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Modelling of Milk Solids Extraction from Tigernut (*Cyperus Esculentus* L) Tubers Using Response Surface Methodology

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Abstract Vegetable milk solids from black and brown varieties of tiger nut (*Cyperus esculentus* L) tubers were extracted using water. Response surface methodology was used to determine the optimum conditions for extraction. The factors and their levels were boiling time (10.125, 240 minutes), milling time (5, 17.5, 30 minutes), and tuber: water (1: 2, 1:5, and 1: 8). The data obtained were fitted to second order polynomial models to enable the determination of variables for the estimation and prediction of the extraction of milk solids. The optimum extraction conditions were for the black cultivar; milling time, 23.8 minutes; tuber: water, 1 g: 7.8 ml; boiling time, 240 minutes and for the brown cultivar, milling time, 22.0 minutes; tuber: water, 1 g: 7.2 ml; boiling time, 10 minutes. Under the experimental conditions the expected milk solids for the black tubers was 65.07 (0.40) % against the predicted value of 70.66 % and that for the brown, 74.84 (1.84) % against the predicted value of 80.99 %.

Keywords Tiger nut tubers, Milk solids extraction, Response surface methodology

1. Introduction

Tiger nuts (*Cyperus esculentus* L) have been cultivated extensively in Africa, Asia and some European countries for centuries [1-2]. It is popularly eaten (raw or roasted) because of its delicious taste as a snack. An aqueous extract is also made from it as tiger nut milk and used for other food products. A popular beverage called *chufa de horchata* in Spain and *atadwe milk* in Ghana is a common product made from the tubers of tiger nuts

[3-6]. The nutritional composition of this drink has been reported as 25.0 g kg-[1] fat, 18.0 g kg-[1] protein, 22.0 g kg[-1] starch, and 4.0 g kg-[1] ash [7]. Tiger nut fat has also been reported to be rich in myristic acid, oleic acid and linoleic acid [8]. It has also been suggested in several reports that tiger nuts have some health benefits, which include reducing the risk of colon cancer, helping to prevent heart attacks, thrombosis and activating blood circulation [4, 7]. Consequently, the application of tiger nuts in food products will have consumer appeal for its nutritional and perceived nutraceutical benefits. Though considerable studies have been conducted into its nutritional quality, sensory characteristics, preservation and shelf life, [7, 9-14] more systematic studies need to be explored for its successful use as an ingredient in some food applications. To achieve this, a clear and efficient extraction protocol of the tiger nuts solids needs to be established. A number of studies have considered various extraction variables for tiger nut milk to include tuber size, soaking, roasting, toasting temperatures, and milling time [12, 15-17]. Several other studies have shown that milling time, cooking time and meal: water ratios are very important processing factors in the aqueous extraction of solids from plant materials [18-22]. The objective of this study was to optimize boiling time, milling time and tuber: water ratio for the production of milk solids from two varieties of tiger nut tubers using response surface methodology (RSM).

2. Materials and Methods

Black and brown varieties of tiger nut tubers were purchased from farmers in Ampenyi in the Komenda Edina Eguafo District and Twifo Praso in the Twifo Hemang Lower-Denkyira District all in the Central Region of Ghana. The tubers were sorted, washed and dried in a Sanyo oven (Model MOV-212, Japan) at 55℃ till moisture was constant between 6 and 8 %. The samples were cooled, packaged and stored until used for the study.

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2.1. Experimental Design and Data Analyses

The independent extraction variables considered were milling time (x_1), tuber: water (x_2) and boiling time (x_3). The variables and their levels are as shown in Table 1. A Face Centered Central Composite rotatable Design [23] was employed in the designing of experiments using MINITAB 14 statistical package (MINITAB Inc., USA). Twenty sample combinations were generated (Table 2) for the extraction of milk.

