

# Extraction of Starch from Mango Seed Using Response Surface Methodology and Its Proximal Characterization

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## Abstract

In the food industry, the various residues and wastes generated during mango processing are a major source of large-scale environmental pollution. Poor management of these wastes in third-world countries is one of the factors hindering their economic development. It is crucial to overcome the source of the problem by proposing innovative and sustainable solutions. Thus, the valorisation of mango seeds into value-added products offers a promising opportunity to partially solve this problem. This study was conducted to extract the maximum starch from mango seeds using a Box-Behnken experimental design, then to analyse the starch extracted by determining its proximate composition according to AOAC recommendations. Based on the findings, the proximal characteristics of mango starch determined in this study show that it is low in protein, fat, and ash, contributing to its high purity. These results show too that mango kernel starch is therefore an attractive alternative source of non-edible starch for various industrial applications.

## Keywords

Mango Seed, Starch, Extraction, Optimization, Proximal Characterization

## 1. Introduction

Generating more than 40 million tonnes each year [1] [2], mango residues and waste (*Mangifera indica* L.) constitute a real environmental challenge. Mango is a fruit well-appreciated worldwide thanks to its nutritional virtues and phytochemical profile that it provides to the body [3]. It is a highly perishable fruit and generates several types of waste throughout its production, marketing, processing, and consumption. Among the various wastes generated by mangoes, the fruit seed alone constitutes 30% to 45% of the total fruit weight. It is estimated that during mango processing, a total of 35% to 60% of waste is produced per gram of fruit processed [4]. It is established that the global production of by-products from the consumption of mangoes (fresh and processed) after peeling and pitting ranges between 14.7 and 25.2 million tonnes annually [5]. In the Ivory Coast, Post-harvest and post-processing and consumption losses in the mango sector are estimated at 70,000 and 63,000 tons, respectively [6]. The seed stands out for its particularity in that it contains several organic molecules of interest, such as cellulose, carbohydrates, fats, secondary metabolites, and especially starch. The valorisation of the mango seed constitutes a path to explore for mitigating the environmental impact caused by its poor management through the exploitation of the precious molecules it contains.

Among the main sources of carbohydrates that exist, starch holds a prominent place due to its abundance, its wide availability in the plant, its low cost, its excellent properties, and especially thanks to its numerous application sectors. The extraction of starch presents a consolidated market with an estimated value of 77 billion dollars for the year 2018 [7]. The starch contained in the mango seed is an alternative source of non-edible starch, not competing with noble sources of human food. Its valorization thus constitutes added value brought to this waste by reducing its environmental pollution impact and, at the same time, lowering the strong dependence on starch from noble sources. Depending on the variety and stage of maturation, the mango kernel can contain more than 50% of starch after extraction [8] [9].

The methods of starch extraction vary widely depending on the source of starch, specifically the processing conditions of mango seed powder (neutral, alkaline, or acidic). A sodium metabisulfite solution is usually used to extract starch from the seed, allowing for significant starch recoveries with very good purity [3]. The concentration of the extraction reagent can vary between 1% and 5%, but generally, a rate of 2% is more commonly used [10]-[12]. During the extraction process, several parameters influence its yield. Some parameters can be mentioned, such as the concentration of sodium metabisulfite, the extraction temperature, the dilution ratio of the biomass, the agitation, etc. [13] [14]. Each factor contributes in its own way to the overall starch extraction process, which *ipso facto* affects the yield. Therefore, these different parameters can be explored using the Design of Experiments (DOE) methodology to understand their contribution to the processes.

Methodologies based on experimental designs are a highly relevant tool used in both research and industry [15] [16]. They allow for the control of all elements that are likely to influence a process. The DOE is widely used in several sectors of activity; it is easy to use, and its great advantage is the control over the number of

experiments, thus avoiding any waste of time, production costs, and materials; its primary role is to optimise a process. Several types of experimental plans are developed according to the field and the targeted objective, offering numerous possibilities to users. Thus, response surface designs stand out for their interpretation and the quality of useful information that can be derived from them. For starch extraction, several experimental designs are employed using different factors to optimise the extraction yield [17]-[20]. According to these previous studies, particle size, solid-liquid ratio, and temperature were chosen in the optimisation of starch extraction yield from mango seed powder using the Box Behnken Design (BBD). The present study aims to optimise the extraction of starch from mango seeds and to determine the proximal chemical composition of the obtained starch, thereby enabling the valorisation of mango waste for different applications.

