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Response Surface Optimization of Bath Type Ultrasound-Assisted Extraction (UAE) of Native Starch from Fresh Cassava Tubers

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Authors' contributions

This work was carried out in collaboration between both authors. Author TK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MSS managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to calculate the optimum conditions for extraction of starch from fresh cassava tubers using response surface methodology (RSM). In this study, Box-Behnken response surface design (BBD) was used to optimize the extraction process conditions (3 independent process factors at 3 levels with 17 runs) and to evaluate the main, linear and combined effects of cassava starch extraction conditions. The independent process variables selected in this study were sonication power (50, 75, 100 W), sonication time (10, 20, 30 min) and solid to solvent ratio (10, 20, 30 g/ml). The non-linear second order polynomial quadratic regression model was used for experimental data to determine the relationship between the independent process variables and response. Design Expert software (version 10.0.2.0) was used for regression analysis and Pareto analysis of variance (ANOVA). Ground cassava paste of 50 g was mixed with a proper quantity of distilled water. The suspension was directly placed in the bath sonicator (operating frequency of 33 ± 3 kHz, input voltage of 240 V and heating strength of 150 W), desired sonication

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power (W), sonication time (min) and solid to solvent ratio (g/ml) were maintained by means of controller. The optimal conditions of the selected variables were obtained using derringer's desirability function as sonication power of 63.32 W, sonication time of 15.59 min and solid to solvent ratio (SS) of 19.19 g/ml) with a desirability value of 0.76. The maximum experimental cassava starch yield was 83.20% which was 8.2% higher than that of conventional wet extraction method. Under the optimized extraction conditions, the selected variables were validated (n=3), a mean starch yield of 82.28 ± 1.12% was obtained with adjusted R^2 value of 0.85. Compared with wet extraction technique, UAE required shorter extraction time and yielded higher percentage of extraction recovery. Thus, UAE could be very effective for increasing the percentage recovery of starch from fresh cassava tubers.

Keywords: Ultrasound assisted extraction; cassava starch; response surface methodology; box-behnken design; extraction.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz), an important food security tuber crop and is widely cultivated in many tropical countries of the world. It is one of the richest sources of starch and considered as poor man's crop in rural areas for the millions of people in tropical countries [1]. In India, it is cultivated about 0.20 million hectares with a total production of 8.13 million tonnes and a productivity of 22.3 metric tonnes per hectare [2]. The dried cassava tubers consists of about 80 to 90% carbohydrate, out of which the most important is starch which ranges from 78 to 90% on dry basis. It is also considered as a good source for minerals such as calcium, iron, magnesium and phosphorus and is richer in calorific value compared to other tubers such as yam, potato and sweet potato. It has nearly the same calorific value as cereals viz., wheat, rice and maize [3]. The native cassava starch is used in the food industry for production of sausage, monosodium glutamate (MSG), glucose and bakery products, whereas modified cassava starch is used for textile, glue, paper, plywood and the pharmaceutical industry [4].

In India, currently small scale cassava starch and sago industries are using conventional wet milling technique for extraction of starch, but there are many improved extraction techniques using saw type blade are being developed now days. The various unit operations involved in the mechanical wet extraction process is shown in Fig. 1.

The traditional mechanical wet extraction applied at industrial level leads to native cassava starch recovery losses up to 20% [5]. Moreover, this method is highly energy intensive and requires large quantities of water for starch recovery. The maximum recovery of extractable starch from fresh cassava tubers (25% starch on fresh weight basis) as measured by chemical method was found as 22.80% and by mechanical wet extraction method using saw type rasper was found as 76-79% [6]. The highest cassava starch recovery of 83.39% which was equal to starch content of 18.98% was reported by [7] using blade type rasper. The treatment of pectinolytic and cellulose enzymes for 2 h for cassava starch extraction resulted to a starch recovery of 21.49% compared with maximum extractable starch content of 80%, which was equivalent to the starch content of 18.20% by mechanical rasper [5]. In India, there are about 300 to 400 small, medium and large scale starch factories are available and most of the starch factories wooden raspers are employed. Though this conventional rasping devices area inexpensive but relatively less efficient than saw and blade type rasper as the rasping sheet must often replaced on account of rapid water [4].

