Optimization of Crude Gum Extraction from Cashew Tree (*Anacardium occidentale* L.) Exudates by Response Surface Methodology Using Box-Behnken Design

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Abstract: - *Response surface methodology using a Box-Behnken Design (BBD) was used to optimize the extraction conditions of crude gum from cashew tree (Anacardium occidentale) exudates. The impact of four independent variables, extracting temperature, agitation speed, water to exudates ratio and extracting time in the process were investigated for their individual, interactive and quadratic effects. Response surface methodology (RSM), based on a three level, four factor Box-Behnken design (BBD), was employed to obtain the best possible combination of extracting temperature (X_1:30-90 °C), agitation speed (X_2:0-300 rpm), water to exudates ratio (X_3:2-6 mL/g) and extracting time (X_4:20-40min)for maximum crude gum extraction yield.The experimental data were fitted to a second-order polynomial equation via multiple regression analysis and also examined with the appropriate statistical methods (ANOVA). The coefficient of determination (R^2) and the adjusted coefficient of determination (Adjusted-R^2) for the model was 0.9409 and 0.8682 respectively. An optimum CCG extraction yield was obtained when the extracting temperature of 75 °C, agitation speed of 225 rpm, water to exudates ratio of 6 mL/g and extracting time of 35 min. Under these conditions, validation experiments were performed, and the mean extraction yield of polysaccharides was 90.76 %, which is well in close agreement with the value predicted by the model 91.03%.*

Key Words: ***Anacardium occidentale*, Box-Behnken Design, crude gum extraction, Optimization, RSM**

1. INTRODUCTION

The cashew tree (*Anacardium occidentale* L) is native to Brazil and grows in many tropical and sub-tropical countries [1]. It is a multipurpose tree crop with great economic importance to third world countries including Benin Republic, Brazil, Côte d'Ivoire, Guinea Bissau, Ghana, India, Mozambique, Nigeria, Philippines, Sri Lanka, Tanzania and Vietnam [2]. The cashew plant is mainly known for its nuts, which are used as food ingredients, but the fruit can also be used in the making of cashew wine. Cashew gum extraction represents one more source of revenue for the producer, in addition to the cashew nut and the peduncle, as well as an alternative for the utilization of the non productive cashew trees in the face of decline or senescence. It is a polysaccharide that is obtained through the exudates. Indeed, the cashew exudates are obtained after mechanical injury of the plant by incision of the bark. Besides, cashew gum is one such versatile naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry [3,4]. It has been used successfully for many years in the food and beverage industry as a thickening agent, a gelling agent and a colloidal stabiliser. Recently the role of these gums in enveloping controlled drug delivery systems has increased significantly and cashew gum is gaining lot of attraction towards this application [5]. There has been great interest in botanical sources of natural gums because plant polysaccharide gums represent one the most abundant raw materials in commercial liquid and semisolid foods [6]. Extraction process is one of the most widely used unit operations in food industry [7]. Generally, hot-water treatment has been used for extraction of hydrocolloid gums [8,9]. Several studies are reported on various gums and the extracting conditions which give the optimal yield varied from one plant species to another [10,11]. Otherwise, hydrocolloids extraction process is influenced by a number of variables, such as extraction temperature, solvent to solid ratio, solvent pH, extraction time, particle size, etc [11-13]. For the crude gum extraction from A. occidentale exudates, [14] showed that the extracting temperature, agitation speed, water to exudates ratio and the extracting time factors were found to be significant in the aqueous extraction process. Therefore, it is important to find the optimum level for each factor in order to obtain the highest yield of crude cashew gum. Little attention has been given to the optimization of the extraction process of the crude cashew gum from A. occidentale exudates. Several studies were carried out on the effects of the different extraction conditions on the gum yield from different sources [15-17] and some of them have used response surface methodology (RSM) to determine the optimum conditions [10,13]. RSM is an alternate strategy involving statistical approach compared to OVAT (one variable at a time), which could represent the effect of interaction between different factors [18]. The main advantages of RSM are the reduced numbers of experimental trials needed to evaluate multiple parameters and their interactions [13,18-19]. It is useful for developing, improving, and optimizing process. This methodology could be used in developing suitable treatment technology considering the effects of operational conditions on the removal process or to determine a region that satisfies the operating specifications [20-21].

Thus, the purpose of this survey was to optimize the process for extraction of crude gum from the cashew tree (*Anacardium occidentale* L.), using response surface methodology (RSM), employing a Box-Behnken Design (4 factors and 3 levels) to study the effects of extracting temperature, agitation speed, water to exudates ratio and extracting time factors on the extraction yield of crude cashew gum (CCG).