2.2. Tiger Nuts Milk Extraction Procedure

One hundred grams (100 g) of the oven dried tubers (W1) with the corresponding volume of water according to the experimental design (Table 2) was used to boil the tiger nuts at the specified time. During boiling, water was topped up in runs where it was depleted due to evaporation. After the required time of boiling, the nuts were drained and weighed. The boiled tiger nuts were then submerged in a volume of water that was twice its weight and then wet milled in a Logik blender (model RSH-005494-018, China) at high speed for the appropriate time according to the experimental design (Table 2). The blended tiger nuts were filtered through a cheese cloth and weighed to obtain tiger nuts milk (W2) which was used for the determination of milk indices.

The effects of the independent variables on extracted milk was expressed as percentage of milk from tubers and determined as follows;

Percentage Milk Extracted (ME) = (W2/W1) x 100
W2 = weight of extracted milk
W1 = weight of tubers

The soluble and insoluble content of the milk was determined by drying a known volume in a calibrated dish in an oven at 105℃. Percentage milk solid (MS) was expressed as W3/W2 x100, where

W3 = Weight of dry powder.

The Percentage expected milk solids (EMS) an estimate of the total obtainable milk solids was then expressed as MS x ME

Each analysis was conducted in triplicate and the results averaged. Response surface regression analyses were used to model the effect of the independent variables on the percentage expected milk solids (g kg-^{1}) using Minitab 14 statistical package. Experimental data were fitted to a quadratic polynomial model. The model proposed for the response (Y) was as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x^2_1 + b_{22}x^2_2 + b_{33}x^2_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

The adequacy of model was checked based on R^2 and adjusted-R^2 as well as the lack of fit error

Table 1. Independent process variables and their corresponding levels

MILK SOLIDS EXTRACTION				
Independent variable	**Levels**			
	Symbol	**-1**	**0**	**1**
Milling time (min)	X_1	5	17.5	30
Tuber: Water (g ml-^{1})	X_2	2	5	8
Boiling Time (min)	X_3	10	125	240

Table 2. Matrix of experimental runs and indices of milk from tiger nut tubers

VARIABLES AND THEIR LEVELS				BLACK TUBERS (g kg-^{1})			BROWN TUBERS (g kg-^{1})		
Run Order	**X_1**	**X_2**	**X_3**	**ME**	**MS**	**EMS**	**ME**	**MS**	**EMS**
1	-1 (5.0)	-1 (2)	-1 (10)	2038.7	239.5	488.3	2075.8	242.6	503.6
2	-1 (5.0)	0 (5)	0 (125)	5336.6	109.9	586.5	5128.3	116.4	596.9
3	0 (17.5)	0 (5)	0 (125)	5763.7	122.7	707.2	5685.7	126.3	718.1
4	1 (30.0)	-1 (2)	1 (240)	1486.4	245.7	365.2	1687.7	253.6	428.0
5	0 (17.5)	0 (5)	-1 (10)	5549.1	119.3	662.0	5720.8	128.7	736.3
6	0 (17.5)	0 (5)	0 (125)	5723.8	121.0	692.6	5800.2	126.0	730.8
7	0 (17.5)	0 (5)	0 (125)	5554.4	118.7	659.3	5602.3	127.6	714.9
8	1 (30.0	1 (8)	-1 (10)	7685.7	81.4	625.6	8991.1	88.2	793.0
9	0 (17.5)	0 (5)	0 (125)	5488.1	123.0	675.0	5558.8	127.6	709.3
10	-1 (5.0)	1 (8)	-1 (10)	8640.9	75.6	653.2	8855.2	79.4	703.1
11	-1 (5.0)	-1 (2)	1 (240)	1434.9	237.1	340.2	1596.6	238.0	380.0
12	0 (17.5)	1 (8)	0 (125)	8727.1	82.0	715.6	8913.9	84.5	753.2
13	0 (17.5)	0 (5)	0 (125)	5428.2	123.0	667.7	5595.8	125.9	704.5
14	-1 (5.0)	1 (8)	1 (240)	8700.8	74.7	650.0	8873.7	76.0	674.4
15	1 (30.0)	1 (8)	1 (240)	8811.3	83.5	735.7	8856.3	86.3	764.3
16	1 (30.0)	0 (5)	0 (125)	5318.1	127.3	677.0	5467.4	128.8	704.2
17	0 (17.5)	0 (5)	1 (240)	5441.7	127.0	691.1	5674.1	128.3	728.0
18	0 (17.5)	0 (5)	0 (125)	5289.3	122.6	648.7	5276.1	125.8	663.7
19	0 (17.5)	-1 (2)	0 (125)	1795.7	247.2	443.9	1843.7	249.5	460.0
20	1 (30.0)	-1 (2)	-1 (10)	1883.0	244.3	460.2	1958.0	247.4	484.4

