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Optimization of Tiger Nut (*Cyperus esculentus*) Roasting Process Using Response Surface Methodology

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Abstract

Tiger nut (*Cyperus esculentus*) is an underutilized tuber and a non-conventional oil seed which are known for their biological effect. The transformation of tubers into nutritional and stable flour could be important for West Africa populations. Thus, roasting is a process that improves the biochemical composition of seeds. The purpose of this study was to optimize temperature and roasting time on Tiger nut's tubers in order to produce high nutritional tiger nut flour. A response surface methodology was used to optimize the roasting process in a temperature range (130˚C - 150˚C) and in a duration range (20 - 35 min). The data are processed by the software Statgraphic Centurion XVI version 16.2.04. Total polyphenols and flavonoids contents, antioxidant activity, titratable acidity, browning index and Maillard reaction products absorbance were evaluated. A second-order polynomial model was established to predict optimal values, included temperature and time. The contents of polyphenols and flavonoids range respectively from 0.196 to 0.891 g GAE/100g and from 0.022 to 0.051 g CE/100g, the antioxidant activity ranges from 25.21 to 57.09%, titratable acidity varies between 0.193 and 0.472 g/100g, the browning index from 28.42 to 56.20 and the absorbances at 420 nm of Maillard Reactions Products ranged between 0.365 and 0.897. The global desirability was 90%. Roasting had significant effects ($p < 0.05$) on titratable acidity, browning index and antioxidant activity. The optimum roasting conditions

were established at the temperature of 147˚C for 38 minutes. Experimental values of parameter for flour obtained in optimal condition were proximate to predict values excepted titratable acidity. The model performance is acceptable for this study. The research for the optimal roasting conditions for tiger nut reveals an appropriate temperature and time combination which could improve the biochemical composition with a more attractive color.

Keywords

Tiger Nut, Roasting, Optimization, Physicochemical And Biochemical Analyses

1. Introduction

Tiger nut (*Cyperus esculentus*) is an underutilized tuber cultivated in West Africa. These tubers are considered to be an excellent mineral and vitamins source [1] [2]. Tiger nuts are a non-conventional oleaginous tuber rich in oils that contain essential fatty acids [3] [4]. They constitute an important source of phytochemicals compounds such as isoflavones, flavonoids, terpenoids, alkaloids, saponins, and polyphenols [5] [6]. Recent studies have proved the biological effects exhibited by the tiger nut tubers, such as anti-diabetic, cholesterol-lowering, hepatoprotective, aphrodisiac [7] [8] [9], antioxidant and antibacterial [10] and galactogenic proprieties [11].

Tiger nut has been the subject of several studies, but few studies have been focused on the roasting of tubers. Roasting is a heat treatment used to induce the development of the typical color, taste and contributes to the formation of the desired aroma; this also changes the chemical composition, modification of nutritional value, inactivation of certain toxins and enzymes, improvement of antimicrobial and antioxidant activity, digestibility and shelf life of product [12] [13]. It also leads to the formation of compounds derived from the Maillard reaction, known for pronounced antioxidant properties, improving the stability of nuts and seeds [14].

This study was focused on the roasting impact on tiger nuts physicochemical and biochemical proprieties using response surface methodology in order to determine the optimal roasting conditions. This methodology has been used in many studies for optimization of seed roasting process.

2. Materials and Methods

2.1. Sample Preparation

Tiger nut (*Cyperus esculentus*) samples were obtained from a local market in Bamako. The samples were separated from stalks and stem and washed thoroughly under running water to remove dirt. They were dried (**Figure 1**) and placed into a plastic bag.

Figure 1. Tiger nut tuber.

2.2. Roasting Process and Experimental Design

The process parameters considered for the study were the roasting temperature and duration. Tiger nuts were placed in an oven (Memmert). Preliminary tests were allowed to fix the limits of these factors. The two independent variables ranges are: (20 to 35 minutes) for roasting duration and (130˚ to 150˚) for roasting temperature (Table 1).

Response surface methodology (RSM) has been used in many studies for optimization of seed roasting process. A central composite design (CCD) of response surface methodology (Table 2) was carried to study the main and combined effects of main roasting conditions on the physicochemical properties of roasted tiger nut flour, to create models between the variables, and to use these variables to optimize the roasting conditions for the production of high-quality nut flour. For the study five levels of roasting temperatures and roasting durations were used and 10 samples were generated (Table 1). Roasting temperatures were 126˚C, 130˚C, 140˚C, 150˚C, and 154˚C, while roasting durations were 17, 20, 28, 35, and 38 minutes.

