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Application of Statistical Experiment Design for Pre-optimization of Crude Gum from Cashew Tree (*Anacardium occidentale* L.) Exudates by Aqueous Extraction

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***Abstract* — A Plackett Burman design (PBD) was used to pre-optimize the yield for cashew gum from *Anacardium occidentale* exudates by aqueous extraction. Various factors influencing cashew gum production were screened using the PBD. These factors included extracting temperature, agitation speed, water exudates ratio, extracting time, pH and average particle size. In this study, the analysis of variance (ANOVA) of our findings revealed that extracting temperature, agitation speed, water exudates ratio and extracting time found to be significant effects ($p \leq 0.05$) on cashew gum production. On the other hand, the average particle size had insignificant ($p > 0.05$) effect and slight effect for pH factor. The established model from ANOVA analysis observed to be meaningful ($p \leq 0.05$) and a coefficient of determination (R^2) value of 0.992.**

***Keywords* — *Anacardium Occidentale*, Cashew Tree Gum, Plackett-Burman Design, Yield.**

I. Introduction

Cashew tree (*Anacardium occidentale*) is native toBrazil and grows in many tropical and sub-tropical countries such as Côte d'Ivoire where its culture has a tremendous social-economical importance especially in the North region [1], [2]. Cashew gum 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. They become hard nodules or ribbons on dehydration to form a protective sheath against microorganism [3]. The exudates form clear glassy masses, which are usually colored from dark brown to pale yellow. However, Cashew gums have been found to have many lucrative possibilities for industrialization [4]. They are highly hydrophilic substances that are soluble or dispersible in water [5]. The major application of gums is in food industry where emulsifying and stabilizing properties are utilized. The gums are also used in the pharmaceutical and medical fields, in addition to other industries (cosmetic, adhesive, paints and inks) [6]. 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 [7].

Cashew gum has been studied widely for various pharmaceutical applications as it is inexpensive, non-toxic, biodegradableand possesses appropriate physicochemical characteristics [4], [8]. 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 [7]. Extraction process is one of the most widely used unit operations in food industry [9]. Generally, hot-water treatment has been used for extraction of hydrocolloid gums [10], [11]. Several studies are reported on various gums and the extracting conditions which give the optimal yield varied from one plant species to another [12], [13]. 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 [13]-[15]. Therefore, It is important to optimize the extraction process in order to obtain the highest yield or/and polysaccharides content to investigate the effect of the different extraction conditions on the gum obtained from different sources [16], [17]. However, little attention has given to the optimization of the extraction process of the crude cashew gum from *A. occidentale* exudates.

Statistical methodologies such as Plackett-Burman Design [18] have shown to be efficient and effective approach to systematic investigation on the target factors. The Plackett-Burman Design (PBD) is an effective screening design which considerably diminishes the number of experiment, time invested and reduce experimental costs and gives information for the evaluation of the target factors as much as possible [15]. Only the most effective factors with positive significance are selected for further optimization. The less significance or high negative effect on response value would be omitted for further experiments [18]. PBD has been widely applied in many fields such as medium optimization, formulation of multi component and so on [19], [20].

The aim of this study was to apply PBD to select the key factors that significantly influenced the yield of crude gum from cashew (*Anacardium occidentale*) exudates by aqueous extraction.

II. Materiel and Methods

A. Materials

Cashew 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.

B. Sample Preparation

The cashew exudates was 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.

C. Aqueous-Extraction Procedure

The flowchart of extraction process of crude cashew gum (CCG) from cashew tree exudates is represented in **Fig. 1**. The dried crude and milled cashew tree exudates powder(CTEP) (40 g) were stirred in distilled water (water to the raw material ratio ranging from 2:1 to 6:1, v/p) at pH 4 to 10. The pH was adjusted with 0.1M HCl or NaOH, while the temperature of the adjustable water bath ranged from 30 to 90°C.Water was preheated to a designated temperature before the CTEP was added.The mixture was stirred with magnetic stirrer at temperatures varying from 30 to 90 °C with the agitation rate ranging from 0 to 300 rpm. The obtainedhomogeneous suspension was centrifuged at 4000 x g for 20 min. The supernatant was concentrated in vacuum oven at 40 °C, lyophilized and weighed. The obtained dry cashew gum was packed and stored at dry conditions.

D. Experimental Design

The present study was aimed at screening the important extraction parameters with respect to their main effects by Plackett-Burman design [18].

The Plackett-Burman experimental design is a two factorial design, which identifies the critical physicochemical parameters required for elevated crude cashew gum yield by screening n variables in n + 1 experiments.