Table represents coded value (and real value)

3. Results and Discussion

Table 3. Coefficients of variables in regression models for expected percentage milk solids (EMS) from tiger nut tubers

SOURCE	d.f.	BLACK TUBERS		BROWN TUBERS	
		Coefficient	P - Value	Coefficient	P - Value
Parameter	9	237.2750	0.000	207.9020	0.000
Linear			0.000		0.000
x_1	1	7.8541	0.110	10.6104	0.002
x_2	1	128.5540	0.000	138.4080	0.000
x_3	1	-1.0199	0.225	-1.1243	0.008
Square			0.000		0.000
x_{11}	1	-0.2721	0.023	-0.3237	0.005
x_{22}	1	-10.5232	0.000	-10.5035	0.000
x_{33}	1	0.0002	0.897	0.0023	0.055
Interaction			0.003		0.073
x_{12}	1	0.2040	0.428	0.5033	0.048
x_{13}	1	0.0145	0.049	0.0058	0.339
x_{23}	1	0.1268	0.001	0.0444	0.097
Residual					
Error	10				
Lack-of-fit	5		0.243		0.451
Pure Error	5				
Total	19				
Adj-R^2		95.20%		96.40%	

From the data in Table 2, the black tiger nut tubers recorded 1434.9 g kg^{-1} to 8811.3 g kg^{-1} percentage extracted milk and 74.7 g kg^{-1} to 247.2 g kg^{-1} percentage milk solids while the brown tubers recorded 1596.6 g kg^{-1} to 8991.1 g kg^{-1} for percentage milk extracted and 76.0 g kg^{-1} to 253.6 g kg^{-1} for percentage milk solids. The observed values of percentage milk extracted were higher than values reported earlier [15]. This can be attributed to the lower volume of water used for the extraction in their study. The ranges of percentage milk solids recorded however were similar to values determined for tiger nut tubers planted at different periods [24] and that determined for non sweetened *Chufa de horchata* [5]. The data obtained for the percentage expected milk solids (EMS) were fitted to regression models, with the extraction variables (milling time, tuber: water ratio and boiling time) as the predictors. The fit statistics of percentage expected milk solids (Y) for the selected quadratic predictive model is shown in Table 3.

Analysis of variance of the full regression models show that in all cases, the models had no significant lack of fit (Table 3) and had adjusted R-squared values of 95.2 and 96.4 for the black and brown samples respectively. The models were therefore adequate to be used to predict the effects of the independent variables milling time (X_1), tuber: water (X_2) and boiling time (X_3) on the percentage expected milk solids (EMS).

Effect of milling time, tuber: water and boiling time on the aqueous extraction of milk solids from tubers

Expected Milk Solids

The percentage expected a milk solid (EMS) is an estimate of the total obtainable milk solids that can be extracted. It was calculated from milk and solids extracted from the tubers. From the regression models for black and brown tubers, the expected milk solids were positively influenced by milling time and tuber: water but negatively by boiling time. Table 3 also shows that while the interaction of tuber: water and boiling time was significant ($P < 0.05$) for black tubers; it was not significant for brown tubers. At low tuber: water, increased boiling time decreased EMS, while at high tuber: water longer boiling times increased the (EMS) for the black tubers. This is very well illustrated in the surface plot in Figure 1.