A higher percentage recovery of starch from tropical tuber crops could be a better way to get higher economic yield for developing countries like India. Ultrasound-Assisted Extraction (UAE) is considered as an emerging green technique and found suitable alternative to conventional techniques, gaining notable attention in recent years because of reduction in solvent usage, low extraction time, increase in extraction yield and improve quality of extracts [8,9,10,11,12, 13,14,15,16]. The technique can accelerate the extraction process at very low temperature, develops negligible damage to the functional and structural properties of extracts [17]. Ultrasound is a sound wave has frequency level above 20 kHz passes in liquid medium creates cavitation. This mechanical action of cavitation with high velocity and shear force lead to high penetration in to cell membranes that causes cell disruption and increase mass transfer that results in higher

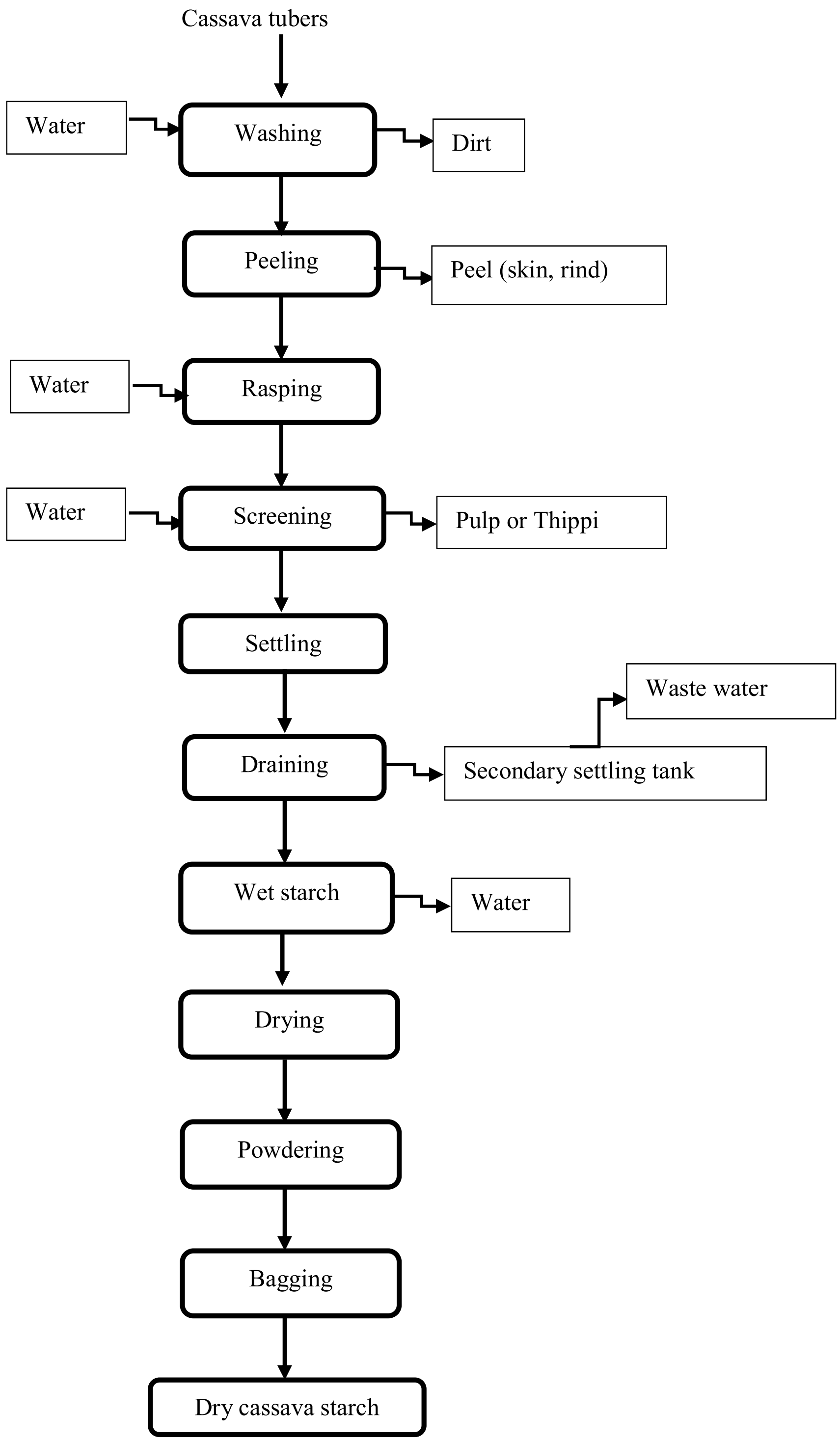

Fig. 1. Flow chart for extraction and isolation of wet cassava starch by mechanical method

efficiency of extraction [18]. UAE found effective for increasing the recovery of extracts from cereals such as maize [19,20] and rice [21].