2. MATERIAL AND METHODS

2.1 Materials

Cashew tree (*Anacardium occidentale* L.) exudates were collected from cashew tree plantation at Korhogo in the Poro region located in northern Côte d'Ivoire. They were obtained after mechanical injury of the plant by incision with knifes on tree trunks previously identified for the quality production of nuts and cashew apple. The harvest of cashew exudates was carried out 21 days after incision. However, the experiments were carried out during February 2016 to March 2016.

2.2 Samples preparation

The cashew exudates were cleaned by removing the bark and other extraneous materials by hand and dried in a hot air oven at 50°C for about 8 h until it became sufficiently brittle. The dried cashew exudates were manually sorted into light coloured and dark coloured grades. The light coloured grade was selected for further processing by milling in a domestic blender into fine powder and sieved. The obtained samples were packaged and stored at room temperature (28 ± 1°C) prior to extraction.

2.3 Extraction procedure

The extraction of the crude gum from cashew tree (*Anacardium occidentale* L.) exudates powders was carried out according to the applied method by [14]. Briefly, the dried and milled (average particle size range from 0.5 mm to 1 mm) cashew tree exudates powder (40 g) were stirred in distilled water (water to the raw material ratio ranging from 2 to 6) at pH 7 (adjusting the suspension pH by 0.1 M NaOH solution), while the temperature of the water bath ranged from 30 to 90±1.0 °C. Water was preheated to a designated temperature before the exudates powder was added. The CTE powder-water slurry was stirred with an electric mixing paddle throughout the entire extraction period (20 to 40 min) with the agitation rate ranging from 0 to 300 rpm. The homogeneous suspension obtained, was centrifuged at 4000 g for 20 min. The supernatant was concentrated, freeze-dried, stored in vacuum oven at 40 °C and weighed to get the crude cashew gum (CCG). The percentage gum extraction yield (%) is calculated as follows (1):

$$Y(\%) = \frac{M_1}{M_0} \times 100 \quad (1)$$

Where Y (%) is the crude cashew gum extraction yield; M0 is the exudates powder weight (g) and; M1 is the lyophilized gum weight (g).

Table -1: Process variables and their levels (coded and uncoded)

Independent variables		Levels		
		-1	**0**	**1**
Extracting temperature (°C)	X_1	30	60	90
Agitation speed (rpm)	X_2	0	150	300
Water-Exudates Ratio (mL/g)	X_3	2	4	6
Extracting time (min)	X_4	20	30	40

2.4 Experimental design

Response surface methodology (RSM) was used to estimate the effect of Extracting temperature, Agitation speed, Water to exudates Ratio and Extracting time factors on the extraction yield of CCG. A three-level Box-Behken Design (BBD) was employed for designing the experimental data [22]. The independent variables and their ranges, which were chosen based on our preliminary experiment results [14] are presented in Table 1. In this study, experiments, which consisted of 27 trials, were randomized and blocked to minimize the effects of unexplained variability in the observed responses due to extraneous factors [23]. The generalized regression second-order polynomial model proposed for predicting the response variables is given as (2) [24]:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_{ii}^2 + \sum \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response (Crude Cashew Gum extraction yield); β0 is the intercept term; βi (i=1, 2, 3 and 4)is linear coefficients; βij (i=1, 2, 3 and 4; j=1, 2, 3 and 4) is interaction coefficients; βii (i=1, 2, 3 and 4) is the quadratic coefficient, and Xi (i=1, 2, 3 and 4) and Xj (j=1, 2, 3 and 4) are the coded factors computed by (3):

$$X_i = \frac{(x_i - x_0)}{\Delta x_i} \quad (3)$$

Where Xi is the coded value of the variable, xi is the independent variable real value, x0 is the independent variable real value at the center point, and Δxi is the step length. In this model, Xi and Xj are independent variables; β0, βi, βii andβij are the different coefficients of the model obtained using polynomial regression.

2.5 Statistical analysis

Statistical analysis was performed using the software STATISTICA (version 7.0) for the experimental design and regression analysis of the experimental data. The analysis of variance (ANOVA) was carried out to show the effects of each variable and their interactions on the extraction yield of crude cashew gum (CCG). This analysis included Fisher's F-test (overall model significance), it's associated probability p-value, determination coefficient (R^2) and adjusted determination coefficient (adj-R^2) which measure the quality of fit of regression model. The significance of the regression coefficients was tested by a t-test. A probability p-value for a given factor less than 0.05 was considered as significant. Otherwise, the correlation between the response and independent variables can be readily seen in the response surface plots (3D) and contour plots (2D), which were generated using STATISTICA software (Version 7.1. Statsoft, USA). These plots showed the simultaneous interaction of two factors on the responses and find the location of optimum experimental variables.

