield (%) = $35.060 + 0.455\,X_1 + 1.113\,X_2 + 1.248\,X3 + 0.728\,X_4 - 0.022\,X_1xX_1 - 0.692\,X_2xX_2 + 0.815\,X3xX3 - 0.192\,X4xX4 - 0.410\,X_1xX_2 - 0.610\,X_1xX_3 + 0.715\,X_1xX_4 + 0.538\,X_2xX_3 + 0.037\,X_2xX_4 - 0.043\,X_3xX_4$ **Eq 3**

To refine the model and find the optimal equation, insignificant terms are eliminated. The result is a new model given by the equation 4

Yield (%) = $35.060 + 0.455\,X_1 + 1.113\,X_2 + 1.248\,X_3 + 0.728\,X_4 - 0.692\,X_2^2 + 0.815\,X^2_3 - 0.610\,X_1xX_3 + 0.715\,X_1xX_4 + 0.538\,X_2xX_3$ **Eq 4**

Table 3 Regression coefficients and P-values for aqueous enzymatic oil extraction

Terms	Coefficient	ES	t-exp	p-value
Constante	35.060	0.228	153.46	0.000
X_1	0.455	0.114	3.98	0.002**
X_2	1.113	0.114	9.75	0.0001**
X_3	1.248	0.114	10.93	0.0001**
X_4	0.728	0.114	6.38	0.0001**
X^2_1	-0.022	0.171	-0.13	0.900
X^2_2	-0.692	0.171	-4.04	0.002**
X^2_3	0.815	0.171	4.76	0.0001**
X^2_4	-0.192	0.171	-1.12	0.284
$X_1 \times X_2$	-0.410	0.198	-2.07	0.06
$X_1 \times X_3$	-0.610	0.198	-3.08	0.009

$X_1 \times X_4$	0.715	0.198	3.61	0.004
$X_2 \times X_3$	0.538	0.198	2.72	0.019
$X_2 \times X_4$	0.037	0.198	0.19	0.853
$X_3 \times X_4$	-0.43	0.198	-0.21	0.834

Enzyme concentration (X_1), Incubation temperature (X_2), Incubation time (X_3) and pH of mixture (X_4); **P ≤ 0.05 indicates the statistical

5.2. Assessment of model fit

The ANOVA on the goodness-of-fit of the Box-Behnken model representing the relationship between oil yield (Y) and the four study factors indicates that the model is significant ($p < 0.001$) Table 4. Model accuracy was assessed by the coefficient of determination (R^2 and adjusted R^2 value). The R^2 and adjusted R^2 values were 0.9681 and 0.9311 respectively. This result indicates that the model explains 97 % of the variability for enzyme-assisted aqueous extraction. Consequently, the developed model is sufficiently applicable to provide the optimal conditions to maximize the oil extraction yield. Our results corroborate those of Liu et al. [26] who in their work found that the coefficient of determination (R^2) of the model was 0.9339, this suggests that our model is applicable with good precision.

Table 4 Analysis of variance for the regression model of oil yield extraction

Source	**df**	**Sum of squares**	**Mean square**	**F -value**	**P-value**
Model	14	57.237	4.088	26.11	<0.0001**
X1	1	2.484	2.484	15.86	<0.0001**
X2	1	14.874	14.874	94.99	<0.0001**
X3	1	18.700	18.700	119.42	<0.0001**
X4	1	6.366	6.366	40.65	<0.0001**
X^2_1	1	0.003	0.003	0.02	0.900
X^2_2	1	2.554	2.554	16.31	0.002*
X^2_3	1	3.546	3.546	22.61	<0.0001**
X^2_4	1	0.196	0.196	1.26	0.284
$X_1 \times X_2$	1	0.672	0.672	4.29	0.06
$X_1 \times X_3$	1	1.488	1.488	9.51	0.009
$X_1 \times X_4$	1	2.045	2.045	13.06	0.004*
$X_2 \times X_3$	1	1.156	1.156	7.38	0.019
$X_2 \times X_4$	1	0.006	0.006	0.04	0.853
$X_3 \times X_4$	1	0.007	0.007	0.05	0.834
Error	12	1.879	0.1566		
Inadequate fit	10	1.776	1.776	3.44	0.246
Total	26	59.1165			
R^2	96.81				
R^2_{ajust}	93.11				

df: degree of freedom; ** Significant at $p < 0.05$

5.3. Effect of parameter interactions on oil recovering rate

The best way to visualize the effect of the independent variables on the dependent variable was to draw three-dimensional (3D) response surface curves of the model [27]. Figure 2 shows the 3D response surface curves for oil extraction yield as a function of enzyme quantity and incubation temperature, hydrolysis time, and pH. The result shows that with increasing enzyme quantity and temperature, extraction yield increased but decreased slightly once these parameters reached high levels figure 2a. A similar result between enzyme amount and extraction pH on yield was obtained, as shown in figure 2c. Figure 2e shows that incubation temperature and pH were favorable for oil extraction. Regarding hydrolysis time and concentration, the influence of these independent variables was not as significant as in previous cases (figure 2b). Oil extraction yield remained virtually unchanged with increasing time and temperature. A similar interaction between pH and extraction time on extraction yield was easily obtained in figure 2f. Figure 2d shows that moderate hydrolysis time and temperature lead to an increase in extraction yield. It is easy to see that extraction yield increased with increasing time and decreasing temperature.