The response surface methodology (RSM), an efficient optimization technique and combination of statistical and mathematical calculations, requires a less number of experimental runs for process optimization [22,23]. It is used as an important tool to analyze the interaction between variables and measure the effect of variables on responses [24,25,26]. The effect of ultrasound on extraction and isolation of native starch from cassava tubers has not been reported in any previous studies. Thus, the objectives of this study was to employ response surface methodology to examine the effect of independent process variables (sonication power, sonication time and solid to solvent ratio) on yield of native cassava starch from fresh cassava tubers and to optimize the extraction variables using bath type ultrasound-assisted extraction technique for native cassava starch yield.

In this study, a three level Box-Behnken design with three factors (sonication power, sonication time and solid to solvent ratio) was used to predict and optimize the effect of process variables on cassava starch yield.

2. MATERIALS AND METHODS

2.1 Raw Materials

Mulluvadi variety of cassava *(Manihot esculenta)* after 10 months of planting was procured without any physical damage from local farmer's field near Thanjavur, Tamil Nadu, India as raw material for extraction of cassava starch.

2.2 Sample Preparation

Cassava tubers were rinsed thoroughly with tap water to remove impurities on the surface. Then skin as well as rind portion of the cassava tubers were removed manually with the help of peeler followed by cutting into small pieces. Then the peeled tubers were ground into a homogeneous paste using a blender (Preethi Blue Leaf Platinum, 750 Watts) without any addition of water. This was immediately used for UAE extraction process.

2.3 UAE Method of Cassava Starch

Ultrasound-assisted extraction (UAE) of cassava starch was conducted as per the method reported by ying et al. (2011), using a bath type ultrasonicator (Life-Care, Model: MT 6) operating frequency of 33 ± 3 kHz, input voltage of 240 V and heating strength of 150 W, attached with digital timer and heater. The volumetric capacity of the bath ultrasonicator was 6.5 litres.

Ground cassava paste of 50 g was mixed with a proper quantity of distilled water. The suspension was directly placed inside the bath sonicator, desired sonication power (W), sonication time (min) and solid to solvent ratio (g/ml) were maintained by means of controller. In order to reduce the variability in conducting the experiment, a randomized order was followed. The coded levels and the corresponding experimental values of independent variables (sonication power, sonication time and solid to solvent ratio) are shown in Table 1. Experiments were carried out twice and the arithmetic mean was considered for estimation of starch yield as per the Table 2.

2.4 Determination of Cassava Starch Yield

After UAE extraction, the starch suspension was centrifuged at 2200 x g for 20 min (Remi R-24 centrifuge, India) and passed through a fine mesh muslin cloth to separate the starch and the pulp. Starch in the filtrate was kept overnight to sediment and then the liquid was decanted. The wet cassava starch was then again washed three times thoroughly for white colour and dried at 50ºC for 12 h to obtain a moisture content of starch sample about 11-12% w.b. It was sieved through standard 72 mesh size BSS sieve and ground into powder using pestle and mortar. The yield of cassava starch (%) was calculated using eq. (1).

$$\text{Starch yield (\%)} = \frac{W_2}{W_1} \times 100 \qquad (1)$$

Where, W_1 was initial starch content in the material (%) and W_2 was the content of starch released after the ultrasonic treatment

2.5 Experimental Design

A four factor three levels completely randomized factorial design (CRFD) was adopted in this present study to examine the effect of ultrasonic treatment on the yield of cassava starch was studied. The factors were sonication power of 50-100 W, sonication time of 10 to 20 min and solid to solvent ratio of 1:10 to 1:30 g/ml) on the extraction yield of starch from cassava tubers. From the preliminary experiments on single

Table 1. Coded levels and the corresponding experimental values of independent variables used in Box Behnken Design

Coded levels	Independent variables		
	Sonication power (W)	Sonication time (min)	Solid-solvent ratio (g/ml)
-1	50	10	10
0	75	15	20
1	100	20	30

factor test, levels for independent experimental process variables were selected. It consisted of 17 experiments with 5 central points for estimating experimental error. The total number of experiments (N) for this study was measured using the eq. (2).

$$N = 2F(F-1) + P_1 \quad (2)$$

Where, F is number of variables; P_1 is the replicate number of centre points.