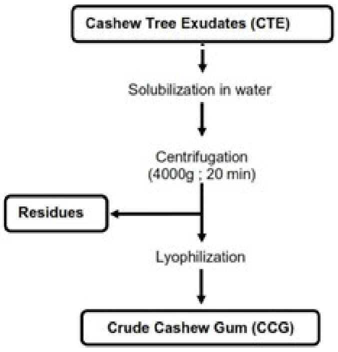

Fig. 1. Flow chart of the unit operations for Crude Cashew Gum (CCG) extraction from Cashew Tree Exudates (CTE)

The selected variables with their levels and the experimental design matrix were shown in the **Table I** and **Table II** respectively. All the variables were denoted as numerical factors and investigated at two widely spaced intervals designated as -1 (low level) and +1 (high level).

The PBD experimental design based on the first order polynomial model with no interaction effect was represented by (1):

$$Y = \beta_0 + \sum_{i=1}^{6} \beta_i X_i \quad (1)$$

Where **Y** is the response or dependent variable, $\boldsymbol{\beta_0}$ is the model intercept, **Xi** is the independent variable, and $\boldsymbol{\beta_i}$ is the linear regression coefficient.

In this fractional factorial design, the main effect of each independent variable (*E*) on crude cashew gum yield was determined using the following (2):

$$E = \left(\sum M_+ - \sum M_-\right)/N \qquad (2)$$

Where E is the effect of parameter under study and M_+ and M_- are responses of trials at which the parameter was at its higher (+) and lower (-) levels respectively and N was the total number of trials.

E. Determination of the CCG Yield

The yield (Y) of the CCG at various extraction conditions was determined according to the method of [21]. It was calculated as the ratio of dry weight of the powder obtained after lyophilization to the exudates powder weight and expressed as follows (3)**:**

$$Y(\%) = \frac{\text{Lyophilized CCG weight(g)}}{\text{Exudates powder weight(g)}} \times 100 \qquad (3)$$

Table I: Independent variables and their levels for the Plackett-Burman Design

Factors	Levels	Units	Coded variables	Coded levels
Extracting temperature	30	°C	X_1	-1
	90			1
pH	4		X_2	-1
	10			1
Water-Exudates Ratio	2	mL/g	X_3	-1
	6			1
Average of particles size	0.5-1	mm	X_4	-1
	1.5-2			1
Agitation speed	0	rpm	X_5	-1
	300			1
Extracting time	20	min	X_6	-1
	40			1

Table II: Experimental design matrix of Plackett-Burman

Trials	Independent variables					
	X_1	X_2	X_3	X_4	X_5	X_6
1	1	1	1	-1	1	-1
2	1	1	1	-1	1	-1
3	-1	1	1	1	-1	1
4	-1	1	1	1	-1	1
5	-1	-1	1	1	1	-1
6	-1	-1	1	1	1	-1
7	1	-1	-1	1	1	1
8	1	-1	-1	1	1	1
9	-1	1	-1	-1	1	1
10	-1	1	-1	-1	1	1
11	1	-1	1	-1	-1	1
12	1	-1	1	-1	-1	1
13	1	1	-1	1	-1	-1
14	1	1	-1	1	-1	-1
15	-1	-1	-1	-1	-1	-1
16	-1	-1	-1	-1	-1	-1

F. Statistical Analysis

Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). This analysis included Fisher's F-test (overall model significance). Significance was judged by determining the probability level that the *F* statistic calculated from the data is less than 5%. Besides, the analysis is associated probability p-values, determination coefficient (R^2) and adjusted determination coefficient (adj-R^2) which measure the goodness of fit of regression model [22]. For each variable, the first order polynomial model was generated using STATISTICA software (Version 7.1. Statsoft, USA) statistical package.

III. Results

A. Screening of Significant Factors for Yield using Plackett-Burman Design

The results showed that there was a variation of total cashew gum yield in the sixteen trials (74.21 to 87.31 %) (**Table III**). These variations reflected the importance of medium optimization to obtain higher cashew gum yield. There is a close agreement between the experimental values of cashew gum yield and theoretical values predicted (74.69 to 87.50%) by PB design model equation for all the medium components (**Table III**).

Table III: Observed and predicted cashew gum yields

Trials	Experimental value (%)	Predicted value (%)	Deviation
1	87.31**	87.50**	-0.19
2	87.08	87.50**	-0.42
3	79.27	79.65	-0.38
4	79.42	79.65	-0.23
5	81.24	81.07	0.17
6	81.50	81.07	0.43
7	85.85	85.82	0.03
8	85.19	85.82	-0.63
9	81.73	81.35	0.38
10	81.58	81.35	0.23
11	86.02	85.84	0.18
12	86.27	85.84	0.43
13	79.56	79.38	0.18
14	79.81	79.38	0.43
15	74.21*	74.69*	-0.48
16	74.56	74.69*	-0.13

