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Optimization of the antioxidants of tigernut (*Cyperus esculentus* L.) during roasting using response surface methodology

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ABSTRACT

Effect of roasting temperature and time on the total polyphenols, flavonoids, total antioxidants and antioxidant properties of tigernut was studied using response surface methodology. Tigernut samples were roasted in an oven at the range of 150 – 200°C for 20 – 50 min. Out of the responses, only radical scavenging activity was not significantly affected by the roasting conditions. Roasting temperature affected the antioxidant contents significantly ($p > 0.05$) than roasting time. The optimum roasting temperature and time obtained was 200 °C for 20 min. The experimental values were very close to the predicted values and were not significantly ($p > 0.05$) different.

Introduction

Roasting is a direct application of high temperature to foods. Apart from cooking the food, it improves palatability, aroma, flavour and colour [1]. Roasting is a non-enzymatic browning reaction which can arise from sugar-sugar reaction (caramelization) or between reducing sugars and protein and its derivatives (Maillard reaction) at high temperature [2]. This results into production of food with acceptable aesthetic and sensory qualities. However, roasting has been reported to affect the nutritional content and phytochemical composition of foods. Roasting reduces the protein, dietary fibre, minerals and phytate contents of roasted maize [3] and polyphenol concentration of tigernut [4].

Polyphenols and flavonoids are bioactive compounds with antioxidative properties. Dietary antioxidants chelate free radicals such as reactive oxygen species and prevent rancidity in foods [5]. Free radicals are known to be a main contributor to major chronic diseases and degenerative diseases of ageing. Phenolic compounds have been associated with health promotion due to their antioxidant activity. They are secondary metabolites with protective power against human chronic degenerative diseases, such as cancer and cardiovascular disease [6]. There are high positive correlations between phenolic acids and antioxidant activity which suggests that these compounds are mainly responsible for the antioxidant activity of many foods [7].

Tigernut (*Cyperus esculentus*) is an under-utilized nut with potential food uses because of its inherent properties which include high dietary fibre and phytochemicals with antioxidative properties [4, 8] among others. It contains polyphenols with high antioxidant capacity [9]. Its extract is also reported to be active against sickle cell haemoglobin gelation [10]. Tigernut in Nigeria are of three types differentiated by their colours (yellow, brown and black) but only two (yellow and brown) are readily available in the market in dried form [11]. Tigernut is consumed in different forms. It can be consumed raw, made into imitated milk ('kunnu ayaya') by soaking and extraction, coated with sugar or roasted for use in 'dakuwa' - a cereal/legume based Nigerian snack [4].

'Dakuwa' is one of the most consumed local snacks in the Northern part of Nigeria. It is consumed by children, adults, male and female. In 'dakuwa' processing, roasting of the major ingredients (maize, tigernut and groundnut) is a critical unit operation which develops the colour and flavour of the product and this affects the consumer acceptability of the product [12]. However, roasting has been reported to affect the antioxidant activity of foods [13, 14]. Although there is information on the effect of roasting on the phytochemicals of tigernut, there is dearth of information on the effect of roasting on the antioxidant content of tigernut. This work therefore evaluated the effect of roasting temperature and time on the antioxidant content of tigernut and studied the condition for optimization of roasted tigernut. This will enable the consumers to benefit more from the functional potentials of tigernut in addition to the nutritional advantages.

Materials and Methods

Sample preparation

Tigernut (*Cyperus esculentus* L.), brown variety, was obtained from a local market in New Bussa, Niger State, Nigeria. They were sorted, cleaned in water, dried in hot air oven at 100°C for 6 h. The dried samples were roasted at different temperature-time regime between 150 – 200 °C for 20 –50 min (Table 1) in a hot air oven, cooled at room temperature, milled with a commercial plate mill into flour which passed through 30mm mesh sieve. The flours were analyzed for total polyphenols, flavonoids, total antioxidants and antioxidant properties (ferric ion reducing antioxidant power, FRAP; radical scavenging activity, RSA).

Experimental design and statistical analysis

The effect of two independent variables, x_1 (roasting temperature) and x_2 (roasting time) on total polyphenols, flavonoids, total antioxidants and antioxidant properties (ferric

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ion reducing antioxidant power – FRAP and radical scavenging activity - RSA) of tigernut were evaluated using the response surface methodology. Central composite design with two variables and five combinations which result in fourteen runs with six centre points was used. Table 1 shows the minimum, medium and maximum coded and the real values of roasting temperature and time while Table 2 shows the roasting temperature-time combination. Models were generated while regression analysis and analysis of variance at 5% level of significance were used to determine fitness of the models. Design-Expert version 6.0.6 (Stat-Ease Inc., Minneapolis, U.S.A) was used for the experimental design and analysis.