The effect of two independent variables X_1 (roasting duration) and X_2 (roasting temperature) on six response variables Y_i (Y_1 - Y_6), namely polyphenols content, flavonoids content, antioxidant activity, titrable acidity, browning index, and absorbance of Maillard Reaction Products were evaluated by using RSM.

2.3. Physicochemical and Biochemical Analysis

2.3.1. Phenolics and Flavonoids Content Analysis

Polyphenols content was determined using Folin-Ciocalteu reagent according to the method described by Georgé *et al.* [15] with little modification. Two (2 g) of flour sample was soaked in 10 mL methanol-water 70% (v/v) and mixing for 30 min and centrifuged at 4000 rpm for 10 min. An aliquot (50 µL) of supernatant and 450 µL of water were mixed and oxidized with 2.5 mL of Folin-Ciocalteu's reagent at 1/10 dilution. The mixture was re-mixed and incubated for 2 min. After the incubation, 2.5 mL of sodium carbonate solution (75 g/L) were added in

Table 1. Level of independent variables, temperature and time used central composite experimental design.

Independent Variables	Symbol	Unit	Level	
			Minimum	Maximum
Roasting duration	X_1	Minutes	20	35
Roasting temperature	X_2	˚C	130	150

test tube. The mixture was vortexed and placed for 30 min in a water bath at 50˚C. After cooling, the absorbance was measured at 760 nm using spectrophotometer (GENESYS 10S UV-VIS, Thermo Scientific). Calibration curve was drawn using the standard drug, gallic acid. The polyphenol content was determined using the calibration curve and expressed as milligrams of gallic acid equivalent (GAE) per gram of the plant extract.

Total flavonoids content was determined using the colorimetric method. Tiger nut tuber flour (0.5 g) was treated with 10 mL of methanol, stirred and centrifuged. Distilled water (400 µL) and 5% sodium nitrate (30 µL) were added to 2 mL of supernatant. The mixture was mixed and incubated at room temperature for 5 min, after which, 20 µL $AlCl_3$ (10%) and 200 µL Na_2CO_3 were added and the mixture was mixed and re-incubated for another 5 min. After the incubation, 250 µL of distilled water were added to the final mixture and the absorbance was determined at 510 nm against a blank (distilled water). The flavonoids content was calculated and expressed as g of catechin equivalent (CE) per 100 g of flour.

2.3.2. DPPH-Radical Scavenging Activity Assay

The free-radical-scavenging activity of tiger nut flour extracts was measured using 1,1diphenyl-2-picrylhydrazyl (DPPH•) according to William Brand's method [16] with some modifications, by soaking 2 g of flour in 20 mL methanol (95%). A volume of 0.1 mL of extract (antioxidant solution in methanol) was added to 2.9 mL methanol DPPH• solution (0.6×10^{-5} mol/L) and was shaken with a vortex. This was allowed to incubate at room temperature for 30 min in a dark place. The intensity of decolorization (absorbance) of purple free radical DPPH• solution was measured at 515 nm using a spectrophotometer (GENESYS 10S UV-VIS, Thermo Scientific). The same procedure was repeated without the tiger nut flour extract for control. Thus, to determine the absorbance at $t = 0$ min, absorbance was immediately taken after adding 0.1 mL methanol to 2.9 mL DPPH• solution. Methanol solution was used as the blank. The antioxidant activity was calculated using the following equation:

$$\%\ \text{DPPH•}\ \text{inhibition} = \left[1 - \frac{\text{A extract solution at 30 min}}{\text{A control at 0 min}}\right] \times 100 \qquad (1)$$

2.3.3. Total Titratable Acidity

Ten grams (10 g) of flour was mixed with 90 mL distilled water in to a 250 mL beaker. The mixture was shaken until particles were evenly suspended and free of lumps and digested for 30 minutes with frequent shaking. The mixture was al-

lowed to stand for 10 mins for the particles to settle. The supernatant was decanted into the 250 mL beaker, and used for the determination of total titratable acidity according the method of Torte *et al.* [17]. Phenolphthalein indicators (3 drops) were added to the solution. Titration was carried out by adding 0.1 M NaOH until the endpoint identification by a color change to pink. The volume of NaOH added was multiplied by 0.09 to obtain the % titratable acidity as citric acid.