**Minimum value ; **Maximum value*

In **Fig. 2**, The Pareto chart was shown. Indeed, The Pareto chart offers a convenient way to view the results obtained by Plackett-Burman design and the order of significance of the variable affecting cashew gum yield. The order of significance ($p \leq 0.05$) as indicated by Pareto chart was extracting temperature (X_1), agitation speed (X_5), water-exudates ratio (X_3) and extracting time (X_6). These factors had significant effect ($p \leq 0.05$)on cashew gum yield with standardized effect of 5.45, 4.04, 32.02 and 2.51 respectively (**Fig. 2**). Besides, the X_1, X_3, X_5 and X_6 factors showed a positive sign of the effect on cashew gum yield. Their percentage contributions were 46.84%, 16.19%, 25.82% and 9.92% respectively.The pH (X_2) factor had not significant effect ($p > 0.05$) on the crude cashew gum yield with contribution and standardized effectof 0.02% and 0.11% respectively. As for the average of particle size (X_4) factor, it influenced slightly the crude cashew gum yield. Its percentage contribution was 1.18%.In addition, the average of particle size having -0.86 of standardized effect had also a negative effect on cashew gum yield (**Fig. 3**).

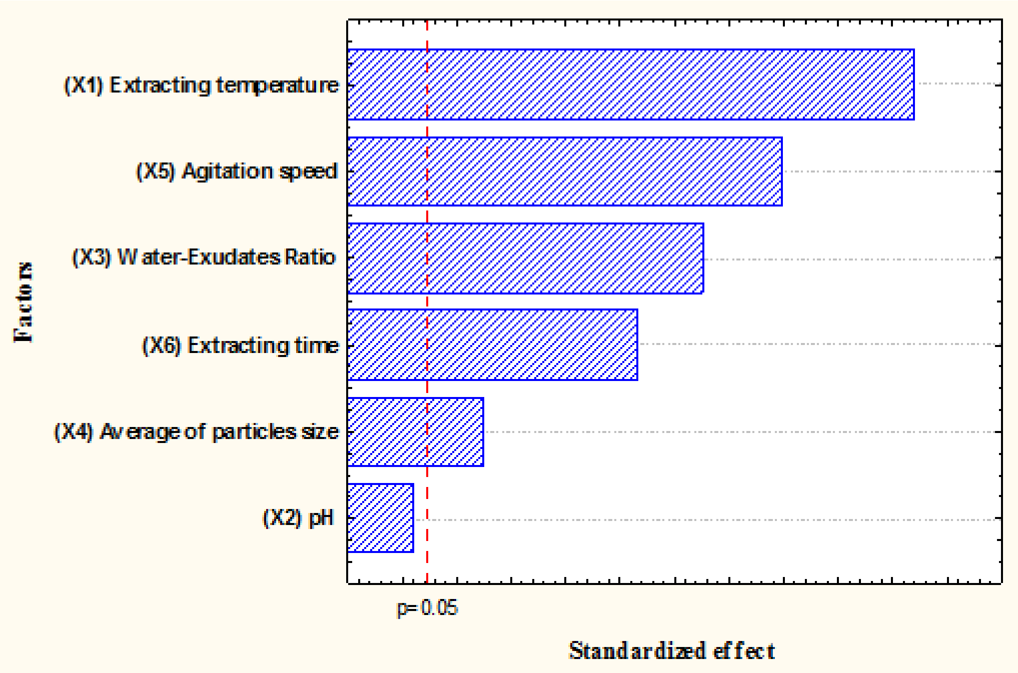

Fig. 2. Pareto chart of Plackett-Burman Design for parameter estimation on cashew gum yields