Optimization and validation of data

Numerical optimization technique was used for simultaneous optimization of the multiple responses. The optimization experiment was designed in such a way to maximize only the significant models: total polyphenol, flavonoids, ferric ion reducing antioxidant power and total antioxidants. After optimization, the temperature and time values obtained were used to roast tigernut which were then analyzed. The data obtained were compared to the optimally suggested values and the percentage error was calculated.

Determination of total polyphenols

The Folin–Denis method [15] was used. Five millilitres of tigernut extracts and Folin–Denis reagent were mixed. After 3 min, 5 ml of 10% Na_2CO_3 was added and the mixture was left at room temperature for 1 h. Absorbance was then measured at 760 nm and the result was expressed in mg of (+)-catechin/100 g dry weight.

Determination of flavonoids

This was determined by a modified version of the technique proposed by Kim *et al*., [16]. Five grams (5 g) of the sample was extracted with 10 ml of 80% ethanol. After filtration, an aliquot (1ml) of extract was added to 10 ml volumetric flask containing 4 ml of distilled water. The following were then added sequentially to complete the system: 0.3ml of 5% $NaNO_2$ at 0 min, 0.3ml of 10% $AlCl_3$ after 5 min and 2 ml of 1M NaOH after 11 min. The total volume was made up to 10ml with distilled water. The solution was mixed very well and the absorbance was measured against blank at 510nm. A standard curve was prepared using various catech in concentrations, in the range of 40–200 µg/ml. Total flavonoids levels in the samples was expressed as mg catechin/100 g sample.

Determination of total antioxidant

Total antioxidant activity of extract was evaluated by the formation of phosphomolydenum complex [17]. Methanolic extract (0.1ml) was added to 1.9 ml of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained only 2 ml of reagent solution. The absorbance was measured at 695 nm after 60 min.

Determination of radical scavenging activity (RSA)

Sample (2 g) was extracted with 10 ml 80% methanol at room temperature with constant stirring for 20 min. After decantation, the antiradical activity of the extracts was determined by spectrophotometry at 515 nm, based on the reaction with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical [18]. A 0.2 ml aliquot of the methanolic extract was mixed with 7.8 ml of 0.1 mM methanolic DPPH and incubated in the dark at room temperature (27 - 30 ^oC) for 30 min and the absorbance was measured at 517 nm. The control sample was 1 ml of 80% methanol. The result was expressed as percentage inhibition calculated as:

RSA (% inhibition) = $(A_o - A_s)/A_o \times 100$

Where A_o = Absorbance of control sample

A_s = Absorbance of sample

Determination of ferric ion reducing antioxidant power

Five grams (5g) of sample was extracted in 10ml of 80% ethanol for 1 h. From the extract, 1 ml was diluted with 10 ml distilled water in a test tube. Dihydrogen orthophosphate solution (2.72%) was prepared and the pH was adjusted to 6.6 with KOH. To 1 ml of the diluted extract, 2.5 ml of the buffer and 2.5 ml 1% Ferricyanide solution were added and the mixture was incubated at 50 ^oC for 20 min. After incubation, 2.5 ml of 10% Trichloroacetic acid solution was added and the mixture was centrifuged at 1500 rpm for 5 min. To 2.5 ml of the supernatant, 2.5 ml distilled water and 0.5 ml 0.1% Ferric chloride solution were added. The solution was allowed to stand for 30 min before measuring the absorbance in a spectrophotometer at 700 nm. Distilled water with added reagents was used as blank [19].

Table 1: Levels of independent variables according to the central composite design

Independent variables	Coded variable levels				
	-1.41	-1	0	+1	+1.41
Roasting Temperature (^oC)	139.64	150	175	200	210.36
Roasting Time (min)	13.79	20	35	50	56.21

Table 2: Central composite design for independent variables (actual and coded values) used for the roasting of tigernut

Run	Coded values		Actual values	
	X_1	X_2	Roasting temperature (^oC)	Time (min)
1	0	0	175	35
2	+1	-1	200	20
3	-1	+1	150	50
4	-1	-1	150	20
5	0	0	175	35
6	0	0	175	35
7	+1	+1	200	50
8	+1.41	0	210.36	35
9	0	0	175	35
10	0	+1.41	175	56.21
11	-1.41	0	139.64	35
12	0	-1.41	175	13.79
13	0	0	175	35
14	0	0	175	35