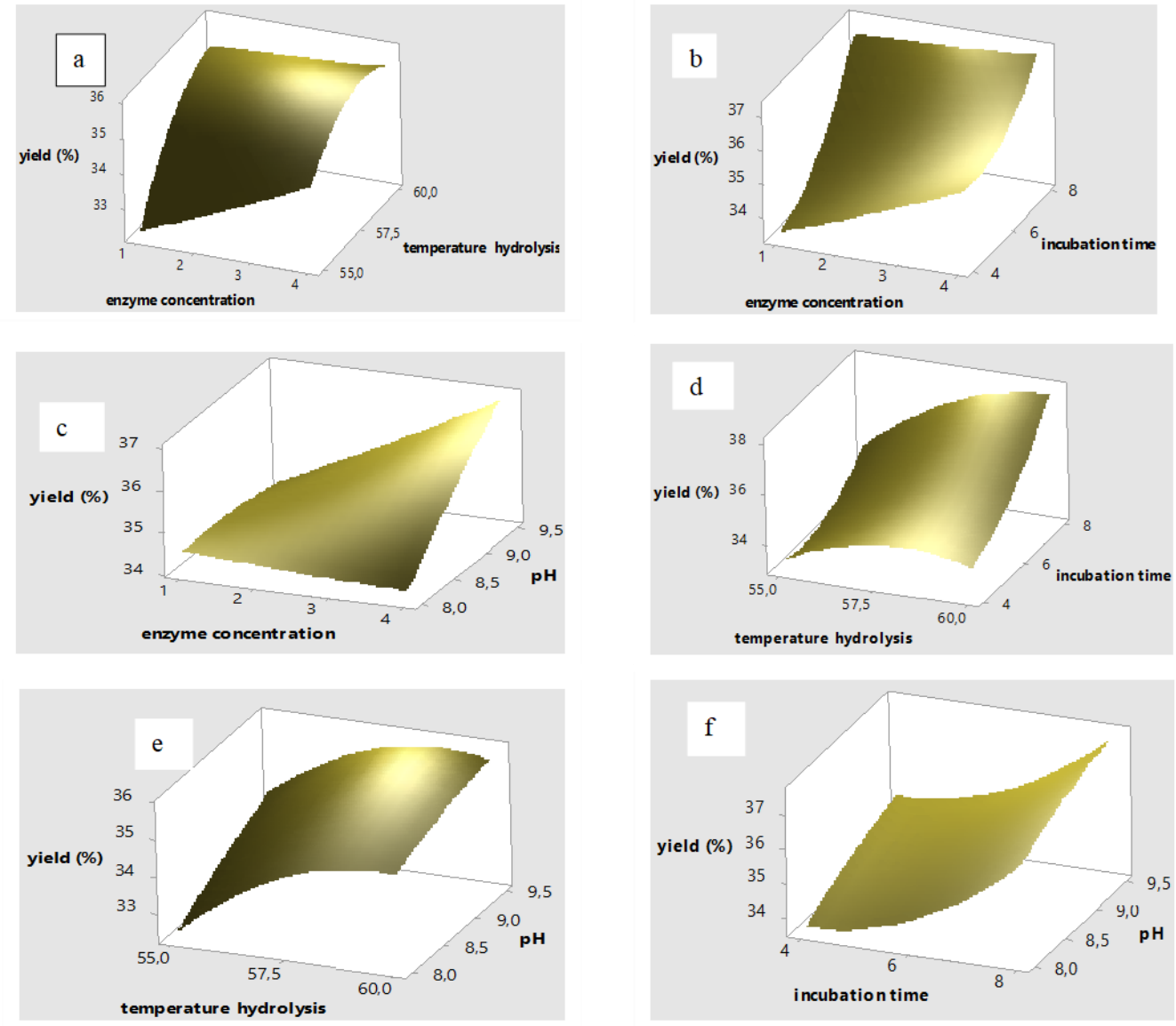

Figure 2 Response surface showing the effects of four variables (Enzyme concentration, Hydrolysis temperature, Hydrolysis Time, and pH) on cashew nut oil yield. a: Enzyme and Temperature dependence in yield, b: Enzyme and hydrolysis Time dependence in yield, c: Enzyme and hydrolysis Time dependence in yield, e: Temperature and hydrolysis time dependence in yield, d: Temperature and hydrolysis Time dependence in yield, f: pH and hydrolysis Time dependence in yield.

5.4. Extraction efficiency by solvent and enzymatic methods

Table 5 shows the yield of the solvent and enzymatic extractions. Enzymatic extraction using alcalase provided a yield of 38.20 % compared to 45 % for solvent extraction, A relative yield of approximately 85 % compared to solvent extraction. Unlike mechanical extraction observed in the literature, this technique is therefore very effective. According to Silué et al. [28] the relative efficiency of mechanical extraction of cashew oil was approximately 60 % compared to solvent extraction.

Table 5 Comparative study of enzymatic and solvent extraction yields

Methods	Enzyme extraction	Solvent extraction
Yield (%)	38.20	45.17
Yield recovery (%)	85	≥95

6. Conclusion

The present study shows the optimization of the extraction yield of cashew kernel oil by the enzymatic method according to the Box-Behnken design. This extraction method using alcalase 2.4 L was found to be better for oil recovery. The experimental oil yield under the optimal conditions obtained was 38.20 % compared to 38.04 % expected with an enzyme concentration (2.5 %), a hydrolysis temperature of 60 °C, a hydrolysis time of 8 hours and a pH of 8.75 with a constant material ratio. with water (1:5). In fact, this process could prove to be an environmentally friendly alternative.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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