The model for brown tubers showed that the effects of tuber: water ratio on EMS were independent of the boiling time, but were significantly influenced by the milling time. It therefore seems that particle size and tuber: water are very important factors in the extraction of milk solids. Indeed for the brown samples, the effects of increasing milling time on EMS were marginal at low tuber: water, while it was profound at high tuber to water ratio (Figure 3). Similar trends were observed for the plots of the black samples. The combined effect of milling time and tuber: water for the tubers (Fig 2 and 3) showed that, the expected solids increased slightly with milling time from 5 minutes for both tubers to 20.6 minutes and 23.4 minutes for black and brown respectively when maximum yield of 720.5 g kg^{-1} and

769.4 g kg-[1] were obtained. The observed increases in EMS after increasing milling time were similar to earlier observations [15]. The subsequent decrease in EMS could be attributed to the finer particles generated from continuous milling plugging the pores of the cheese cloth thus slowing down the passage of more solids. Increases in EMS were however more pronounced as tuber: water increased with maximum EMS obtained at tuber: water of 1:7.1 and 1:7.4 for black and brown tubers respectively. This phenomenon has also been observed in aqueous extraction studies predicting yield quality of soymilk, extracting reducing sugar from cashew apple bagasse and for crude polysaccharides from *Plantango asiastica* [21, 25, 26]. It has been attributed to the availability of more liquid which increases the driving force of solids out of the plant material [22]. The coefficient of the reduced model EMS after re-evaluation of the full model with respect to the significance of parameters at $p \leq 0.05$ is shown in Table 3. The reduced model statements for EMS for black and brown varieties are as follow;

$$Y_{black} = 237.27 + 128.55X_2 - 0.03X^2_1 - 10.52X^2_2 + 0.12X_2X_3$$

$$Y_{brown} = 207.90 + 10.61X_1 + 138.40X_2 - 1.12X_3 - 0.32X^2_1 - 10.50X^2_2 + 0.50X_1X_2$$

Where Y_{black} is the predicted (EMS) for black tubers, Y_{brown} is the predicted (EMS) for brown tubers, X_1 is milling time (min), X_2 is tuber: water and X_3 boiling time (min).

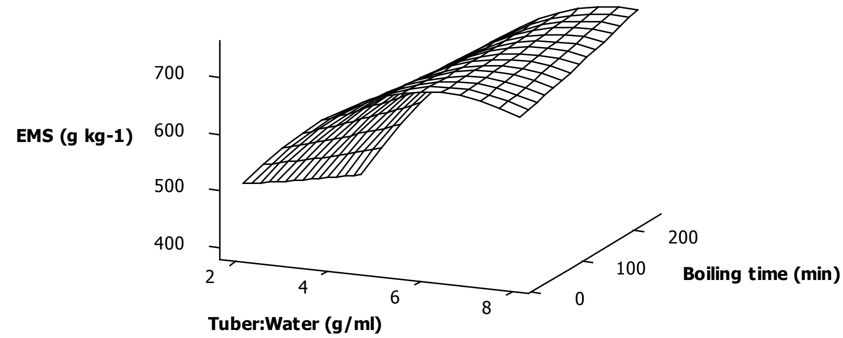

Figure 1. Surface plot for EMS (g kg-[1]) as a function of tuber: water and boiling time for black tubers

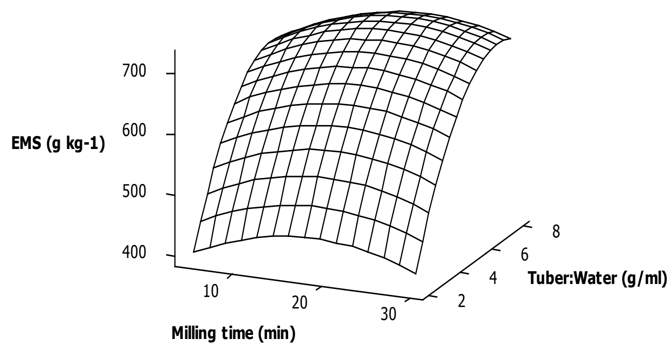

Figure 2. Response surface plot of EMS (g kg-[1]) as a function of milling time and tuber: water for black tubers