2.3.4. Browning Index

The colour of the flour was measured with a chromameter (Minolta CR-310 Osaka, Japan). The color index (browning index (IB) was determined by the L^* a^* b system.

$$\mathrm{IB} = \frac{100(X - 0.31)}{0.17} \tag{2}$$

$$\text{with} \quad X = \frac{a + 1.175L}{5.46L + a - 3.012b} \tag{3}$$

2.3.5. Maillard Reaction Product Absorbance

Maillard reaction was analyzed by reading the absorbance at 420 nm, according to [18] method. The measurements were made on an extract obtained by mixing 0.25 g of nut flour with 10 mL distilled water for 10 min. The suspension was stirred for 15 minutes using a mechanical stirrer (Heidolph MR Hei-Standard, Germany) and centrifuged at 5000 rpm (Universal 16A, D-78532 Tuttlingen, Germany). The supernatants were recovered and the absorbances at 420 nm (A420) were read on a Spectrophotometer (GENESYS 10S UV-VIS, Thermo Scientific).

2.4. Statistical Analyses and Optimization

Statgraphic centurion XVI version 16.2.04 is used for statistical data analyses. The variance analysis (ANOVA) and regression surface analysis were conducted to determine the regression coefficients and statistical significance of model terms and fit the mathematical models to the experimental data, aiming at an overall optimal region for the response variables. The terms statistically found non-significant ($P > 0.05$) were dropped from the initial models the experimental data refitted only to significant ($P < 0.05$) independent variable effects to obtain the final reduced model. The optimization process was designed at finding the levels of roasting temperature and roasting duration, which could maximize flavonoids, polyphenols contents and antioxidant activity, minimize titratable acidity, browning index and absorbance of Mailliard reaction products.

The response surface analysis allowed the development of an empirical relationship where each response (Y_i) was assessed as a function of time (X_1), temperature (X_2).

$$Y_i = b_0 + \sum_{k=1}^{2} b_i X_i + \sum_{i=1}^{2} b_i X_i^2 + b_{ij} X_I X_J \tag{4}$$

Y: the response calculated by the model,

b_0: a constant,

b_i, b_{ii}, b_{ij}: linear, squared and interaction coefficient, respectively,
X_1: roasting time linear factor,
X_2: roasting temperature linear factor,
X_1^2: roasting time squared factor,
X_2^2: roasting temperature squared factor,
X_1X_2: roasting time and temperature interaction factor.

3. Results and Discussion

The experimental values of response are presented in Table 2. The polyphenols and flavonoids contents ranged from 0.196 to 0.896 gEAG/100g and 0.022 to 0.051 gEC/100g respectively. DPPH• radical inhibition percentage ranged from 25.21% to 57.09%. Titratable acidity ranged from 0.193 to 0.468 g/100g, and the browning index (BI) ranged from 28.42 to 56.20. The absorbance of the Maillard Reaction Products ranged from 0.365 to 0.897. The R^2 values for the response variables vary between 32.91% and 97.10% (Table 3).

Table 2. Central composite design: real variables and response.

Essay	Temperature (˚C)	Time (minutes)	Polyphenols (gEAG/100g)	Flavonoids (gEC/100g)	Activity antioxidant %	Titratable acidity (TTA) (g/100g)	Browning index (BI)	Absorbance of MRA (A_{MRP})
1	140	28	0.506	0.028	53.44	0.468	40.16	0.897
2	154	28	0.196	0.034	52.57	0.234	50.97	0.37
3	130	20	0.256	0.029	34.00	0.45	31.2	0.645
4	150	35	0.867	0.051	56.97	0.432	56.2	0.768
5	130	35	0.734	0.048	56.81	0.382	40.07	0.502
6	126	28	0.349	0.034	53.51	0.306	31.51	0.635
7	150	20	0.797	0.024	27.21	0.193	39.98	0.365
8	140	28	0.585	0.022	49.59	0.472	35.47	0.401
9	140	38	0.746	0.026	57.09	0.463	49.18	0.7
10	140	17	0.891	0.040	25.21	0.441	28.42	0.792

Table 3. Analysis of variance of response.