For statistical measurements, the process independent variables were coded with three levels between -1, 0 and +1 and the coding was performed by using the eq. (3).

$$Y_i = \frac{y_i - y_z}{\Delta y_i} \quad i = 1,2,3, \ldots\ldots\ldots\ldots\ldots. k \quad (3)$$

The percentage recovery of cassava starch yield (%) was measured using second order polynomial quadratic response model. The generalized form of the non-linear quadratic second order polynomial response model is presented in the eq. (4).

$$\text{ResponseY}) = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_i \sum_{<j=2}^{k} \beta_{ij} X_i X_j \quad (4)$$

Where Y indicates starch yield; X_i and X_j denotes process independent variables (i and j range from 1 to k) and β_0 represents interception coefficient of regression model; β_j, β_{jj}, β_{ij} are linear, quadratic and interaction coefficients; *k* indicates the number of independent process variables (k =3).

2.6 Determination of Desirability and Validation of Optimized Conditions

Optimization of multiple responses for various independent process variables is performed by derringer desirability function [27]. This is one of the most widely used techniques for multi response optimization. In this technique, the predicted response (starch yield) is transformed into a dimensionless partial desirability function (g_i), which varies from 0 to 1. The required goals of response and independent process variables were chosen. For maximizing the response, the independent process variables were kept within range, where the response was maximized with the help of desirability function (D).

$$D = (g_1 \times g_2 \times g_3 \times \ldots\ldots\ldots\ldots.. \times g_{n)}^{\frac{1}{n}} \quad (5)$$

Where, g_i is desirability of response; n is number of responses. If any one of the variable response is outside the desirability, the total function will be converted into 0. g_i ranges between completely undesired response to fully desired response (0 to 1). The maximization and transformation of response into a multi-response dimensionless desirability (T_i) was done using the eq. (6).

$$T_i = \frac{Z_i - Z_{min}}{Z_{max} - Z_{min}} \quad (6)$$

Where, Z_{min} is the minimum value of response; Z_{max} is the maximum value of response; Z_i is the weight of individual response.

Triplicate processing experiments were conducted to confirm the results under the optimal conditions and its mean values were compared with the predicted values at the same conditions to validate the developed regression model.

2.7 Statistical Analysis

Experimental data were analyzed by least square method of multiple regression analysis. Pareto analysis of variance (ANOVA) at 95 % level of confidence ($p<0.05$) was applied to calculate linear, quadratic and interaction coefficients of regression model so as to measure the significance of process variables. F-value, predicted error sum of squares (PRESS) and predicted R^2 was considered to check model adequacy. RSM was applied using a Design Expert statistical package version 10.0.2.0 (Stat Ease Inc., Minneapolis, MN, USA) to determine the optimal response (starch yield).

Table 2. Box-Behnken response surface design (BBD) with the experimental and predicted values on starch yield

Run order	Sonication power (W) X_1	Sonication time (min) X_2	Solid –solvent ratio (g/ml) X_3	Starch yield (%)		Residual error	% Error	Absolute error
				Experimental	Predicted			
1	75	70	15	79.03	79.82	-0.79	-0.99	0.79
2	75	50	20	81.15	81.68	-0.53	-0.65	0.53
3	100	90	15	80.69	80.16	0.53	0.65	0.53
4	100	70	20	83.23	82.44	0.79	0.94	0.79
5	100	70	10	80.03	80.08	-0.045	-0.05	0.04
6	75	70	15	82.38	82.68	-0.30	-0.36	0.30
7	75	70	15	80.32	80.02	0.30	0.37	0.30
8	50	70	20	81.59	81.55	0.045	0.05	0.04
9	50	70	10	82.88	82.04	0.84	1.01	0.84
10	75	90	20	81.18	81.67	-0.49	-0.60	-0.49
11	75	70	15	81.01	80.52	0.49	0.60	0.49
12	100	50	15	81.15	81.99	-0.84	-1.03	-0.84
13	75	90	10	82.98	83.06	-0.082	-0.09	0.08
14	50	50	15	82.96	83.06	-0.10	-0.12	0.10
15	75	50	10	83.18	83.06	0.12	0.14	0.12
16	50	90	15	82.99	83.06	-0.072	-0.08	0.07
17	75	70	15	83.20	83.06	0.14	0.16	0.14

3. RESULTS AND DISCUSSION

3.1 Experimental Data Analysis Using BBD

Experiments were conducted so as to study the linear, cubic, quadratic and interaction effect of independent process variables (sonication power, sonication time and solid to solvent ratio) and optimize the response (starch yield) and the results are listed in Table 2.

The experimental data were fitted to various polynomial models viz., linear, interactive (2FI), quadratic and cubic models. Different statistical tests performed to calculate the suitability models for higher response (starch yield) were viz., sequential model sum of squares, lack of fit tests and model summary. The analyzed parameters are presented in Table 3.