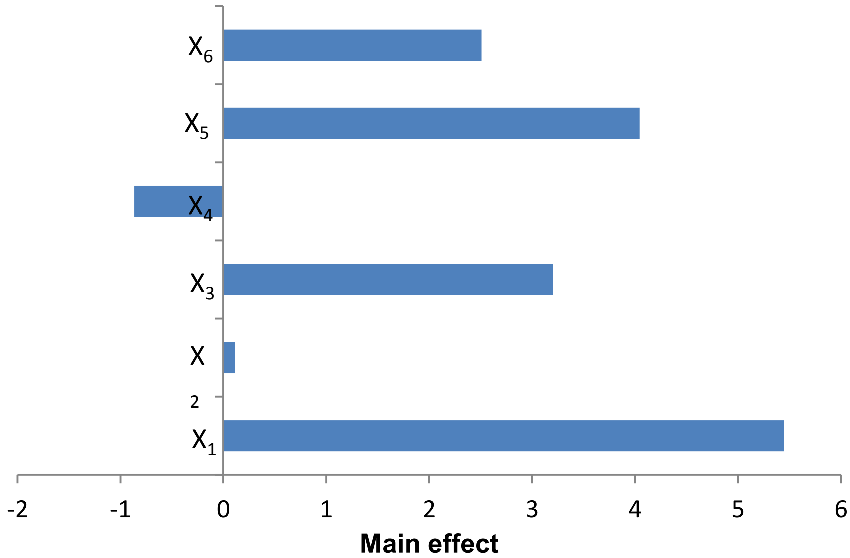

Fig. 3. Estimate of the effect of factor on cashew gum yields

B. Statistical Validation of the Postulated Model

According to ANOVA for the model (**Table IV**), the model of regression was significant ($p \leq 0.05$) which inferred that extracting temperature (X_1), agitation speed (X_5), water exudates ratio (X_3)and extracting time (X_6) as most significant variables influencing cashew gum yield (**Table V**).Otherwise, the significance of the model was confirmed by the calculation of $F_{\text{Ratio}(R/r)}$ (203) which showed that it is higher than the theoretical value of F(0.05;6,9) at 95% confidence level (3.314).The regression coefficient for cashew gum yields indicated 99.26 % of variability around the mean. Correlation between the study factors on the response is shown in first-order polynomial equation (4):

$$Y = 81.91+2.72X_1+ 1.60X_3+2.02X_5+1.25X_6 \quad (4)$$

Where Yis the cashew gum yield.

The obtained regression coefficient (R-squared) showed a relatively high correlation between experimental and predicted value. The adjusted R-squared statistic of 0.9877 was also high enough.

Table IV: Statistical analysis of the model

Source	**SS**	**DF**	**MS**	**F-value**	***p*-value**
Model	253.374	6	42.229	203.00	0.0000
Error	1.876	9	0.208		
Total	255.250	15			

Table V: Estimated regression coefficients for the Plackett-Burman design

Terms	Coefficient	Coefficient values	SD of coefficient	Effect	SD of effect	*t*-value	*p*-value
Constant	β_0	81.91	0.11	81.91	0.11	717.59	0.000000*
Extracting temperature(X_1)	β_1	2.72	0.11	5.45	0.23	23.86	0.000000*
pH(X_2)	β_2	0.06	0.11	0.11	0.23	0.50	0.626552
Water-Exudates Ratio (X_3)	β_3	1.60	0.11	3.20	0.23	14.03	0.000000*
Average of particles size (X_4)	β_4	-0.43	0.11	-0.86	0.23	-3.79	0.004290**
Agitation speed (X_5)	β_5	2.02	0.11	4.04	0.23	17.72	0.000000*
Extracting time (X_6)	β_6	1.25	0.11	2.51	0.23	10.98	0.000002*

IV. Discussions

The findings of this work showed that the factors such as extracting temperature, agitation speed, water-exudates ratio and extracting time affected meaningfully ($p \leq 0.05$) the production of crude cashew gum from *Anacardium occidentale* exudates by aqueous extraction. Similar significant effect of extracting temperature and water seeds ratio on yield was indicated by [23] in extraction of Qodume Shirazi gum. These authors revealed less effect of pH on yield. This result was agreed with our findings. The effect of pH on the yield reported for some gums origins revealed minor effect in agreement with our finding [10].

Besides, [24] observed a significant effect of ratio, temperature, and pH on the extraction yield of gum from durian (*Durio zibethinus*) seeds. According to these authors, the difference in the effect of factors on the responses variables may reflect the chemical structure of the gum.

Concerning extracting time, it an important parameter in the extraction process in order to optimize the yield of the cashew gum from *Anacardium occidentale* exudates. The effect of extraction time on extraction of antioxidant, flavonoids and phenolic content from *Averrhoa bilimbi*was indicated by [25] who reported the extracting time ranging from 15 to 240 min.

As for the agitation speed, our results were closed to the reports of [25] who showed the effect of agitation speed in action of antioxidant, flavonoids and phenolic content from *Averrhoa bilimbi*. In this study, the optimum agitation speed value was 300 rpm. Indeed, High agitation rate leads to a high mass transfer coefficient and improves the convective mass transfer rate leading to increase in extraction yields [25]. Similar finding was also reported by [26], which implied that agitation increased the extraction yield by accelerating the disruption and dissolution of active compounds bound to the sample matrix to the fluid.

Otherwise, the coefficient of determination R^2 value of 99.26%, which indicates that this rate of the variability in the response could be explained by the model, and it indicates an acceptable agreement between experimental and predicted values. Thisimplies that the mathematical model is very reliable for cashew gum production in the present study.

V. Conclusion

In this investigation, Plackett-Burman design offers a good and fast screening procedure to test the relative importance of medium components on the production of crude cashew gum. Among the variables extracting temperature, water exudates ratio, agitation speed and extracting time were found to be the most important variables. The optimum levels of the variables can be determined using response surface methodology in further research.

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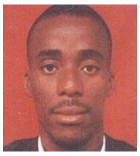

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