Results and discussion

The mean values of polyphenols, flavonoids, FRAP, RSA and total antioxidants are presented in Table 3 while the regression coefficients, R^2 and statistical significance are presented in Table 4. The total polyphenol content ranged between 95 and 396 mg/100 g sample. Control sample had the least while the sample roasted at 200 ^oC for 50 min had the highest value. The total polyphenol of the roasted tigernut was significantly ($p<0.05$) affected by roasting. Only roasting temperature affected the polyphenol significantly ($p<0.05$). Increase in roasting temperature increased the polyphenols of tigernut. The polyphenol content was influenced by the linear and quadratic effect of temperature. Roasting temperature had more effect on the samples compared to the roasting time. High polyphenol content was obtained at higher temperature and time. From the surface plot (Fig. 1), the minimum and the maximum values for polyphenols were 22.69 and 460.04 mg/100 g obtained at 157.46 ^oC, 23.40 min and 199.83 ^oC for 32.54 min, respectively. Therefore, a high content of polyphenols can be obtained at a high temperature and time. These results agree with the previous research findings [20, 21] with report of an increase in phenolics of roasted grape seed extract and peanut hulls, respectively, with increase in temperature. At high temperature e.g. roasting temperature, usually ≥100^oC, phenolics

can be liberated from residual sources thereby increase phenolic content of a sample [21]. The increase in total polyphenols in tigernut from this study may be due to the development of Maillard reaction products and/or liberation of phenolic compounds. In Maillard reaction, highly reactive radicals are formed in the early phases whereas strong antiradical properties are attributable to the high molecular weight brown compounds formed in the advanced phases of the reaction [22, 23].

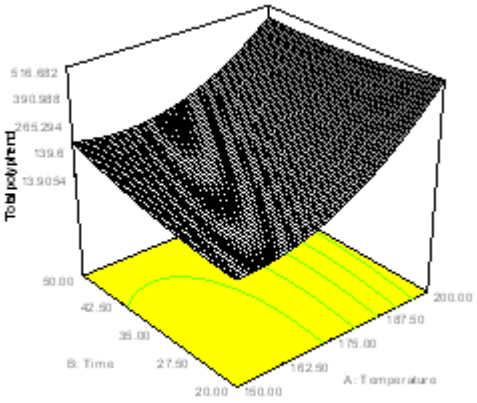

Figure 1: Surface plot of the effect of roasting temperature and time on the total polyphenol of roasted tigernut

Phenolic acids are powerful antioxidants with antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory powers [24]. Phenolic compounds are contributors to the antioxidant and antiproliferative activities of materials. They aid the death of human hepatocellular liver carcinoma and colon cells [25, 26].

The quadratic model equation fitted for the total polyphenol is highly suitable with R^2 of 0.94.

$Y_{TP} = 4597 – 62.73T + 16.84t + 0.22T^2 + 0.14t^2 + 0.14T – t$

Where TP = Total polyphenol, T = Temperature, t = time

The flavonoid content of the roasted tigernut was affected significantly ($p<0.05$) by the linear effect of temperature and time (Table 4). The values fall between 48 and 196 mg/100 g sample. The control sample had higher flavonoid content than the roasted samples. Roasting time had more influence on the flavonoid content of tigernut than roasting temperature. Increase in roasting temperature increased the flavonoid content while increase in time decreased it. Decrease in flavonoid content with increase in roasting time had been reported for roasted sweet potato [27] and dried tomato [28]. Roasting time, according to the results is a key factor in the reduction of flavonoids. Total phenol and flavonoids are intermediate or final products of browning reaction produced by the reaction of aldehydes and ketones [29] and are susceptible to degradation at high temperature. From Figure 2, flavonoid content was highest (101.81 mg/100 g) and lowest (50.03 mg/100 g) at 199.27 ^oC for 20.21min and 150.21 ^oC for 49.54 min respectively. The R^2 (0.61) was low and this indicates that the quadratic model may not be adequate for the prediction of the effect of roasting conditions on the flavonoids of tigernut.

Ferric ion reducing antioxidant power (FRAP) of the roasted tigernut was significantly ($p<0.05$) affected by roasting. The FRAP content of the roasted tigernut was higher compared to control. Tigernut roasted at 210 ^oC for 35 min was highest in FRAP value (258.41 mg/100 g sample). The FRAP of tigernut was significantly ($p<0.05$) influenced by the linear and quadratic effect of temperature, quadratic effect of time and interaction effect of temperature and time (Table 4). Roasting temperature had more influence than time. Increase in roasting temperature and time increased FRAP. A similar result was reported by Woffenden *et al.* [30]. The Maillard reaction products generated during roasting contribute to enhancement of reducing power. The model equation for predicting the effect of roasting temperature and time on FRAP of tigernut is adequate with high R^2 of 0.98.