Factors	Polyphénols		Flavonoids		Antioxidant activity		Titratable acidity		Browning index		Absorbance of MRA (A_{MRP})	
	F-v	P-v	F-v	P-v	F-v	P-v	F-v	P-v	F-v	P-v	F-v	P-v
X_1	0.30	0.6149	0.58	0.4894	147.40	**0.0003**	6.10	0.0690	66.09	**0.0012**	0.05	0.8266
X_2	0.62	0.4758	0.01	0.9450	1.10	0.3537	16.14	**0.0159**	60.31	**0.0015**	0.53	0.5066
X_1X_2	1.06	0.3610	0.99	0.3750	0.00	0.9879	53.41	**0.0019**	4.16	0.1109	0.84	0.4114
X_1^2	2.72	0.1744	0.86	0.4062	17.33	**0.0141**	0.23	0.6535	1.28	0.3205	0.14	0.7291
X_2^2	0.98	0.3779	0.11	0.7579	1.56	0.2801	28.62	**0.0059**	2.48	0.1905	1.70	0.2623
R^2	66.92		32.91		97.76		96.50		97.10		48.24	
Probability	0.3308		0.8336		**0.0021**		**0.0052**		**0.0036**		0.6292	

3.1. Polyphenols Content Variation

The obtained R^2 value was 66.92% (Table 3). The response surface plot of the interaction is shown in Figure 2 and a second-order polynomial regression model was used to describe the relationship between the roasting conditions (roasting temperature and time) and the total phenol content of nut flour (Equation (5)). Roasting factors have not significant effect ($p > 0.05$) on polyphenols content (Table 3). The total phenol content decreases with roasting time, which was more pronounced with increasing roasting temperature (Figure 2). The second step is an increasing of polyphenols according to with increasing temperature up to 140˚C and then decreasing at higher temperatures. These results

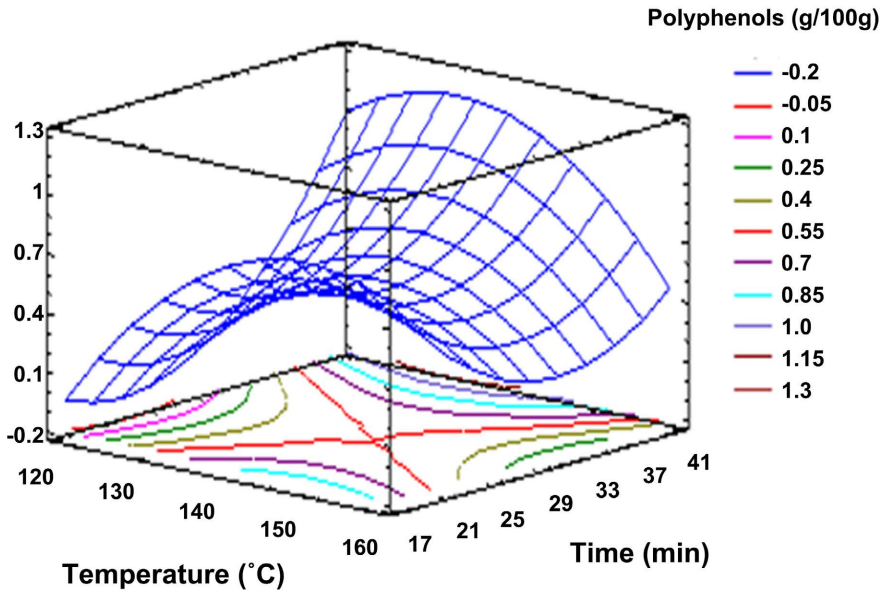

Figure 2. Response surface plot for polyphenols contents.

are in agreement with those of Lee *et al.* [19], Xu *et al.* [20]; however, this decrease of the content is due to the sensibility of the polyphenols with the high temperatures [21].

The increasing in total phenol content could be explained by many facts: 1) The roasting partially destroyed the cell structures, resulting in the release of certain phenolic compounds, which could then become more extractable [22] [23]; 2) Compounds derived from Maillard reactions such as pyrrols and furans which could react with the Folin-Ciocalteu reagent [24] and other compounds with polyphenolic structures [25] could increase phenolic compound content. The availability of certain phenolic compounds released from certain polymers and in free form [24]. However, this increase of phenolic compounds by the presence of Maillard reaction products [25].

$$\text{Polyphenols content} = -24.8436 + 0.0402857X_1 + 0.347427X_2 + 0.00307097X_1^2 - 0.00107605X_2^2 - 0,0014536X_1X_2 \quad (5)$$