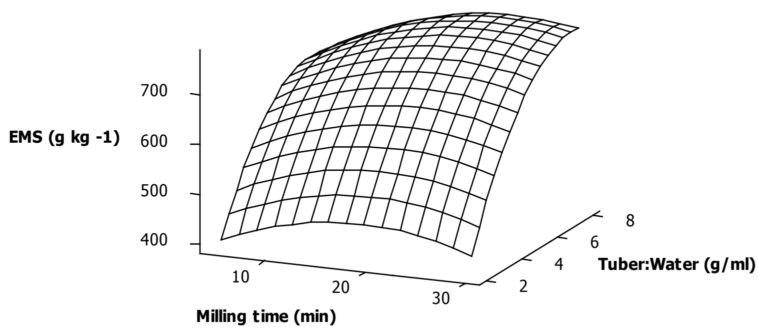

Figure 3. Response surface plot of EMS (g kg-[1]) as a function of milling time and tuber: water for brown tubers

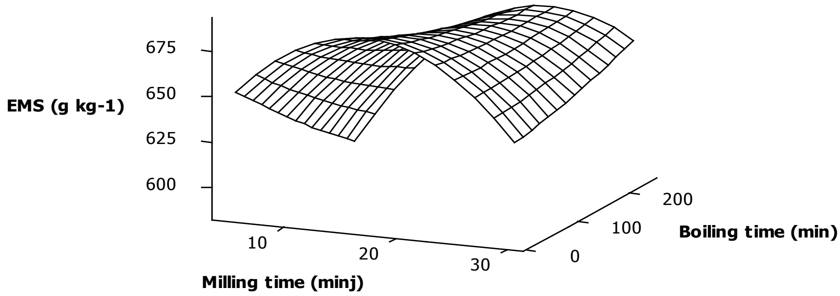

Figure 4. Response surface plot of EMS (g kg-[1]) as a function of milling time and boiling time for black tubers

Boiling time also had significant effects on the solids extracted from the tiger nut tubers (Figure 4). Increasing the boiling time increased the EMS for both samples. For black tubers, maximum EMS of 687.7 g kg-[1] was obtained after 10.7 minutes of boiling time and 16.0 minutes of milling time while for brown tubers, increasing boiling time increased milk solids till maximum yield of 762.3 g kg-[1] at 10.7 minutes of boiling time and 20.6 minutes of milling time. The increase could be attributed to the softening of hard tissues of the tuber to facilitate milling into finer particles [27]. Figure 4 also show that there were significant interaction effects of milling time and boiling time on EMS of black tubers. Significant interaction suggests that the EMS for black tubers at any milling time depended on the time period for which it was boiled. The figure again shows that there is an optimum milling time at which maximum EMS were obtained. Boiling time on the other hand decreased extraction yield at low milling times (i.e. larger particles) while it increased extraction yield at longer milling times (smaller particle size).

Determination of the factor settings for optimum yield of milk solids

Contour plots obtained from regression models were overlaid to determine the region of the variable combinations that yielded the optimum EMS, using Minitab (version 14). The optimum region was where the combinations of the variables settings of milling time, tuber: water and boiling time met the minimum criteria of 700 g kg-[1] EMS (Figure 5). The minimum criteria for the EMS were derived from preliminary extractions of black and brown tiger nut tubers. Various combination of factors that resulted in EMS above 700 g kg^{-1} are shown in Table 4.

The table shows that the optimum extraction conditions were for the black cultivar; milling time, 23.8 minutes; tuber: water, 1 g: 7.8 ml; boiling time, 240 minutes and for the brown cultivar, milling time, 22.0 minutes; tuber: water, 1 g: 7.2 ml; boiling time, 10 minutes.