The results showed that quadratic model was statistically highly significant and showed higher value of R^2, adjusted R^2 and predicted R^2 and also exhibited a low *p*-value (Table 3). Thus, the developed quadratic model found to be best suitable for higher starch recovery from fresh cassava tubers.

3.2 Statistical Analysis

Pareto analysis of variance (ANOVA) and the model regression coefficients for the experimental data were compared by their corresponding *p*-values mentioned in the Table 4. By comparing the *p* values, it could be mentioned that linear coefficient (X_1) was found to be highly significant. The adequate precision (*F*-value) of the model was 24.70, which indicated the model was significant at $p<0.0016$. The goodness of fit of the developed model was estimated by regression coefficient (R^2=0.9355) and adjusted regression coefficient (R_a^2= 0.8527) and coefficient of variance (CV= 0.66 %). This CV value shows the difference between the predicted and experimental values. The developed quadratic model showed a high degree of accuracy with good deal of adoptability. The adequate precision of model compares the range of predicted values to the mean prediction error at the design points. In general, F value greater than 4 is acceptable and the present model got F value of 16.09, which shows that this model is significant for higher starch recovery from cassava tubers.

Table 3. Sequential model fitting for cassava starch yield

Source	Sum of square	Mean square	DF	F value	Prob>F	Remarks
Sequential sum of squares						
Mean	82895.89	82895.89	1			
Linear	7.09	2.36	3	1.96	0.1702	
2FI	0.35	0.12	3	0.077	0.9713	
Quadratic	13.88	4.63	3	22.04	0.0006	Suggested
Cubic	1.43	0.48	3	48.49	0.0013	Aliased
Residual	0.039	0.0098	4			
Total	82918.68	4877.57	17			
Lack of fit tests						
Linear	15.66	1.74	9	177.01	<0.0001	
2FI	15.31	2.55	6	259.54	<0.0001	
Quadratic	1.43	0.48	3	48.49	0.0013	Suggested
Cubic	0.000	0				Aliased
Pure error	0.039	0.0098	4			
Model summary statistics						
Source	**SD**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**Press**	
Linear	1.10	0.31	0.81	0.15	24.60	
2FI	1.24	0.32	-0.07	-0.95	44.50	
Quadratic	0.46	0.93	0.85	-0.00	22.94	Suggesetd
Cubic	0.09	0.99	0.99	-	+	Aliased

Table 4. Analysis of variance of the regression coefficients of the fitted polynomial quadratic model of starch yield

Source	Coefficient estimate	Sum of squares	Degree of freedom	Standard error	Mean square	*F* value	*p*-Value
Model	71.14	21.32	9	0.20	2.37	11.29	0.0021
X_1	0.81	5.18	1	0.16	5.18	24.70	0.0016
X_2	-0.46	1.71	1	0.16	1.71	8.15	0.0246
X_3	0.16	0.20	1	0.16	0.20	0.95	0.3633
Residual		1.47	7		0.21		
Lack of fit		1.43	3		0.48	48.49	0.0013
Pure error		0.039	4		0.0098		
Cor total		20.58	16				
Std. dev.	0.46			R^2		0.9355	
Mean	69.83			Adj- R^2		0.8527	
C.V. %	0.66			Pre- R^2		0.7201	
Press	22.94			Adequate Precision		16.098	

3.3 Fitting of Non-linear Quadratic Polynomial Model to the Uniformity Response

The purpose of optimizing the process using various independent process variables is to increase the response. The suitability of model for estimating the optimal response (starch yield) was tested under optimal conditions. The effects of sonication power, sonication time and solid to solvent ratio on the starch yield were studied. The experimental data obtained were assessed by design expert software and fitted to quadratic regression model for response (starch yield) in terms of actual factors. The predicted second order non- linear quadratic model in terms of coded factors is shown in the eq. (7).

$$Starch\ yield\ (\%) = 83.06 + 1.04\,X_1 + 0.27\,X_2 - 0.37\,X_3 + 0.11\,X_1X_2 - 0.27\,X_1X_3 + 0.46\,X_2X_3 - 1.26\,X_1^2 - 0.78\,X_2^2 - 0.73\,X_3^2 \quad (7)$$