3.2. Flavonoids Content Variation

The analysis of variance showed a value of R^2 equal to 32.91% (Table 3). The flavonoids contents are in the same interval found by Imam *et al.* [5]. As shown in Table 3, the model has not significant affect ($p > 0.05$) on flavonoids content. The high flavonoids content is given at 150˚C for 35 minutes (Table 1). Figure 3 describes the variations of flavonoids content with the time-temperature pair and the Equation (6) represents the mathematical model reflecting these interactions. Two steps were noticed: an early decreasing of flavonoids contents with increasing temperature to a minimum value and then an increasing with temperature and time increasing. The flavonoids content varies like total polyphenol. The production of phenolics and flavonoids compounds during roasting in this study may have been related to the apparition of new phenolic compounds and to the increasing production of Maillard reaction products (MRPs) produced during roasting [26]. This increase in the flavonoids content is in agreement with the results of Lee *et al.* [19], where the flavonoids content increases with the rise in temperature and duration and Mohamed *et al.* [27].

$$\text{Flavonoids content} = 1.32337 - 0.00857718X_1 - 0.0169224X_2 + 0.0000956538X_1^2 + 0.0000576885X_2^2 + 0.0000268276X_1X_2 \quad (6)$$

3.3. Antioxidant Activity Variation

The variance analysis shows that the coefficient of determination R^2 is of the order 94.97% (Table 3). Statistical data also show that roasting process factors are significant ($p < 0.05$) on antioxidant activity (Table 3). It was significantly ($p < 0.05$) affected by both the linear and quadratic terms of roasting time parameters (Table 3). Response surface plot of the relationship is shown as Figure 4. As observed in Equation (7), a full quadratic term was fitted for predicting the antioxidant value and the highest DPPH• radical inhibition was obtained at 150˚C for 35 min. The increase in roasting time is associated with an increase in antioxidant activity. It can be associated with a release of phenolic compounds and an

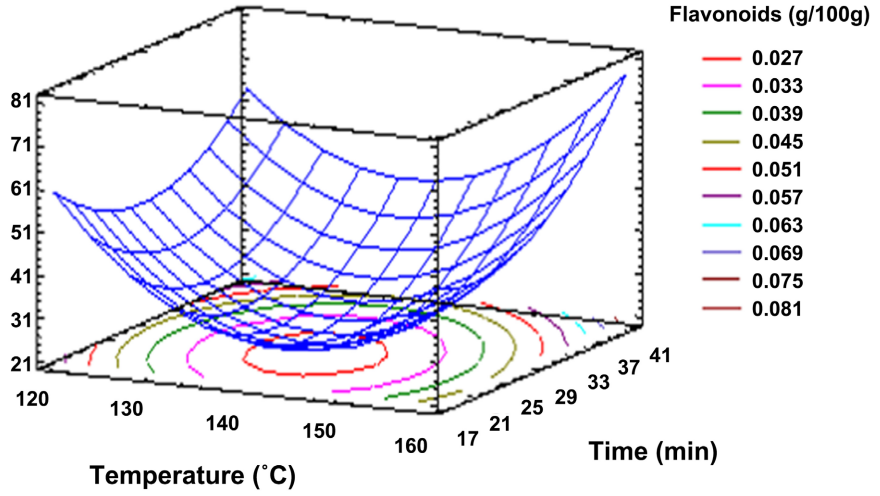

Figure 3. Response surface plot for flavonoids.