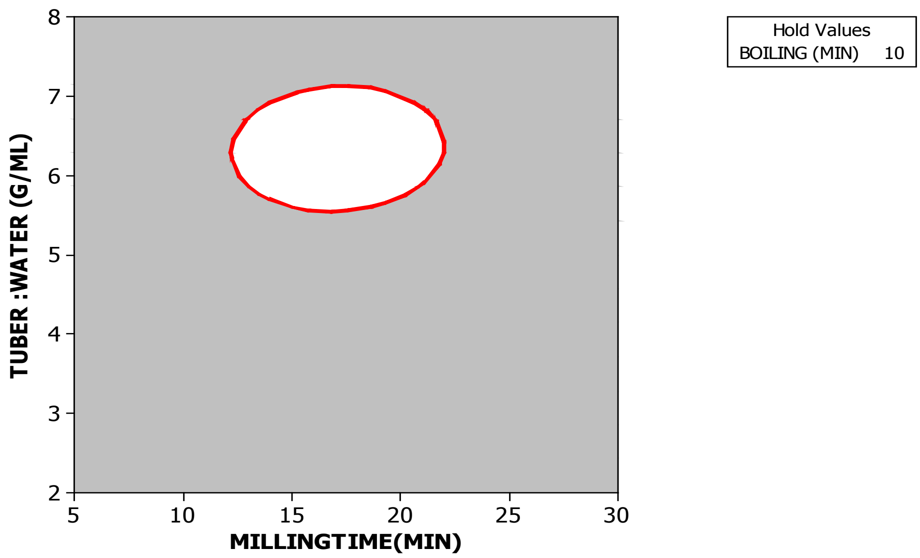

Figure 5. Overlaid cotour plot of expected milk solids for black tubers

Table 4. Summary of optimum EMS (g kg^{-1}) as a function of treatment variables

TUBERS	Milling time (X_1)	Tuber: water ratio (X_2)	Boiling time (X_3)	EMS (g kg-^{1})
Brown	22.0	1:7.2	10	809.9
Brown	5.0	1:7.9	239.3	717.5
Brown	23.4	1:7.4	125	769.4
Brown	17.5	1:7.0	10.2	803.3
Brown	22.6	1:8	10.4	802.0
Brown	24.2	1:7.6	240	793.1
Brown	30.0	1:7.4	10.2	789.6
Black	17.2	1:6.4	10	706.6
Black	20.6	1:7.1	125	720.5
Black	17.5	1:7.7	239.9	744.3
Black	23.6	1:8	239.1	754.0
Black	23.8	1:7.8	240	754.9
Black	30.0	1:7.9	239.3	743.9

Verification of predictive model

Table 5. Predicted and experimental values of the responses at the selected factor levels

Tiger Nut Cultivar	Milling time (min)	Tuber: water (g/ml)	Boiling (min)	Milk Extracted (g kg^{-1})	Milk Solids (g kg^{-1})	Predicted Value (g kg-^{1})	Experimental Value (g kg-^{1})
Black	17.0	1:6.4	10.0	689.56 (2.93)	94.4 (0.3)	706.6	650.7 (4.0)
Brown	22.0	1: 7.2	10.0	785.75 (3.36)	95.2 (1.9)	809.9	748.4 (18.4)

The reliability of the model in predicting the EMS of tiger nut milk solids was verified in separate extraction runs, using the extraction variable combinations within the optimum region in Figure 5. The two data sets (ie data obtained from using the models to predict the yield and experimental data obtained to verify the model adequacy) are shown in Table 5.

The results indicate that the predicted values of the yield (g kg^{-1}) of milk solids obtained within the optimum region were close to the experimental values, thus confirming the adequacy of the predictive models.

4. Conclusions

The yield of EMS from tiger nut tubers was determined by variables such as the degree to which the nuts were boiled before crushing, the extent to which the crushed nuts were milled, and the tuber: water during extraction. The interactions of the variables influenced the yield of EMS. Optimization of the processing conditions using Response Surface Methodology techniques showed estimated boiling time of 10 minutes, crushing and milling for about 17-22 minutes and then extracting with a 1:7 (tuber: water) will yield optimum EMS. These findings could be used as a basis for establishing scale up criteria for the extraction of tiger nut milk solids for industrial applications.

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