3.4 Adequacy of the Developed Quadratic Model

Checking of model adequacy is to calculate whether the developed regression model is adequate or not and how best the developed model predicts the response. Analysis of variance was used to check the adequacy of the developed polynomial quadratic model. The diagnostic residual analysis for validating the model is presented in the Fig. 2. The predicted and the experimental data points were close enough and showed a straight line relationship, which confirms the good agreement between the experimental independent process variables and the response (Fig. 2A). This clearly depicts that the developed model is successful to capture the correlation between the independent process variables on the response (starch yield). The normal probability/test plot is a graphical tool suitable to determine residuals normality. The goodness of fit model is a component of regression analysis and calculated using the values of internally studentized residuals and normal % probability. In this present study, all the data points were within acceptable limits as they lie on a straight line. This confirms that experimental data are normally distributed. Since all the leverage values of the model falls below 1, there were no unexpected errors occurred (Fig. 2C). However, Fig. 2D shows that no excessive deviation or influence in the experimental data based on the beta values plot.

3.5 Effect of Experimental Process Independent Variables on Response

The various process variables (sonication power, sonication time and solid to solvent ratio) were studied on the response (starch yield). A BBD design under three variables at three levels was performed to assess the effects of experimental process variables on starch yield. To understand the interaction between the process variables and the response, a three-dimensional (3D) response surface and contour graphs were drawn by keeping the third variable constant at the "0" level and adjusting the other two variables. This is variably used to find out the optimal conditions. The response surface plot estimating the starch yield against independent process variables are presented in Fig. 3.