Table 2: Box–Behnken design (coded and uncoded) and results (observed and predicted values) for extraction yield of CCG.

Runs	Blocks	Independent variables				Experimental value (%)	Predicted value (%)	Deviation
		X_1	X_2	X_3	X_4			
1	1	-1 (30)	-1 (0)	0 (4)	0 (30)	61.55	58.76	2.79
2	1	1 (90)	-1 (0)	0 (4)	0 (30)	65.09	69.82	-4.73
3	1	-1 (30)	1 (300)	0 (4)	0 (30)	63.26	61.09	2.17
4	1	1 (90)	1 (300)	0 (4)	0 (30)	65.37	70.72	-5.35
5	1	0 (60)	0 (150)	-1 (2)	-1 (20)	58.72	61.61	-2.89
6	1	0 (60)	0 (150)	1 (6)	-1 (20)	88.33	88.91	-0,58
7	1	0 (60)	0 (150)	-1 (2)	1 (40)	59.88	61.86	-1.98
8	1	0 (60)	0 (150)	1 (6)	1 (40)	89.81	89.49**	0.34
9 (C)	1	0 (60)	0 (150)	0 (4)	0 (30)	81.57	81.56	0.01
10	2	-1 (30)	0 (150)	0 (4)	-1 (20)	62.14	58.67	3.47
11	2	1 (90)	0 (150)	0 (4)	-1 (20)	81.03	76.32	4.71
12	2	-1 (30)	0 (150)	0 (4)	1 (40)	62.01	66.38	-4.37
13	2	1 (90)	0 (150)	0 (4)	1 (40)	66.28	69.42	-3.14
14	2	0 (60)	-1 (0)	-1 (2)	0 (30)	56.65	58.11	-1.46
15	2	0 (60)	1 (300)	-1 (2)	0 (30)	59.22	60.15	-0.93
16	2	0 (60)	-1 (0)	1 (6)	0 (30)	87.25	85.99	1.26
17	2	0 (60)	1 (300)	1 (6)	0 (30)	88.98	87.18	1,80
18 (C)	2	0 (60)	0 (150)	0 (4)	0 (30)	80.25	81.56	-1.31
19	3	-1 (30)	0 (150)	-1 (2)	0 (30)	51.22*	50.73*	0.49
20	3	1 (90)	0 (150)	-1 (2)	0 (30)	56.19	49.42	6.77
21	3	-1 (30)	0 (150)	1 (6)	0 (30)	61.97	66.53	-4.56
22	3	1 (90)	0 (150)	1 (6)	0 (30)	90.26**	88.53	1.73
23	3	0 (60)	-1 (0)	0 (4)	-1 (20)	74.13	76.53	-2.40
24	3	0 (60)	1 (300)	0 (4)	-1 (20)	74.27	76.58	-2.31
25	3	0 (60)	-1 (0)	0 (4)	1 (40)	79.89	75.36	4.53
26	3	0 (60)	1 (300)	0 (4)	1 (40)	83.16	78.55	4.61
27 (C)	3	0 (60)	0 (150)	0 (4)	0 (30)	82.85	81.56	1.29

(C): center point; * Minimum value; ** Maximum value

3. RESULTS AND DISCUSSION

Statistical designs are effective tools that can be used to account for the main as well as the interactive influences of extraction parameters on the process performance. Among them, response surface methodology (RSM) is a collection of certain statistical techniques for designing experiments, building models, evaluating the effect of the factors and searching for optimal conditions for desirable responses [23]. In this study, Box–Behnken Design was employed to investigate the interactions among the selected factors (Extracting temperature, Agitation speed, Water-exudates Ratio and Extracting time) on the extraction yield of CCG and to establish their optimum levels for maximum crude cashew gum production. The experimental and predicted values for response (CCG) under different combination of extraction conditions were given in Table 2. The results indicated that there was a variation of total cashew gum yield in the twenty-seven trials (51.22 to 90.26%) (Table 2). These variations reflected the importance and influence of the studies factors on the production of gum to obtain the higher yield [16]. Otherwise, the predicted values (50.73 to 89.49%) are close to those of the experimental values.

3.1 Optimization of the extraction conditions

Exploration of the response surfaces is a relevant aspect in optimization studies using experimental design. Thus, to illustrate the correlations between the independent and dependent variables, three-dimensional (3D) and two-dimensional (2D) plots were used to represent the response in function [23]. Therefore, contour plot shapes indicated whether the mutual interactions between the variables are significant or not [25]. The significance of the interactions between the corresponding variables was indicated by an elliptical or circular nature of contour plots [26]. The circular contour plots meansthe interactions between the corresponding variables are negligible, while elliptical contour plot suggests the interactions between the corresponding variables are significant [25]. Besides, these plots revealed the optimum conditions for maximizing or minimizing the response.In this study, the response surfaces (3D) and contours plots (2D), which represented the mutual interaction effect of the selected factors (Extracting temperature, Agitation speed, Water-exudates Ratio and Extracting time) on the crude cashew gum extraction yield, are shown in Fig. 1 and Fig. 2 respectively.