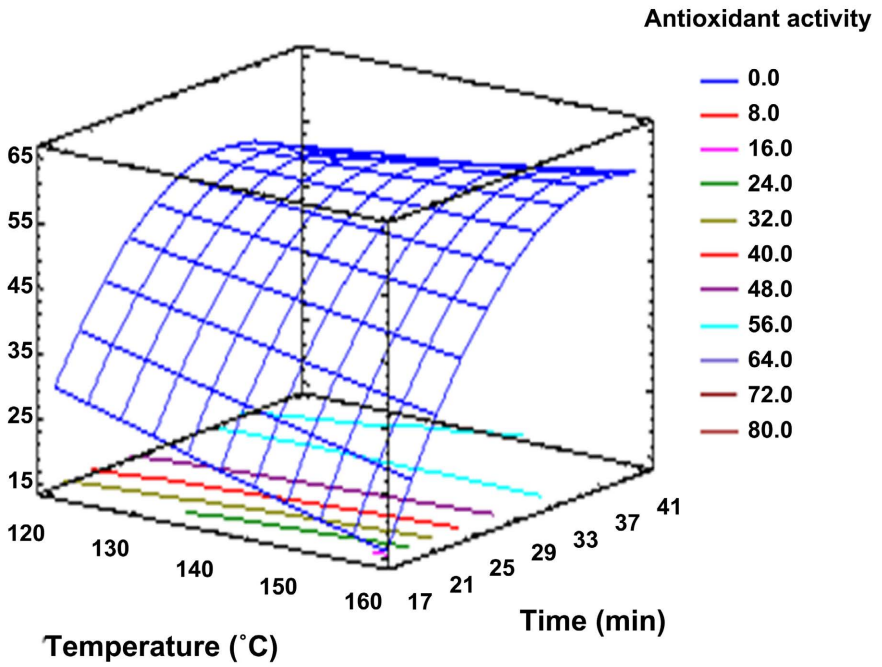

Figure 4. Response surface plot antioxidant activity.

increase in the brown color; therefore, compounds with antioxidant properties resulting from the Maillard reaction. These results are in agreement with those of Fazli Aghdaei *et al.* [28]. In their study, Boublenza *et al.* [25] notice that roasting increases antioxidant activity mainly due to Maillard reaction products which have antioxidant activity [29] [30]. In fact, some compounds of the Maillard reaction products have phenolic structures that may have antioxidant activity [31]. However, there was a slight decrease in antioxidant activity with increasing temperature, thus showing the compounds' sensitivity concerning temperature.

$$\text{Antioxidant Activity} = 40.2382 + 3.83761X_1 - 0.819111X_2 - 0.100275X_1^2 + 0.000218798X_2^2 + 0.023693X_1X_2 \quad (7)$$

3.4. Titratable Acidity (TTA) Variation

The variance analysis indicates that the coefficient of determination is 96.06% (Table 3). The regression model is statistically significant for titratable acidity (TTA) ($p < 0.05$) (Table 3). The regression equation (Equation (7)) predicted the relationship between titratable acidity and variables. A surface plot displays the effect of roasting temperature and time on the titratable acidity (Figure 5). It was shown a significant effect ($p < 0.05$) of the temperature linear factor, temperature quadratic factor and the combined factor between temperature and roasting time on roasted tiger nut (Table 3). The acidity increases with the increase of roasting time. With roasting temperature, the acidity increases up to a maximum value of 0.47 g reached at a temperature of 140˚C beyond which, it decreases. The decrease in titratable acidity could be explained by thermal sensibility of certain acidic compounds [32]. The acidity increase could be done to

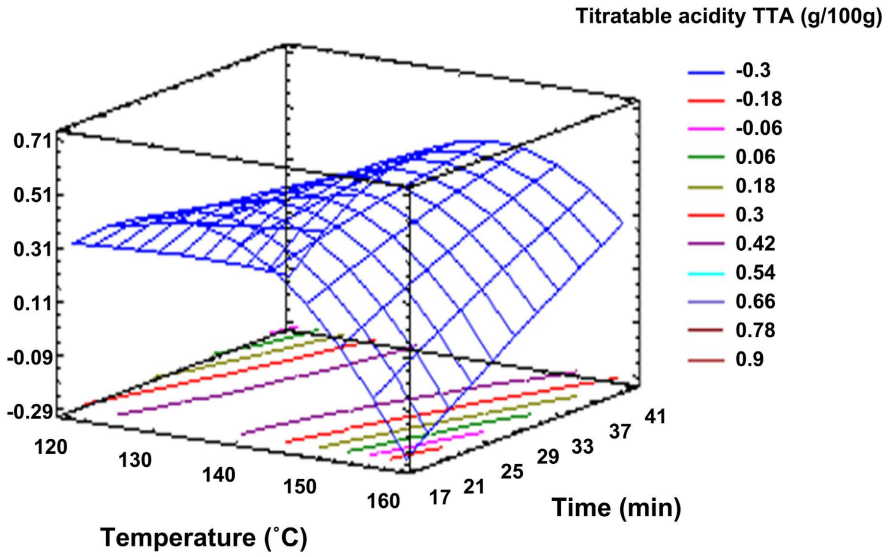

Figure 5. Response surface plot for titrable acidity.

release new organic acid compounds (formic, acetic, glycolic, and lactic acids) from the sugar conversion and caramelization of sugars [33]. The increase in acidity could also be due to oxidation of the oil contained in the tubers during roasting. These are also confirmed that the heating process resulted in a loss of phenolic acid content of flaxseed oil, confirming that the heat treatment causes oxidation [34].