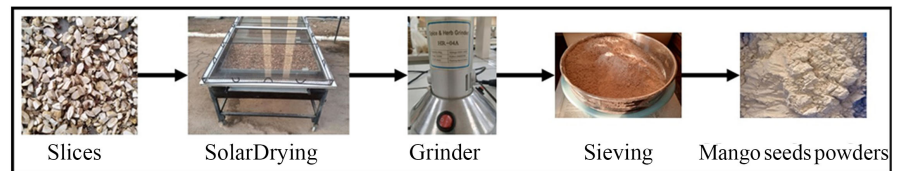
## 2. Material and Methods

### 2.1. Collection, Pretreatment, and Obtaining Mango Seed Powder

The ripe local variety mangoes (**Figure 1(a)**) that are abandoned or downgraded under the tree were collected, during periods of abundance, in the 227-unit housing complexes of the city of Yamoussoukro and then transported to the INP-HB site. They were stored in bags. After one week of storage, the pulp and skin are removed from the mangoes (**Figure 1(b)**), and the seeds are washed and then sun-dried for a week. With the help of a knife and a chisel, the hard shell was removed from the kernel (**Figure 1(c)**), and the inner layers were also removed. The kernel of the seed (**Figure 1(d)**) was cut into small slices of one centimetre in length for drying. The sliced almonds were then dried in a solar dryer for a week. The temperature in the solar dryer varied between 40°C and 60°C depending on the sunlight. The slices of dry biomass kernels were ground in a knife grinder (Spice & herb grinder HR-04A), then sieved using sieves of different mesh sizes (Retsch GmbH; 1.6 mm, 800 µm, and 400 µm mesh). The different samples obtained were placed in airtight bags and then stored in the laboratory for later use. The process of obtaining the powders from the seeds is represented in **Figure 2**.



**Figure 1.** a) Mango fruit residues; b) mango seeds; c) mango seed shells; d) mango seed kernels.



**Figure 2.** Process of obtaining the mango seed powder (MSP).

## 2.2. Starch Extraction

The starch extraction method applied in the current study was described by [21], but with some modifications made by [12]. The experiment consisted of infusing 25 g of mango seed powder in 250 mL of an aqueous solution of sodium metabisulfite (0.2%) for 16 to 24 h at a temperature of 50°C under continuous stirring at 500 rpm. The above solution was then ground using a Moulinex for 10 to 15 minutes, and 150 mL of distilled water was added during grinding. The resulting milled liquid was filtered twice using a 150 µm pore size sieve, topped with a nylon cloth to retain any solid particles that might contaminate the starch. The collected filtrate was left to settle for 4 hours, then the supernatant was removed. The starch pellet was washed with distilled water in an Erlenmeyer flask of 250 mL. The operation was repeated three times until a white layer of starch was obtained. The washed starch layer was then distributed into 50 mL Eppendorf tubes and centrifuged at 4000 rpm for 15 min at 25°C. After the separation of the solid and liquid phases, the top layer of the non-white solid phase was removed, while the bottom white layer was resuspended in distilled water and re-centrifuged to repeat the operation 3 times as planned before. The completely bleached starch was then collected and dried until 48 hours in an oven ventilated (70%), where the temperature was set at 40°C [12] [21]. The dry starch thus obtained was collected in a hermetically sealed bottle and stored in the laboratory for subsequent analysis.

## 2.3. Determination of the Starch Extraction Yield

The starch extraction yield (Y%) was determined using Equation (1).

$$Y \left( \frac{g}{g} \cdot \frac{100}{g} \right) = \frac{S_{dc} \times m_{ad} (g)}{D_{sp} \times m_{sp} (g)} \times 100 \quad (1)$$

With:

$S_{dc}$ : Starch dry matter content.

$m_{ad}$ : Mass of starch obtained after drying.

$D_{sp}$ : Dry matter content of MSP.

$m_{sp}$ : Mass of starting MSP.

## 2.4. Optimization Plan for Starch Extraction from Mango Seed Powders

A Box-Behnken Design (BBD) was used to determine the optimum conditions for starch extraction from MSP. In this response of surface methodology, three inde-

pendent factors are considered: particle size ( $X_1$ ,  $\mu\text{m}$ ), solid-liquid ratio ( $X_2$ ,  $\text{g/mL}$ ), and temperature ( $X_3$ ,  $^\circ\text{C}$ ). These factors are used at three levels of variation, coded as  $(-1, 0, +1)$ . Low and high levels are coded as  $-1$  and  $+1$ , respectively, while the midpoint is coded as  $0$ . In this study, a total of 17 randomized experiments were carried out, including five repetitions at the central points of the experimental domain, to calculate the repeatability of the method [22]. Each experiment was carried out in duplicate, with the mean values entered in the matrix. Data were analysed, using Design Expert software (Version 13, State-Ease, Inc., Minneapolis, USA), to obtain optimum extraction conditions. Level graphs, coefficient of variation, and analysis of variance (ANOVA) were used to study the response. The results of these analyses were used to determine the coefficient of Equation (2).

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} X_i X_j \quad (2)$$

With:

$Y$ : Response or dependent variable (starch extraction yield);

$\beta_0$ : Constant coefficient of interception.

$\beta_i$ : Linear coefficient.

$\beta_{ij}$ : Interaction coefficient.

$\beta_{ii}$ : Quadratic coefficient.

$X_i$  and  $X_j$ : Coded levels of independent variables (particle size, solid-liquid ratio, and temperature).

The three coded independent factors of the experiment with the range of the experimental domain are summarized in **Table 1**. The range of the values for each factor was defined based on results published in previous work [13] [14] [18] and preliminary results by varying one factor at a time and expanding it to study their influence on starch extraction.