Thus, the response surface (Fig. 1a) and the contour plot (Fig. 2a), representing CCG extraction yield as a function of extraction temperature (X_1) and agitation speed (X_2) with the water to exudate ratio (X_3) (4 mL/g) and extracting time (X_4) (30 min)(level 0), showed that CCG extraction yield increased when the extracting temperature (X_1) and the agitation speed (X_2) increased from 30 to 75 °C and from 0 to 250 rpm respectively, but beyond 75 °C and 250 rpm, extraction yield decreased. Furthermore, the maximum extraction efficiency of the cashew gum was obtained for an extracting temperature (X_1) between 55-75 °C and the agitation speed of between 75-250 rpm. The mutual interaction effect of extracting temperature (X_1) and water to exudate ratio (X_3) on CCG extraction yield with the extracting time (X_4) and the agitation speed (X_2) fixed at 30 min and 150 rpm respectively, is also illustrated by the 3D (Fig. 1b) and 2D (Fig. 2b) graphical representations. The analysis of Fig. 1b showed that the crude CCG extraction yield increased rapidly when extracting temperature (X_1) and the water to exudate ratio (X_3) increased in the range of 60-85 °C and 5-6 mL/g respectively; but beyond 85°C and 6mL, extraction yield decreased slightly. This demonstrates that the effect of extracting temperature (X_1) and water to exudate ratio (X_3) on CCG extraction yield is significant. Moreover, the elliptical contour plots in Figure 2b mean that there is a significant interaction between the two variables. As shown in Fig. 1c, CCG extraction yield according to extracting temperature and extracting time with the water to exudates ratio (X_3) and agitation speed (X_2) fixed to 4 mL/g and 150 rpm (level 0) respectively, increased with extracting temperature and extracting time increasing, and reached the peak value rapidly at extracting temperature varying from 60°C to 78 °C. It can be seen that the mutual interactions between extracting temperature (X_1) and extracting time (X_4) are significant due to the elliptical contour plots shown in Fig. 2c. From figure 1d, both agitation speed (X_2) and water to exudates ratio (X_3) had mutual interaction effect on CCG extraction yield with the extracting time and extracting temperature fixed at 30 min and 150 rpm (level 0) respectively. CCG extraction yield increased with increasing of the two parameters and a maximum extraction yield is achieved when agitation speed (X_2) and water to exudates ratio (X3) are 150 rpm and 6 mL/g, respectively. It can be seen that the mutual interactions between agitation speed (X_2) and water to exudates ratio (X_3) are significant due to the elliptical contour plots shown in Fig. 2d. Otherwise, there appeared significant mutual interactions between agitation speed (X_2) and extracting time (X_4) due to the elliptical contour plots presented in Fig. 2e. CCG extraction yield increased quickly with increasing of the both parameters agitation speed (X_2) and extracting time (X_4) to achieve a maximum of 80% with an extracting temperature (X_1) and the water to exudates ratio(X_3) fixed at 60 °C and 4 mL/g respectively (Fig. 1e). In Fig. 1f, the results showed that CCG extraction yield increased, when both water to exudates ratio (X_3) and extracting time (X_4) increased with extracting temperature (X_1) and the agitation speed (X_2) fixed at 60°C and 150 rpm respectively. This confirmed in Fig. 2f by meaningful mutual interactions between water to exudates ratio and extracting time due to the elliptical contour plots. Similar effects of the process parameters on the gum extraction yield from *Qodume Shirazi* seeds, was indicated by [27].