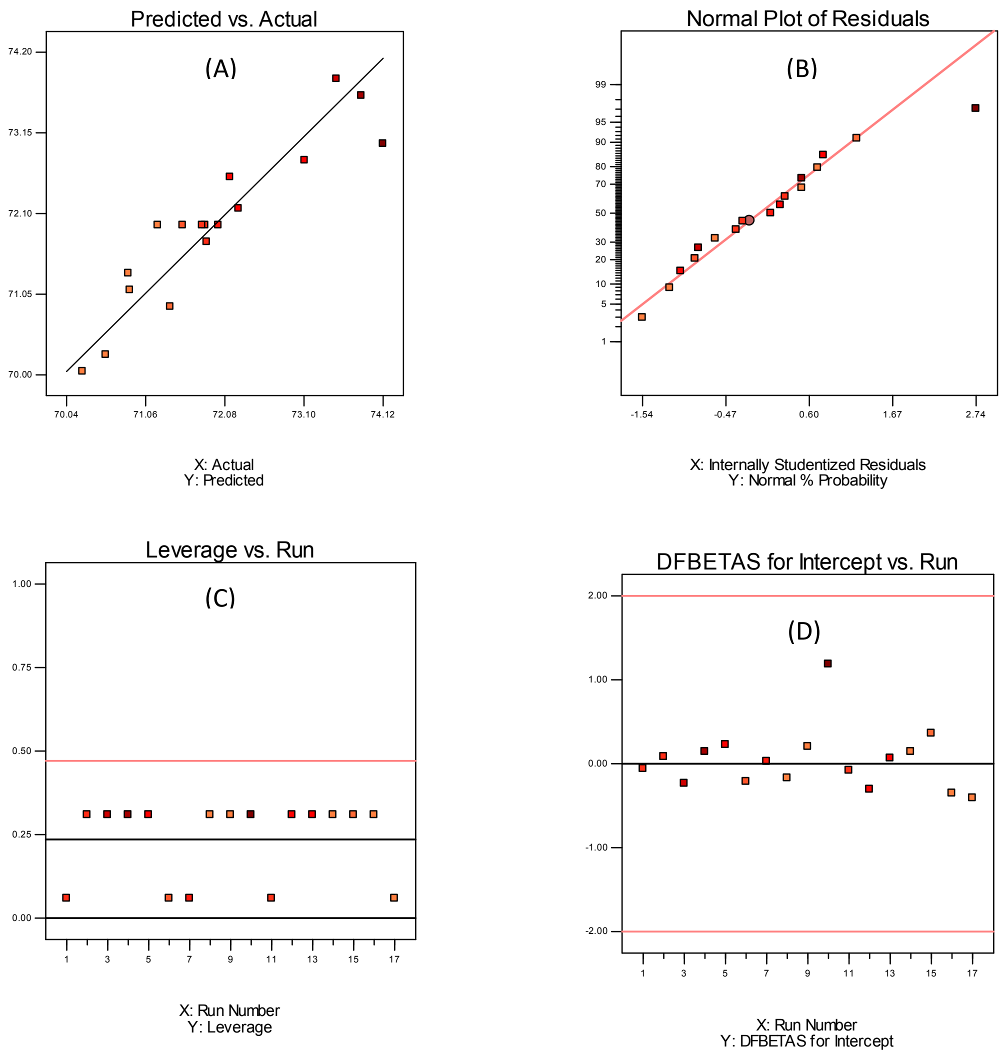

Fig. 2. Diagnostic residual plots for the response surface model

3.5.1 Effect of sonication power

In this study, the potential effect of different sonication power (50-100 W) on the response (starch yield) was studied. The cassava starch yield was increased with increase in sonication power up to 87.5 W and then decreases with higher sonication time and solid to solvent ratio (Fig. 3A, 3B). As larger ultrasound wave passed over the liquid medium, harsh shock waves and high speed jets were developed by ultrasonic waves, stimulates the swelling of matrix and increase of micro bubbles in the matrix, which permits higher diffusivity in the cassava mash and increase the starch extraction yield [28,29,30]. Sonication power has a greater influence on cavitational effect. Furthermore, beyond sonication power of 87.5W could affect the increase of number of bubble formation in solvent (water) during cavitation, which might diminish the working potential of ultrasound power transferred into the liquid medium and reduced the extraction yield of cassava starch [31]. Based on the present study, the optimum sonication power for higher starch yield was 63.32 W.

3.5.2 Effect of sonication time

Sonication time is an important and highly influencing variable for extraction of the cassava starch. The influence of sonication time on cassava starch yield was studied and the results are presented in the Fig. 3. The starch yield was gradually increased with increase in time up to 17.50 min due to swelling and hydration of starch was initiated simultaneously by cavitational effect of ultrasonic waves [32] and then slowly decreases with higher solid to solvent ratio (Fig. 3A, 3C). The diffusion of ultrasonic waves improves the leaching of starch from cassava mash into the surrounding solvent and enhances the extraction yield [33]. Nevertheless, the penetration and heating effect of ultrasonic treatment for longer duration might cause the structural changes of starch that reduces the recovery of cassava starch due to frequent asymmetric collapse of micro-bubbles. Therefore based on the results the sonication time of 15.59 min was found to be optimum for higher starch yield.

3.5.3 Effect of solid to solvent (SS) ratio

The SS ratio is also one of the important factors responsible for enhancing the starch yield. In this study, a different SS ratio ranged from 1:10 to 1:30 g/ml was studied and showed that the starch yield was highly influenced by SS ratio. The higher ratio of solid to solvent means higher concentration difference which in turn facilitates the mass transfer rate results and yield higher starch recovery [34]. However, the high ratio of raw material to water beyond the required level would not change much of driving force (diffusion rate) furthermore as transfer of mass is mostly confined towards interior solid tissues [35]. Further, this is associated with the cavitation formation which invariably requires negative