**Table 1.** Independent factors and their different levels  $(-1, 0, +1)$ .

	Code	Factor Levels		
Independent factors		$-1$	$0$	$+1$
Particle size ( $\mu\text{m}$ )	A	$\leq 400$	$\leq 1000$	$\leq 1600$
Solid-liquid ratio ( $\text{g/mL}$ )	B	1:5	1:10	1:15
Temperature ( $^\circ\text{C}$ )	C	10	30	50

## 2.5. Optimization of Extraction Parameters and Validation of the Developed Model

Starch extraction parameters were optimised to achieve maximum starch yield. The numerical optimization method of the Design Expert software was employed using the desirability function  $D$  in the interval of  $0 \leq D \leq 1$ . The optimisation objective is intended to obtain the highest yield while keeping the variables in optimal conditions [23]. The optimal extraction condition was selected from solu-

tions with a desirability function of 1.

To validate the model, the theoretical response value predicted by the software's numerical optimization was compared with the experimental value using the predicted variable conditions. A difference in error between experimental and theoretical data should be relatively small. In the circumstances, a difference of less than 10% is acceptable for good accuracy and adequacy of the developed model [24].

## 2.6. Proximal Analysis of Mango Seed Powders and Starch

### ✓ Moisture

Water and volatile matter content or moisture (H%) was determined according to the AOAC (2001) standard (Equation (3)).

$$H(\%) = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad (3)$$

where:

$W_0$ : Weight of empty crucible (g);

$W_1$ : Weight of powdered sample + empty crucible (g);

$W_2$ : Weight of dried sample + empty crucible (g).

### ✓ Ash content

Ash content (A%) was determined by calcination in a muffle furnace in accordance with the recommendations of AOAC (2001). The result was expressed using Equation (4).

$$A(\%) = \frac{W_3 - W_2}{W_2 - W_1} \times 100 \quad (4)$$

where:

$W_1$ : Weight of empty crucible (g);

$W_2$ : Weight of crucible with anhydrous sample (g);

$W_3$ : Weight of crucible with ash (g).

$A(\%)$ : Ash content.

### ✓ Crude fibre content

For crude fibre content (CF%), the gravimetric method was used in accordance with the AOAC (2001) standard. Equation (5) was used for the calculations.

$$CF(\%) = \frac{W_2 - W_3}{W_1} \times 100 \quad (5)$$

where:

$W_2$ : Weight of residues (g);

$W_3$ : Weight of the ashes (g);

$W_1$ : Weight of the sample (g).

### ✓ Lipid content

The Soxhlet solvent extraction method was applied, using 2 g of powder. The lipid content (F%) was calculated based on Equation (6).

$$F(\%) = \frac{W_2}{W_1} \times 100 \quad (6)$$

where:

$W_2$ : Weight of fat obtained (g);

$W_1$ : Weight of the sample (g).

#### ✓ Protein content

Protein content (P%) was assessed using the Kjeldahl method [25] which consists of converting the total amount of nitrogen present in a sample into ammonia ( $\text{NH}_3$ ). From the nitrogen content calculated (N%) with Equation (7), P (%) was deduced according to Equation (8).

$$N(\%) = \frac{(V_1 - V_0) \times 1.4 \times X}{W} \times 100 \quad (7)$$

$$P(\%) = N(\%) \times 6.25 \quad (8)$$

With:

$V_1$ : Volume of HCl solution used for sample titration (mL).

$V_0$ : Volume of HCl used for blank titration (mL).

$X$ : Correction factor for 0.01 mol/L HCl.

$W$ : Sample mass (g).

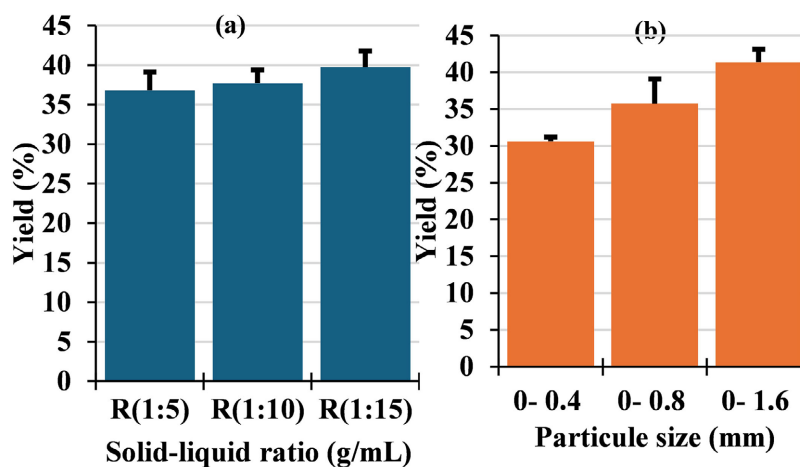
#### ✓ Total carbohydrate content

Generally, the total carbohydrate content (G%) is calculated by deducting the percentages of water (H%), protein (P%), crude fibre (CF%), fat (F%), and ash (A%) from 100% (Equation (9)).

$$G(\%) = 100\% - [H(\%) + P(\%) + CF(\%) + F(\%) + A(\%)] \quad (9)$$

## 3. Results and Discussion

### 3.1. Preliminary Study of the Parameters Influencing Extraction Yield



**Figure 3.** One-factor variation of the parameters on the mango starch yield extraction, a) solid-liquid ratio (g/mL), b) particle size (mm).