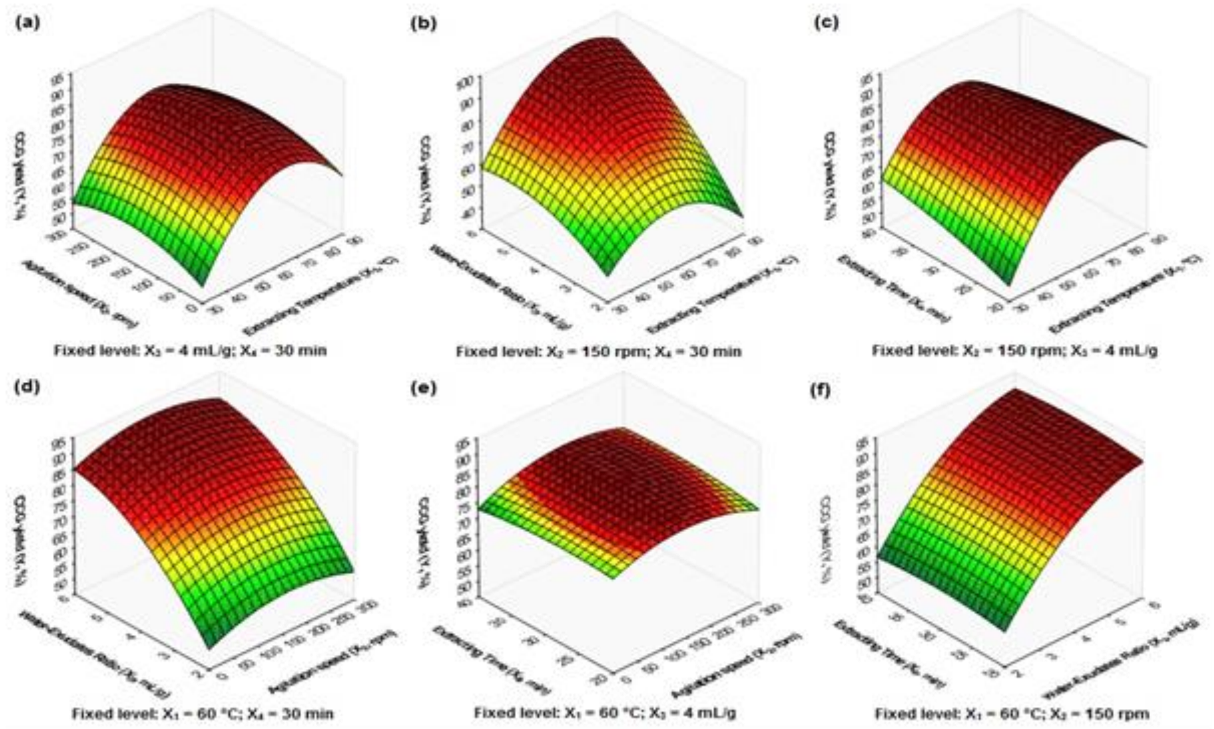

Fig -1: Response surface showing the effect of extracting temperature and agitation speed (a), extracting temperature and water to exudates ratio (b), extracting temperature and extracting time (c), agitation speed and water to exudates ratio (d), agitation speed and extracting time (e), and water to exudates ratio and extracting time (f) on the Crude Cashew Gum (CCG) extraction yield.