$$\text{TTA content} = -14.8333 - 0.134638X_1 + 0.248515X_2 - 0.000118615X_1^2 - 0.00100376X_2^2 + 0.0010325X_1X_2 \quad (8)$$

3.5. Browning Index and Maillard Reaction Products (MRP) Absorbance Variation

Color is an important parameter in the roasting process; it is one of the most important appearances in Maillard reactions. Hence the importance of it's study by monitoring is the index of browning. The coefficient of determination R^2 obtained is 97.01% (Table 3). It was shown that roasting process globally affects the browning index ($p < 0.05$). Roasting temperature and time linear factors showed significant ($p < 0.05$) effects on the browning index. However, time-temperature interaction factors and quadratic factors have no significant impact ($p > 0.05$) on the browning index. The Equation (9) is the mathematical model reflecting the interactions between the parameters on the browning index and Figure 6 were the response surface plot which displays roasting effects of temperature and time.

Soluble Maillard reaction's products play an important role in the preparation of foods. Statistical analysis reveals a value of 48.24% for the coefficient of determination R^2 (Table 3). As shown in Table 3, no significant effect was noticed by different roasting factors and a second-order polynomial regression (Equation (10)) was fitted for predicting Maillard reaction product absorbance. The

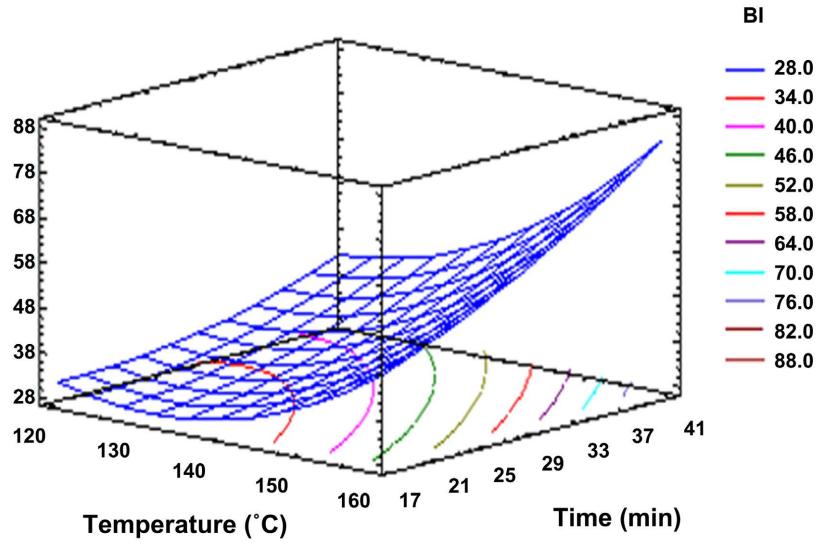

Figure 6. Response surface plot for browning index.

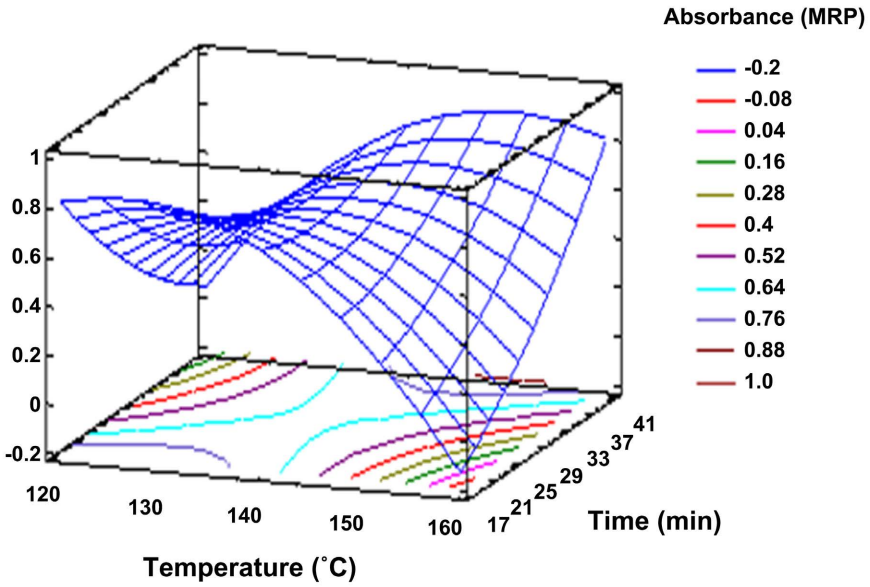

Figure 7. Response surface plot for Maillard reaction products absorbance.

interactions between the roasting parameters on the Maillard Reaction Product were given by a response surface plot (Figure 7).