The factors influencing the starch extraction yield from mango seed, such as the solid-liquid ratio and particle size, are illustrated in **Figure 3**. The solid-liquid ratio presented in **Figure 3(a)** shows that there is no significant difference between the three ratios used. This indicates that more extraction solvent does not necessarily lead to a significant release of starch. Between the ratios of 1:5 and 1:15, there is a slight increase in extraction yield. These observations are consistent with the work of [13] [26].

Three particle sizes of seed powder were used for starch extraction, and it was observed that the yield increases proportionally with the particle size. This is illustrated in **Figure 3(b)**, and a significant difference between the three particle sizes is noted. The mixture of particle sizes ranging between [0 - 1600  $\mu\text{m}$ ] allows for the highest starch yield in these experiments. These two factors studied individually inform about their influence on starch extraction yield, but a simultaneous variation of these factors combined with others will allow to better understand the effect of each factor, its interactions, and their importance, which are not observable when they are taken separately. This preliminary study justifies the basis of the field of the current study for the use of a response surface design that allows to vary the three factors simultaneously.

### 3.2. Approach of Response Surface Methodology (RSM) on Extraction Yield

The Box-Behnken design was used to optimise the extraction yield of starch from the mango seed. Three factors were varied, namely particle size (A), solid-liquid ratio (B), and temperature (C). The measured response was considered as the extraction yield. In total, 17 experiments were generated by the Design Expert version 13 software with 5 repetitions at the centre of the experimental domain. The results obtained, as well as the ones predicted by the software and the residuals, are presented in **Table 2**.

In this table, the extraction yields range from 28.40% (experiment 4) to 45.67% (experiment 11). Each experiment was conducted in duplicate, and the averages are recorded in **Table 2**. However, the values predicted by the software range from 31.28% to 45.49%, which fall within the experimental range. The residual values, which are simply the difference between the experimental values and the predicted ones, show more negative values than positive, indicating that the prediction is superior to the experiment.

The analysis of variance (ANOVA) shows that the experimental results were perfectly in line with the second-order quadratic model proposed by the software, with an  $R^2$  of 0.9784. Indeed, three static models were tested: the linear model with  $R^2$  of 0.1475, the 2FI model with  $R^2$  of 0.0561, and the quadratic model, which only gives the highest  $R^2$  of 0.9784. Only 2.16% of the variations were not explained by the quadratic model. The F probability, the p probability, and the lack of fit are respectively 35.27;  $<0.0001$  ( $p < 0.005$ ); and 0.2214, indicating that the model is very significant and does not suffer from any lack of fit. The final equation of the

model in terms of coded variables is given as Equation (10):

$$Y (\%) = +31.28 - 1.43 \times A + 2.06 \times B + 2.72 \times C - 1.84 \times AB - 2.40 \times AC + 6.10 \times A^2 + 2.79 B^2 - 1.59 \times C^2 \quad (10)$$

**Table 2.** Extraction parameters and the experimental response, predicted, and residual.

	A ( $\mu\text{m}$ )	B (g/mL)	C ( $^{\circ}\text{C}$ )	Yield	Predicted	Residual
1	1000	10	30	30.67	31.28	-0.6060
2	400	10	50	42.7	42.34	0.3588
3	1000	15	10	32.47	31.63	0.8425
4	1000	5	10	28.40	27.86	0.5350
5	1000	10	30	30.3	31.28	-0.9760
6	1000	5	50	32.11	32.95	-0.8425
7	1000	15	50	36.89	37.43	-0.5350
8	1600	10	50	35.69	34.67	1.02
9	1600	15	30	38.47	38.95	-0.4837
10	1000	10	30	32.02	31.28	0.7440
11	400	15	30	45.67	45.49	0.1763
12	400	10	10	31.08	32.10	-1.02
13	1600	10	10	33.67	34.03	-0.3588
14	1000	10	30	32.25	31.28	0.9740
15	400	5	30	38.19	37.71	0.4838
16	1600	5	30	38.33	38.51	-0.1762
17	1000	10	30	31.14	31.28	-0.1360

A: particle size, B: Solid-liquid ratio, C: temperature.