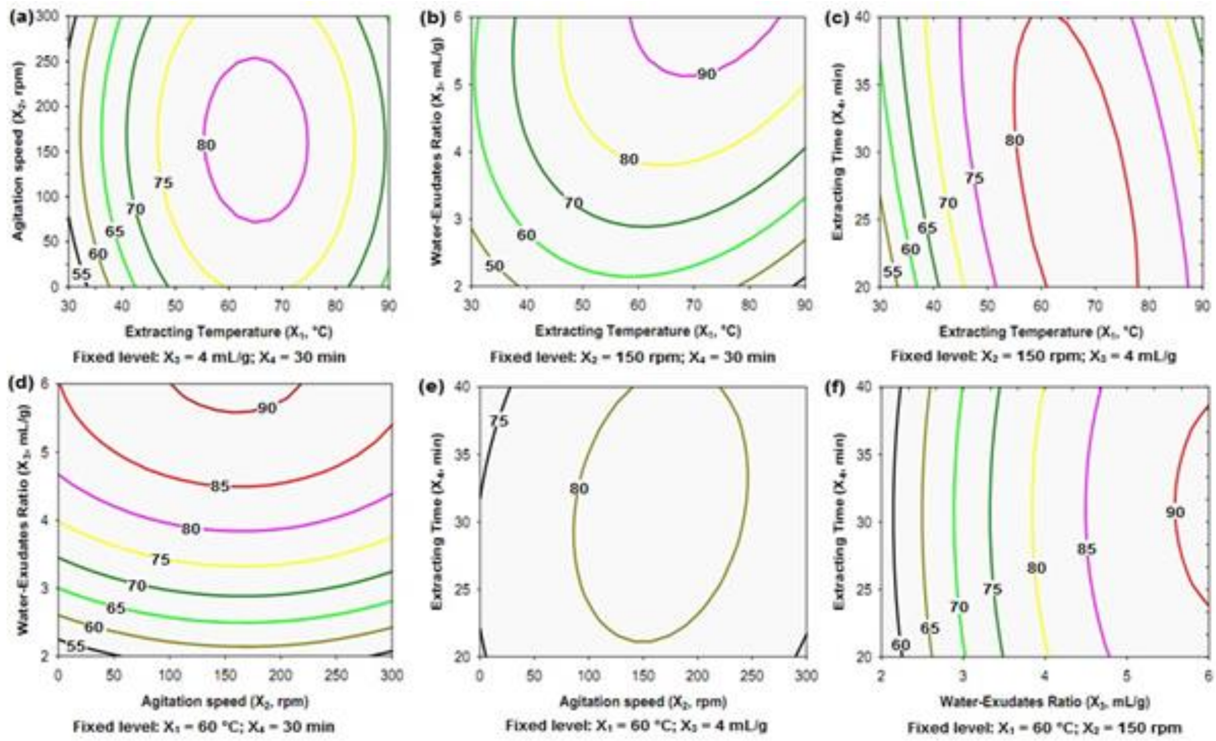

Fig -2: Contour plot showing the effect of extracting temperature and agitation speed (a), extracting temperature and water to exudates ratio (b), extracting temperature and extracting time (c), agitation speed and water to exudates ratio (d), agitation speed and extracting time (e), and water to exudates ratio and extracting time (f) on the Crude Cashew Gum (CCG) extraction yield.

Table -3: Regression coefficients of polynomial function of response surface describing Crude Cashew Gum yield.

Terms	Coeff	SE	*t*-test	p-value
β_0	81,56	0,751	108,6575	<0,0001 (**)
β_1	5,17	0,375	13,7826	0,005223 (*)
β_2	0,81	0,375	2,1539	0,164083 (ns)
β_3	13,73	0,375	36,5759	0,000747 (**)
β_4	0,20	0,375	0,5351	0,646090 (ns)
β_{11}	-12,76	0,563	-22,6660	0,001941 (*)
β_{22}	-3,70	0,563	-6,5786	0,022335 (*)
β_{33}	-4,99	0,563	-8,8746	0,012460 (*)
β_{44}	-1,10	0,563	-1,9533	0,190011 (ns)
β_{12}	-0,36	0,65	-0,5500	0,637550 (ns)
β_{13}	5,83	0,65	8,9689	0,012204 (*)
β_{14}	-3,66	0,65	-5,6229	0,030203 (*)
β_{23}	-0,21	0,65	-0,3231	0,777296 (ns)
β_{24}	0,78	0,65	1,2038	0,351815 (ns)
β_{34}	0,08	0,65	0,1231	0,913303 (ns)

Coeff: regression coefficient; SE: standard error; *t*.exp: Student's *t*-test value; p-value: probability value; (**): highly significant (p≤0.001); (*): slightly significant (0.001<p≤0.05); (ns): not significant (p>0.05).

3.2 Model development

For the model development, the multiple regression analysis was used to estimate the regression coefficients of the second-order polynomial equation. The values of the regression coefficients obtained and recorded in Table 3, allowed us to write an initial model (in terms of the coded levels) expressing the crude cashew gum extraction yield (Y) as a function of the independent variables as follows (4):

$$\begin{aligned} Y &= 81.56 + 5.17X_1 + 0.81X_2 + 13.73X_3 \\ &+ 0.2X_4 - 12.76X_1^2 - 3.7X_2^2 - 4.99X_3^2 \\ &- 1.1X_4^2 - 0.36X_1X_2 + 5.83X_1X_3 \\ &- 3.66X_1X_4 - 0.21X_2X_3 \\ &+ 0.78X_2X_4 + 0.08X_3X_4 \end{aligned} \quad (4)$$

Where X_1, X_2, X_3 and X_4 are coded values of Extracting temperature (°C), Agitation speed (rpm), Water-exudates ratio (mL/g) and Extracting time (min), respectively.

The p-values were then used as a tool to check the importance of each regression coefficient at 0.05% confidence level. Thus, for the smallest value of P (P≤0.05), the corresponding regression coefficient is significant [13]. In this case (Table 3), it could be concluded that the quadratic terms (X_1^2, X_2^2 and X_3^2); two linear terms for the extracting temperature (X_1), and the water to exudates ratio (X_3); and the interaction between extracting temperature and water to exudates ratio (X_1X_3) and extracting temperature and extracting time (X_1X_4) were significant (P≤0.05) for CCG extraction yield (Table 3). By considering the significant factors and including the linear effects of non-significant factors (P> 0.05), whose interaction with another factor is statistically significant; the CCG extraction could be predicted by the following equation (5):

$$\begin{aligned} Y &= 81.56 + 5.17X_1 + 13.73X_3 + 0.2X_4 \\ &- 12.76X_1^2 - 3.7X_2^2 - 4.99X_3^2 \\ &+ 5.83X_1X_3 - 3.66X_1X_4 \end{aligned} \quad (5)$$