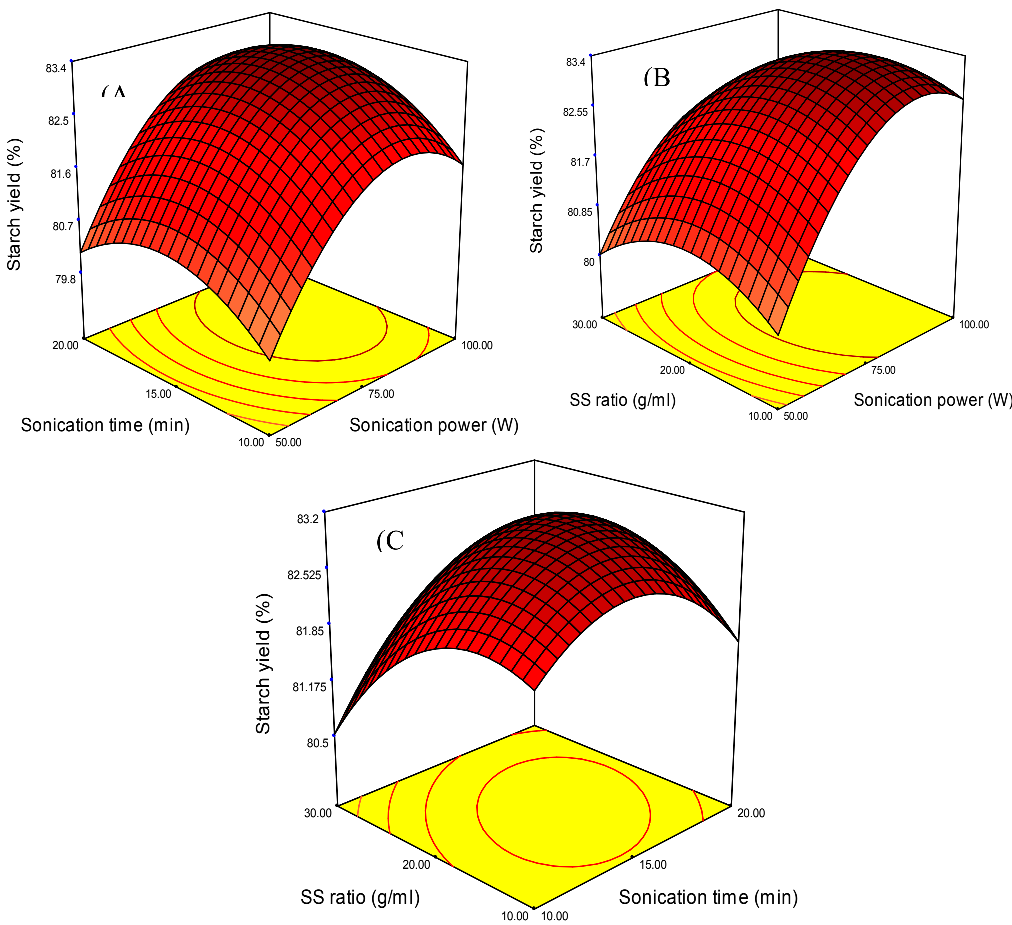

Fig. 3. Response surface plot for interaction effects of independent process variables on starch yield

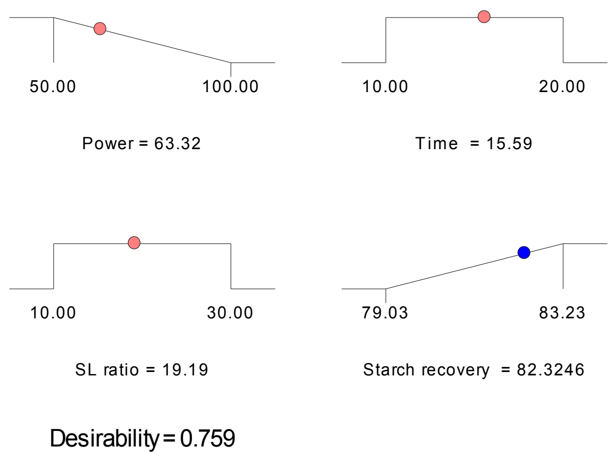

Fig. 4. Desirability ramp for numerically optimized process conditions

pressure in the rarefaction cycle to overcome the natural cohesive forces [36]. In consideration of the optimum conditions of other two independent process variables (sonication power of 63.32 W and sonication time of 15.52 min), a solid to solvent ratio of 1:19 g/ml was selected an optimum for higher cassava starch yield.

3.6 Determination and Validation of Optimized Extraction Process Conditions

To achieve a maximum starch yield from fresh cassava tubers, a multivariate regression model (Eq. (4)) was employed. To optimize the process conditions, derringer's desired methodology was adopted and as follows: sonication power of 63.32 W, solid to solvent ratio of 19.19 g/ml, and sonication time of 15.59 min. Under these optimum conditions, the predicted cassava starch yield was 82.32% with desirability value of 0.759. For validating the optimum extraction process conditions, confirmatory experiment in triplicates (n=3) was successfully performed under the optimal conditions. The mean starch yield from the confirmatory trial was found to be 82.28±1.12%. The experimental results obtained were closely associated to the data obtained from the optimized conditions of the model. The desirability ramp for optimal points was generated by numerical optimization technique (Fig. 4).

4. CONCLUSIONS

In this present study, a three level Box-Behnken design (BBD) with three factors was successfully applied to optimize the independent process variables (sonication power sonication time and solid to solvent ratio) for extraction of cassava starch. The design of experiments, second order polynomial nonlinear regression models and numerical optimization were performed using commercial statistical software (Design-Expert®). A maximum determination coefficient (R^2) of 0.935 was obtained for second order polynomial quadratic model from ANOVA and confirmed the developed model was in best fit with the experimental runs. Based on the derringer's desired function, the optimal conditions were found to be, sonication power of 63.32 W, solid to solvent ratio of 19.19% and sonication time of 15.59 min resulted in maximum starch recovery percentage of 82.32%. Under these optimized conditions, the experimental results (83.20%) were in close relationship with the predicted results of starch yield (82.32%). Thus, the extraction of starch from fresh cassava tubers for various industrial applications could be successfully extracted through bath type UAE system.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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