In this Equation (10), the ANOVA confirmed that the term BC is not present in the model, so it was removed and has a non-significant effect on the overall process. The model coefficients are expressed by the constants A, B and C, representing the linear coefficients of the independent variables, respectively, particle size, solid-liquid ratio, and temperature. AB, AC and BC were the coefficients of the interaction terms, while  $A^2$ ,  $B^2$  and  $C^2$ , the coefficients of the quadratic terms. The constant 31.28 represents the starch extraction yield based on the conditions corresponding to the coded variables at the centre of the experimental domain (value 0). The lack of fit of the model has a probability value of 0.2214 and an F value of 2.28, which was non-significant compared to the pure error of all the variables. The interaction terms BC have a probability value of 0.7449, confirming their non-significance, and are removed from the overall model equation. The coefficients of  $R^2$  determination of 0.9784, of adjustment ( $R^2$  adjust) of 0.9507, and of prediction ( $R^2$  predicted) of 0.7698, showed the accuracy of the developed model, with a difference between the adjusted  $R^2$  and the predicted  $R^2$  of 0.1809,

which was less than 20%. The model is ideal for describing the chosen response in this study. The precision adequacy (AP) of the model was 21.9213, which was greater than 4, also indicating a good signal-to-noise ratio; the AP measures the signal-to-noise ratio, making a comparison between the range of predicted values and the average prediction error. The overall prediction of the model strongly depends on the  $R^2$ , which was calculated from the residuals of the sum of squares of prediction errors (PRESS). Here, the value of PRESS was 82.12. In this study, the coefficient of variation (CV%) was 3.02%, which is less than 10%, indicating the high reproducibility of the model and a good fit of the model with the experimental results [18].

**Table 3.** Analysis of variance (ANOVA) of the quadratic model.

Source	Sum of Squares	df	Mean Square	Coef	F-value	p-value	
<b>Model</b>	348.99	9	38.78	31.28	35.27	<0.0001	significant
A	16.47	1	16.47	-1.43	14.98	0.0061	
B	33.91	1	33.91	2.06	30.84	0.0009	
C	59.24	1	59.24	2.72	53.89	0.0002	
AB	13.47	1	13.47	-1.84	12.25	0.0100	
AC	23.04	1	23.04	-2.40	20.96	0.0026	
BC	0.1260	1	0.1260	0.1775	0.1146	0.7449	Not significant
A <sup>2</sup>	156.84	1	156.84	6.10	142.66	<0.0001	
B <sup>2</sup>	32.68	1	32.68	2.79	29.72	0.0010	
C <sup>2</sup>	10.70	1	10.70	-1.59	9.73	0.0168	
<b>Residual</b>	7.70	7	1.10				
Lack of Fit	4.86	3	1.62		2.28	0.2214	Not significant
Pure Error	2.84	4	0.7101				
<b>Cor Total</b>	356.68	16					
<b>Std. Dev</b>	1.05				<b>R<sup>2</sup></b>	0.9784	
<b>Mean</b>	34.71				<b>R<sup>2</sup> adjusted</b>	0.9507	
<b>C. V (%)</b>	3.02				<b>R<sup>2</sup> Predicted</b>	0.7698	
<b>PRESS</b>	82.12				<b>Adeq. precision</b>	21.9213	

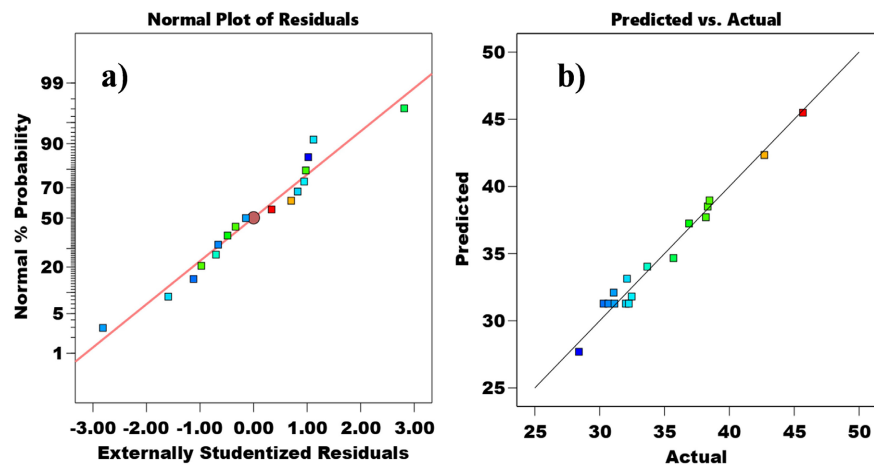
A: particle size, B: Solid-liquid ratio, C: temperature.

The overall prediction of the model strongly depends on the  $R^2$ , which is calculated from the residuals of the sum of squares of prediction errors (PRESS); here, the value of PRESS is 82.12. The coefficient of variation (CV%) is 3.02% in this study, which is less than 10%, indicating high reproducibility of the model and a good fit of the model with the experimental results [17].

### 3.3. Model Adequation

In **Figure 4(a)** is shown the probability of the normal distribution of residuals in

percentage (internal studentized residues versus normal % probability plot). This allows us to verify if the residuals follow the normal distribution; on this graph, we can see that we can draw a line passing through the maximum number of points. This confirms that the residuals followed a normal distribution. There were very few points that deviated from the line, and the presence of outliers was very minimal. The accuracy of the model was excellent, and only a few very small or unexpected errors could be attributed to this proposed model [17].