3.3 Model fitting

The statistical significance of the mean square ratio variation due to regression and residual errors of the mean square were evaluated using the ANOVA technique. ANOVA describes a statistical algorithm that subdivides the total variation in a set of data into element items relating to sources of variation for testing hypotheses on the parameters of the model [23,28]. The analysis of variance (ANOVA) which was tested using Fisher's statistical analysis was presented in Table 4. The model fitted the experimental data well when the determination coefficient (R^2) and the adjusted determination coefficient (adj-R^2) approach unity [28-29]. For this model fitted, the determination coefficient (R^2) was 0.9317, which showed that only 6.83% of the total variations were not explained by the model (Table 4). The model *p*-value (p<0.05) was very low (<0.0001), suggesting the model was significant. The value of the adjusted determination coefficient (adj-R^2 = 0.8520) also confirmed that the model was highly significant (Table 4). Earlier studies have reported similar values of R^2= 0.9372 and adj-R^2= 0.9040, for pectin from *Abelmoschus esculentus* [30] and of R^2=0.9893 and adj-R^2=0.9755, for polysaccharides extraction from *Tricholoma mongolicum* [31]. Otherwise, the F-value and P-value for the lack of fit were 15.966 and 0.0604, respectively (Table 4). The result showed that the lack of fit was insignificant (p>0.05), which was an indication of the validity of the model. Indeed, the significance of the model was also judged by a lack-of-fit test. A model will be well-fitted to the experimental data if it presents a non-significant lack of fit [23,28].

Table 4 Analysis of variance for the fitted model of CCG extraction.

Source	DF	SS	MS	F-value	p-value
Model	14	3726.001	266.143	11.689	<0,0001 **(*)**
Residual	12	273.230	22.769		
Lack of Fit	10	269.850	26.985	15.966	0.0604 **(ns)**
Pure error	2	3.380	1.690		
Total	26	3999.231			
R^2 = 0.9317			**adj-R^2 = 0.8520**		

SS: Sum of square; DF: Degree of freedom; MS: Mean square; (*): significant p≤0.05); (ns): not significant (p>0.05).

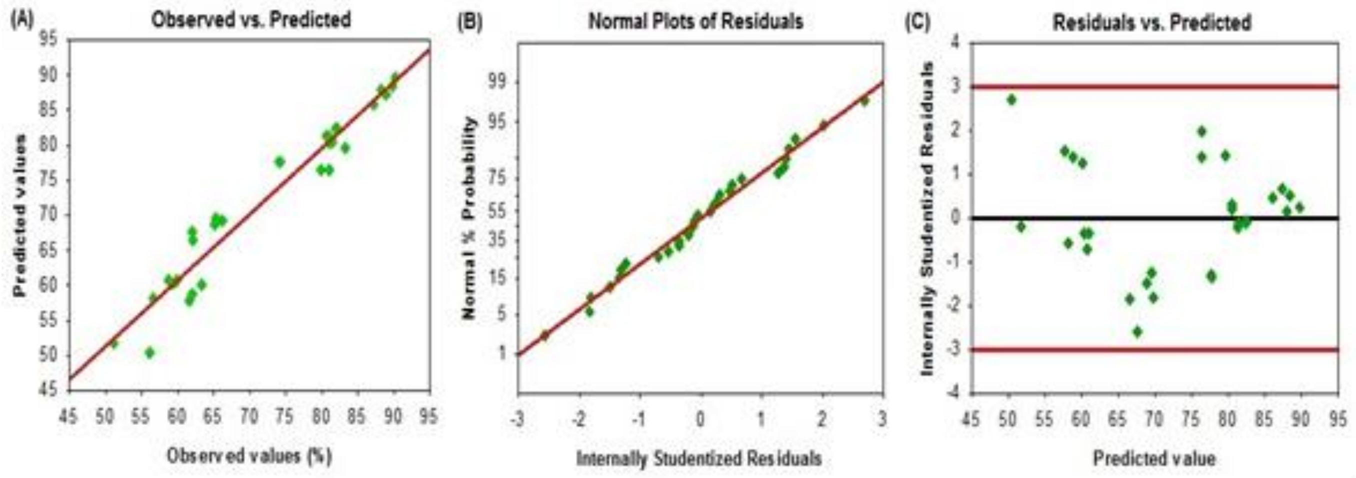

Fig -3: (A) Predicted response versus actual value, (B) Normal probability of internally studentized residuals and (C) Plot of internally studentized residuals vs. predicted response for model adequacy checking.