$Y_{FRAP} = 4029.85 – 58.26T + 48.65t + 0.21T^2 – 0.15t^2 – 0.22T - t$

In radical scavenging activity (RSA), the % inhibition of the roasted tigernut was higher compared to unroasted tigernut. The least and the highest values were 14 and 61% for the unroasted tigernut and tigernut roasted at 175 ^oC for 13.79 min. Roasting increased the RSA of tigernut more than four folds i.e. from 14% to 61% (about 400% increase). The Radical Scavenging Activity (RSA) of the roasted tigernut was affected significantly ($p<0.05$) only by the linear effect of temperature. Increase in roasting temperature and time increased the RSA. Roasting temperature had a greater influence compared to time (Fig. 4). Increase in scavenging activity due to roasting of peanut kernels and barley was reported by Hwang *et al.* [31] and Sharma and Gujral [32], respectively. The antioxidant activity of thermally processed material can increase or remain unchanged. This is due to the fact that during roasting, both primary and secondary products produced through non-enzymatic browning reaction, especially Maillard reaction, contribute to the antioxidant property of roasted products [33]. Also, degradation of polyphenol compounds by thermal process may result in releasing antioxidant compounds that have different chemical and biological properties [34]. The RSA has low R^2 (0.43) therefore, cannot be predicted by model equation.

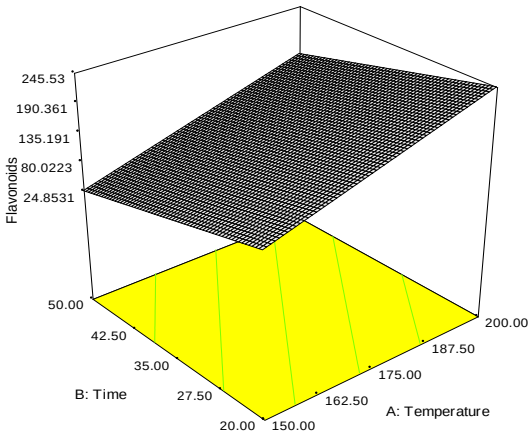

Figure 2: Surface plot of the effect of roasting temperature and time on the flavonoids of roasted tigernut

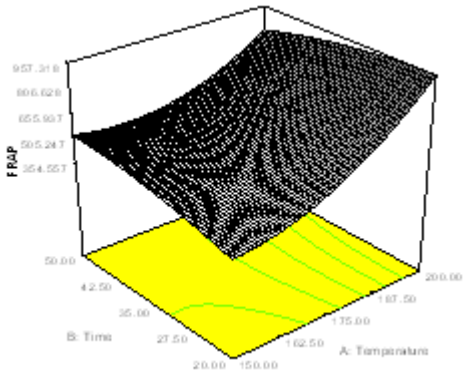

Figure 3: Surface plot of the effect of roasting temperature and time on the reducing power of roasted tigernut

Table 3: Concentrations of antioxidants in the roasted tigernut

Run	Independent variables values		Dependent variables values				
	Roasting temperature (^oC)	Roasting Time (min)	Total polyphenols (mg/100g)	Flavonoids (mg/100g)	Ferric ion reducing power (mg/100g)	Radical scavenging activity (% inhibition)	Total antioxidants (mg/100g)
1	175	35	320.67	70	170.16	50.96	790
2	200	20	208.33	93	187.62	53.5	608.33
3	150	50	110.33	58	132.38	53.11	818.33
4	150	20	233.17	78	151.91	53.3	664.17
5	175	35	176.67	48	146.83	52.13	453.33
6	175	35	281	82	174.92	53.85	683.33
7	200	50	396.17	66.7	176.83	53.58	468.33
8	210.36	35	318.33	137.7	258.41	54.67	320
9	175	35	320.67	48	174.92	50.96	470.9
10	175	56.21	388.5	64.3	179.52	55.18	536.67
11	139.64	35	128.17	69.7	160.79	56.08	707.5
12	175	13.79	202.83	96.3	180.16	60.92	820.83
13	175	35	176.67	82	170.16	53.85	796.3
14	175	35	281	70	146.83	52.13	680
Control	-	-	95.2	194.67	47.78	14.42	315.6

Table 4: Regression coefficients, R^2 and P-values for the model of the antioxidants of roasted tigernut