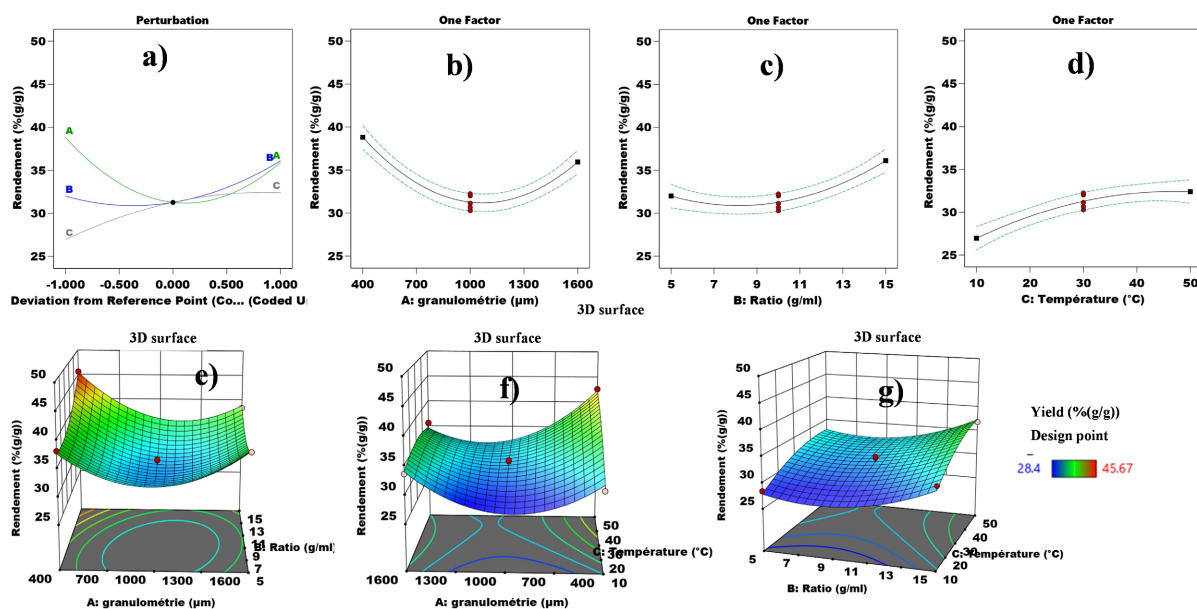


**Figure 4.** a) The diagnostic plots of internally studentized residuals versus normal % probability and b) Predicted extraction yield versus actual extraction yield.

The distribution between the experimental values and the predicted ones (predicted vs actual) is presented in **Figure 4(b)**, where a good match between these two values is observed. The experimental and predicted values must describe a straight line with the equation  $y = x$  [16]. The distribution obtained in this study showed that a few points deviate from this line ( $y = x$ ). The adjusted  $R^2$  of this study was 0.9784, showing that the model explained 97.84% of the variation in extraction yield based on the independent factors and their interactions. When the study yields an  $R^2$  and adjusted  $R^2$  value, respectively, greater than 0.9 and 0.7, these two values are sufficient for the model to be accepted.

### 3.4. Effects of Different Parameters on Starch Extraction Yield

The perturbation plot of the extraction yield is presented in **Figure 5**. In this Figure, the effect of each independent factor on the extraction yield (**Figures 5(b)-(d)**), and the response surfaces (**Figures 5(e)-(g)**) of the interactions of the factors taken two at a time. **Figure 5(a)** shows that temperature and the solid-liquid ratio had an increasing effect on extraction yield compared to particle size, which had a decreasing effect. The coefficients of the factors presented in **Table 3** confirm that temperature and the solid-liquid ratio had a positive effect on the yield. The coefficient of particle size was negative, showing a decreasing effect on starch extraction yield.



**Figure 5.** a) Perturbation plot of the yield (% (g/g)); One-factor plots of yield (%): Effect of b) granulometry at ratio of 1:10 and temperature of 30 °C; c): Effect of ratio at granulometry of 1000 µm and temperature of 30 °C; d): Effect of temperature at granulometry of 1000 µm and ratio of 1:10; 3D response surface plots of yield (%): Effect of e): granulometry ratio at 30 °C; f): granulometry and temperature at 1: 10 of ratio; g): temperature and ratio at 1000 µm granulometry. Colour gradients indicate the level of optimisation (red = high, green = intermediate, and blue = low).

#### ✓ Effect of particle size on yield

The particle size of the mango seed powder is essential for achieving good penetration of the extraction solvent and releasing starch granules. The effect of particle size taken over a range of 400 µm to 1600 µm was presented in **Figure 5(b)**. According to **Figure 5(b)**, the particle size described a parabolic curve with an upward concavity, admitting a minimum around 1000 µm. At both ends of this curve, the yield would seem to be high; the particle size of 400 µm has a greater effect than that of 1600 µm when the solid-liquid ratio and temperature are set to their coded values at level zero (1:10 g/mL, 30 °C). When the particle size increased to 1070 µm, the extraction yield was lower, reaching its minimum with a value of 31.20%. The response surface plot in **Figure 5(e)** shows the interaction between particle size and the solid-liquid ratio, and it was observed that it followed the same pattern as the particle size perturbation plot. The coefficients of the two factors were opposite, and their interaction could only be antagonistic on the response.