Moreover, these values showed that the proposed mathematical model could be used to predict the yield of CCG according to the extraction conditions investigated. The obtained results indicated a high degree of correlation between the experimental and predicted values of CCG extraction yield values by the regression equation.

3.4 Diagnostics of model adequacy

It is usually necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface is likely to give poor or misleading results [23]. In Fig. 3, the study showed that the polynomial regression model (Eq. (4)) agreed with the experimental results. There didn't appear significant difference (P>0.05) between experimental and predicted values of CCG extraction yield (Fig. 3A). By constructing a normal probability plot of the residuals, a check was made for the normality assumption. As shown in Fig. 3B, the residual plot approximates along a straight line, which is an indication that the normality assumption is satisfied. In addition, Fig. 3C presents a plot of residuals vs. the predicted response. The residuals scatter randomly at around ±1.5, suggesting a fit of the experimental data with the predicted ones (obtained from the model). Both plots (Fig. 3A-C) are satisfactory, so we securely conclude that the empirical model is adequate to describe CCG yield by response surface. The results suggest that the used model in this research was able to identify operating conditions for extraction of crude gum from cashew tree (*Anacardium occidentale* L.) exudates.

3.5 Determination of optimum extraction conditions

Optimum extraction conditions were estimated by the desirability method using a Statistica Software. An extracting temperature of 75 °C, agitation speed of 225 rpm, water to exudates ratio of 6 mL/g and extracting time of 35 min, were found to be optimal for crude gum extraction from cashew tree exudates. In order to validate the adequacy of the model (5), a verification experiment was carried out under the optimal conditions (within the experimental range): extracting temperature of 75 °C, agitation speed of 225 rpm, water to exudates ratio of 6 mL/g and extracting time of 35 min. Under these conditions, the model predicted a maximum CCG yield of 91,03%, while a mean value of 90,76 ± 0,13% (n = 3) was obtained from real experiments (Table 5).

Table -5: Predicted and experimental values of the responses at optimum conditions.

Indépendant variables	Optimum levels	Extraction yields (Y, %)	
		Experimental	Predicted
Temperature (X_1, °C)	75	90,76 ± 0,13[a]	91,03
Agitation speed (X_2, rpm)	225		
Water to exudates ratio (X_3, mL/g)	6		
Extracting time (X_4, min)	35		

Mean ± standard deviation (n=3).

The validation result revealed that there was not meaningful difference set of mean between experimental and predicted values, suggesting that the response model was adequate for reflecting the expected optimization. This result of analysis indicated that the experimental values were in agreement with the predicted ones, and also suggested that the model (5) is satisfactory and accurate. Studies of the extraction process of crude cashew gum from *Anacardium occidentale* exudates could not be traced, but similar studies using other materials have been reported. [10] studied the extraction of polysaccharide gum from flaxseed (*Linum usitatissimum* L.) and concluded that the maximum flaxseed gum yield was obtained by extracting whole seed with a temperature of 85-90 °C, pH of 6.5-7.0 and water/seed ratio of 13:1. Furthermore, [32] worked on polysaccharide extraction from Lingzhi (*Ganoderma lucidum*) and found significant effects of extraction temperature and concluded that optimum conditions were: extraction temperature of 95 °C, extraction time of 3 h, and solvent/meal ratio of 12:1 (v/w). Besides, [13] studied also the crude polysaccharide extraction from the Iranian *Abelmoschus esculentus* using the central composite rotatable design (CCRD) and concluded that the maximum crude polysaccharide yield was obtained at the extraction time of 4.94 h, the extraction temperature of 94.97 °C, the number of extraction cycle of 4 and water to raw material ratio of 21.74. [19] optimized the extraction process of polysaccharides gum from Lemongrass (*Cymbopogon citratus)* using the central composite rotatable design (CCRD) and obtained 13.24 ± 0.23% as maximum gum yield at the optimum extraction conditions were as follows: extraction time was around 113.81 min, extraction temperature at 99.66 °C and the ratio of water to raw material was 33.11 g/mL.

4. CONCLUSION

The RSM using Box-Behnken Design for optimizing the bioprocess variables for crude cashew gum extraction from *Anacardium occidentale* exudates, is an effective and reliable tool to finding the optimal level of those factors. This study showed that the effects of linear terms (X_1 and X_3), interaction terms (X_1X_3 and X_1X_4) and quadratics terms (X_1^2, X_2^2 and X_3^2) were significant and quadratic models were obtained for predicting the crude cashew gum yield. Also, this work revealed that the maximum of CCG yield (90.76 %) was obtained at the extracting temperature of 75 °C, agitation speed of 225 rpm, water to exudates ratio of 6 mL/g and extracting time of 35 min. This servey showed that the CCG can be used as a potential, low cost source of hydrocolloid.

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