Regression coefficients	Total polyphenol	Flavonoids	FRAP	RSA	Total antioxidant
a_o	4597.18	-201.4998	4029.85	14.9899	-13396.52
Temperature	62.7310*	2.5464*	58.2561*	0.0352*	87.7705
Time	16.8359	-3.1120*	48.6497	0.0336	487.64
Temp-temp	0.2161*	-	0.2133*	-	-
Time-time	0.1418	-	-0.1472**	-	-
Temp-time	-0.1427	-	-0.2164*	-	-2.7111*
R^2	0.9367	0.6126	0.9775	0.4266	0.6212
P-value	0.0005	0.0087	<0.0001	0.0620	0.0272

Table 5: Validation of the predicted values for the antioxidants

	Predicted values	Actual values	Percentage error
Total polyphenol (mg/100g)	516.68	490.00	5.44
Flavonoids (mg/100g)	245.53	252.30	2.68
FRAP (mg/100g)	957.30	942.01	1.62
Total antioxidant (mg/100g)	3065.93	3050.23	0.50

The total antioxidants (TA) content of the roasted tigernut varied and ranged between 315 and 820 mg/100 g sample. Tigernut roasted at 175^{o}C for 14 min was highest in TA content while the unroasted tigernut was lowest.

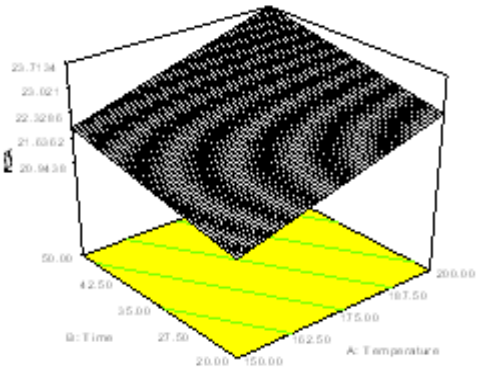

Figure 4: Surface plot of the effect of roasting temperature and time on the radical scavenging activity of roasted tigernut

The TA of the roasted tigernut was significantly ($p<0.05$) affected by roasting temperature and time. The content was not affected significantly ($p<0.05$) by the linear and quadratic effect but with interaction effect of temperature and time. Increase in roasting temperature and time increased the total antioxidant content (Fig. 5). Also, increase in temperature - time interaction decreased the total antioxidant content. Roasting time had a greater influence compared to temperature. A similar result was reported for antioxidant activity of sand roasted and microwave cooked barley [32]. Total antioxidants is the aggregate of the antioxidant activity of a food. This could include the activity of both determined and undetermined components with antioxidant capacity.

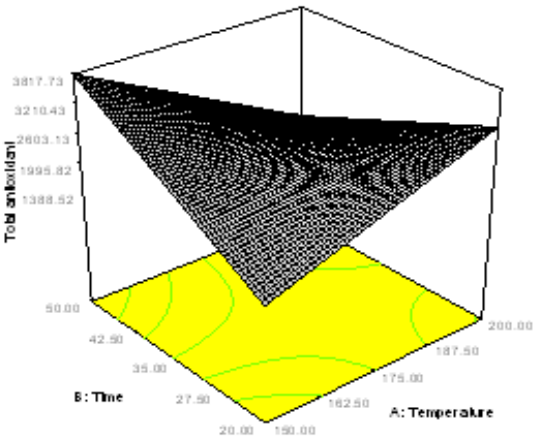

Figure 5: Surface plot of the effect of roasting temperature and time on the total antioxidants activity of roasted tigernut

Optimization

The optimization experiment was designed in such a way to maximize only the significant responses: total polyphenol, flavonoids, ferric ion antioxidant power and total antioxidants. The optimum roasting temperature and time obtained were 200 ^{o}C for 20 min with the values 516.68, 245.53, 957.30 and 3065.93mg/100g for total polyphenol, flavonoids, ferric ion antioxidant power and total antioxidants, respectively. When tigernut was roasted with the optimum temperature and time, the values obtained were compared to the predicted values. The percentage errors were 5.44, 2.68, 1.62 and 0.50 respectively. Generally, a percentage error less than 10 indicates reproducibility and can be used to develop a satisfactory model.

Conclusion

Roasting affected antioxidant activities of tigernut significantly ($p<0.05$). Increase in roasting temperature and time increased the total polyphenols, flavonoids, total antioxidant, RSA and FRAPs except flavonoid content which reduced with increase in roasting time. Roasting temperature affected the antioxidants significantly ($p<0.05$) than time. High temperature is required for high polyphenol and flavonoid content. It could be inferred that both flavonoids and polyphenols contributed to the antioxidant activity of roasted tigernut since the two increased during roasting. Temperature-time regime of 200 ^{o}C for 20 min is advised to be used for roasting of tigernut for optimum antioxidant activities.

Conflict of interest

There is no conflict of interest.

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