#### ✓ Effect of the solid-liquid ratio on yield

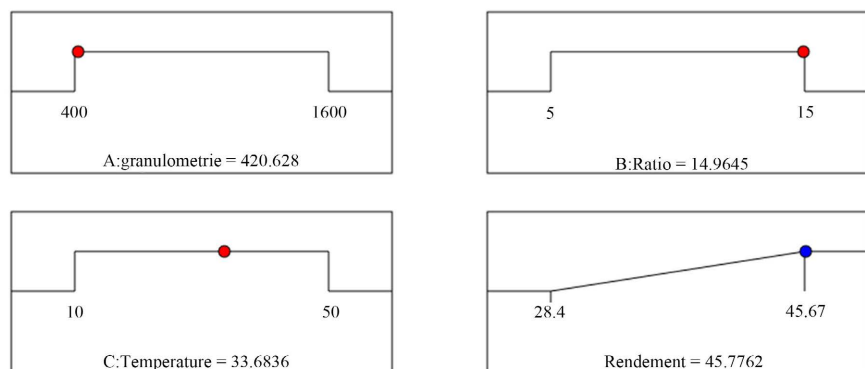
The solid-liquid ratio is a biomass dilution factor. It positively contributes to the extraction yield, its coefficient being positive in **Table 3**. **Figure 5(c)** expresses the disturbance of the solid-liquid ratio on the yield. The higher is the ratio, the higher is the yield. At the beginning, it experienced a slight decrease, but after that, it remained increasing in yield. The response surface plot in **Figure 5(g)**, showing the interaction between the solid-liquid ratio and particle size, reveals an opposite effect on the extraction yield. The ratio has a positive effect, while the particle size

has an antagonistic effect. In general, increasing the solid-liquid ratio allowed to promote contact between the biomass and the solvent and leads to the rupture of the cell membrane; this favours the release of starch granules into the medium. The high ratio promotes good mixing and uniform heat and mass transfer, as well as a decrease in the mixture's viscosity [27].

#### ✓ Effect of temperature on yield

In **Figure 5(d)** is shown the curve of the evolution of starch extraction yield as a function of temperature. The shape of this curve indicates that as the temperature increased, the extraction yields also increased. The temperature promoted the release of starch granules in this experiment. **Figure 5(f)** displays that temperature and particle size interacted with a coefficient whose value was negative (**Table 3**), thus expressing an antagonistic effect on extraction yield. The temperature increased the extraction yield, while the particle size lowered it. In the work of [11], they used a temperature range from 25 °C to 50 °C and observed that the extraction yield was higher at a temperature of 50 °C, and that temperature was the second factor influencing the response of their study. In this work, a temperature ranged from 10 °C to 50 °C, was chosen to observe the effect of temperature on the extraction yield. It follows that the higher was the temperature, the greater was the increase in extraction yield.

### 3.5. Optimization of Starch Extraction from Mango Kernel



**Figure 6.** Numerical optimization of the yield of extraction (desirability: 1).

To experimentally confirm the model, the previously achieved results in this research were utilized by taking advantage of the ideal conditions of the three examined variables: particle size, solid-liquid ratio, and temperature. To maximise the extraction yield, numerical optimisation of the software was used, and no constraints were imposed on the three factors. Several solutions were predicted by the model with a desirability of 1, among which a yield of 45.78% was taken as the ideal solution. The predicted experimental conditions of the three factors, as well as the obtained results, are illustrated in **Figure 6** and recorded in **Table 4**. The average of the three observations made was  $45.33\% \pm 1.06\%$ ; the difference between the predicted and experimental value is 0.45%, which was less than the rec-

ommended 10% for the validation of numerical optimisation. This yield of 45.33% was close to the yield obtained by [26], who achieved a value of 47.45% with the Totapuri variety; it was close to that of [12] whose yields range from [39.35% - 44.96%] on the Uba variety, However, it remained higher than the values of 38.8 obtained by [28]; and of 27.35% found by [29]. This yield remains lower than the values of 53.89% obtained by [30], of 56% obtained by [31], and the values of 55.17% and 57.05% obtained by [8], respectively, on the Sugar and Tommy varieties. [9] obtained yields ranging from [44.49% - 62.11%] with two mango varieties and using two extraction methods. Ultimately, the optimisation of these three factors allowed for a yield close to that reported in the literature, once again confirming the use of the response surface method.

**Table 4.** Predicted and experimental values of the yield at optimum extraction conditions.

Essay Numbers	A ( $\mu\text{m}$ )	B (g/mL)	C ( $^{\circ}\text{C}$ )	Observed value (%)	Predicted value (%)	Errors (%)
1	420.6	1:14.96	45.78	44.14	45.78	$\pm 1.64$
2	420.6	1:14.96	45.78	45.69	45.78	$\pm 0.09$
3	420.6	1:14.96	45.78	46.17	45.78	0.39

A: particle size, B: Solid-liquid ratio, C: temperature.