The browning index increases with the temperature rise in the same way for the roasting time. Similar results were obtained by [35] on roasting peanuts. These observations show that a long roasting operation with a high temperature can affect flour quality. Sugar content in tiger nut is important [1]. Therefore, an increase of temperature for a longer time leads to an increase of the sugar and amino group reactivity [36]. This browning is a non-enzymatic reaction that occurs when reducing sugar and protein are heated together and enhances the roasted products' antioxidant capacity. Maillard reactions are known sometimes for their biological effect. They have high antioxidant activity.

$$\text{Browning index}(\text{IB}) = 480.883 - 3.80101X_1 - 6.42107X_2 + 0.0226115X_1^2 + 0.0228302X_2^2 + 0.0247634X_1X_2 \quad (9)$$

$$\text{Absorbance MRP} = -8.75425 - 0.281689X_1 + 0.194515X_2 + 0.000642109X_1^2 - 0.000888056X_2^2 + 0.00177599X_1X_2 \quad (10)$$

3.6. Optimization and Verification of the Model

Multi-response optimization of RSM was applied to determine the optimal combination of roasting time and temperature variation. The optimum roasting conditions of tiger nut predicted by the regression model were as follows: for duration (38 min) and temperature (147˚C) (Figure 8). The overall desirability is 90%, and this shows that it is fit to explain this study. The predicted are shown in Table 4. Flour samples were prepared using the derived optimum formulation conditions to check the surface response model's validity.

The experimental data were compared with predicted values to verify the adequacy of the final reduced flour (Table 4). Also, close agreement and no significant difference existed between experimental and predicted values.

Table 4. Predicts values and desirability.

Responses	Predicts Values	Experimental values	Limit inferior at 95.0%	Limit superior at 95.0%	Desirability
Polyphénols (g EAG/100g)	0.83	830.570 ± 4.80	0.26	1.40	0.85
Flavonoids (g EC/100g)	0.04	0.043 ± 0.008	0.012	0.08	0.76
Antioxidant Activity (%)	57.89	59.25 ± 1.25	50.48	65.30	0.99
Titratable acidity (g/100g)	0.49	0.819 ± 0.01*	0.42	0.57	1.0
IB	56.47	60.32 ± 0.01*	50.33	62.60	1.0
MRP	0.79	0.764 ± 0.081	0.26	1.32	0.80

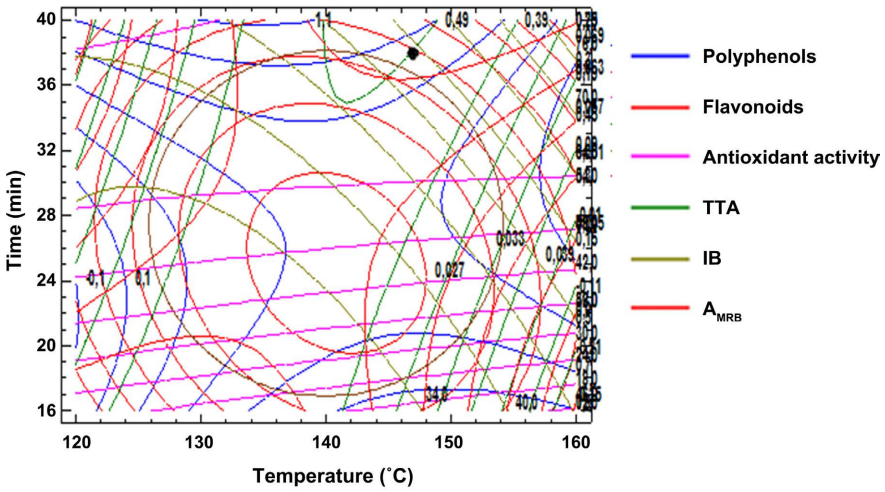

Figure 8. Response surfaces superposition plot.

4. Conclusion

The response surface methodology was used to model the roasting process of tiger

nut. The different parameters studied were influenced by the temperature-time pair. The experimental values were found to be in close agreement with the predicted values. They were within acceptable limits indicating the model's suitability in predicting quality attributes of tiger nut flour. In further research, it will be necessary to do a comparative study on the proximate composition and biochemical proprieties between the flour obtained with raw nut and the nut flour obtained in optimal roasting condition.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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