### 3.6. Proximal Analyses of Mango Seed Powders and Starch

The results of the proximal analysis of mango seed powders as well as the extracted starch are presented in **Table 5**. The moisture content of the powder and the starch is 6.24% and 5.47%, respectively. These humidity values are low compared to those of the fresh biomass from which they are derived. The moisture content of the fresh biomass was 47.6%. The moisture content found in this work is higher than the moisture values of 4.26% reported by [12], 4.8% reported by [29], and 5.85% reported by [32]. The moisture content of the obtained starch is close to that obtained by [9], which falls within the range of [4.23% - 5.89%]; they used two extraction methods on two different varieties of mango. The moisture content of the starch is higher than the value of 4.9% obtained by [29]. It remains lower than the moisture values of 9.86% obtained by [30]. The moisture content provides information on the drying state of the product and its proper preservation, and values below 20% are ideally recommended.

The ash, fat, protein, and total carbohydrate contents (%) were, respectively, 6.24, 2.11, 8.33, and 80.74 for mango seed powder, and 5.47, 1.82, 0.75, and 90.25 for mango seed starch. The ash content of the seed starch was slightly lower than that of the seed powder. These ash contents remained higher than the values ranging from 0.12 to 0.14% for mango seed starch reported by [9]. Ferraz *et al.* (2019) [12] reported an ash content in the seed powder of 1.96%, which remains lower than that found in this work. Ash contents of 0.03% and 0.12% were reported by [30] and [28], respectively. Ash content value of 1.77% reported by [29] was close

to the ash content of mango seed starch in this work. Generally, this biomass does not contain a large amount of mineral matter.

**Table 5.** Proximal analysis of mango seed powder and starch.

Parameters	PNM	AMS
Moisture (%)	6.24	5.47
Ash (%)	2.11	1.82
Fat (%)	8.33	0.75
Proteins (%)	2.58	1.50
Carbohydrate (%)	80.74	90.46

The fat content of 8.33%, found in this study, was higher in mango seed powder. [29] reported a fat content of  $11.69\% \pm 2.06\%$  in the Tommy Atkins variety of mango seed powder, while [12] found a content of 10.78% in their work. This made the seed powder of the mango, a biomass of choice for fat extraction. The amount of mango seed starch remained very low because, during extraction, a significant portion was eliminated during purification process. Here, these levels are estimated to 0.75% in mango seed starch, which remained higher than the values of 0.22% and 0.24% found by [9]; and the value of 0.38% found by [33]. The value in this study was lower than the 4.63% found by [30]. The mango seed powders being 2.58% remained lower than the values of 4.57% found by [12], of  $3.36\% \pm 1.58\%$  found by [29]. Similarly, the protein content of the seed starch remains higher than the values ranging between 0.67% and 0.69% reported by [9] in their study. [30] found a protein content value of 3.63%, which remained higher than the 1.50% of the present work. Very low respective values of 0.02% and 0.043% were found by [28] and [33], while that of [29] was 0.68%.

The total carbohydrate content calculated by difference with the other constituents of the matter gave very high values. Thus, a content of 90.46% was obtained with mango seed starch, followed by that of the powder, which is 80.74%. [29] reported a total carbohydrate content of 66.88% in mango seed powder, considering a crude fibre content of 11.5%; [32] reported a value of 92.03% for total carbohydrates on mango seed powder. As for the work of [12], they found a total carbohydrate content of 78.43%, which was close to that found for mango seed powders in this study. For the starch from the seed, total carbohydrate contents ranging from [93.70% - 95.25%] were mentioned in the work of [9] on mango kernel starch. A high total carbohydrate content showed that mango seed powder is a biomass of choice for the extraction of carbohydrates such as starch. This higher content in the extracted starch indicated the purity degree of the starch extracted and that the extraction method used was satisfactory. The qualities of the extracted mango starch meet the recommendations of this sector; it can then be an excellent candidate for various applications.

## 4. Conclusions

Mango residues, mainly the seed, constitute a significant source of non-edible starch. Other times considered as waste and causing numerous problems for the various stakeholders in the mango industry, as well as the environment in general, its valorisation would be a major asset for them. Therefore, mango seed starch is becoming an alternative for the constantly growing starch market. The response surface design, such as Box-Behnken, was used to optimise the starch extraction yield from mango seed in this study. The model established by the Design Expert software was statistically significant and can be used to predict yield based on the three independent factors chosen in this study. The validation of the established model was consistent with the experimentation, with a difference between the predicted and obtained values that was less than 10%, showing the relevance of the model. A starch yield of  $45.33\% \pm 1.06\%$  was obtained at the end of the experiment. The proximal analysis of the obtained starch showed that it was low in fats and proteins with equally low ash content, indicating the excellent purity of the starch.

In subsequent studies, further characterisations will be conducted, and the application of the starch will be investigated, such as its use for bioenergy production such as bioethanol.

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## Conflicts of Interest

The authors have declared no conflicts of interest.

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