ANTI NUTRIENT CONTENT AND HAEMAGGLUTININ ACTIVITY IN FOOD GRADE FLOUR OF ROASTED AFRICAN BREAD FRUIT SEEDS PRODUCED UNDER EXTREME CONDITIONS

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Abstract

The Anti-nutrient composition of food grade flour produced from African breadfruit (*Treculia africana*) seeds under modulated roasting variable conditions was studied. The study was designed to test the relationship between treatment variables of roasting temperature, roasting time and feed quality and maximum detoxification of Anti nutrients. The concept of three factors – five levels Central composite Rotable Design, regression analysis and Response surface methodology was employed. Results showed decreased values of anti-nutrients, which were reflective of the significant effect of treatment variables. Regression model predicted minimum anti-nutrient values of Total phenol 0.61mg/100g, Tannin 0.24mg/100g, HCN 0.05mg/100g, Alkaloid 0.01mg/100g, Oxalate 0.44mg/100g and Phytate 0.52mg/100g at Optimum condition of Roasting Temperature 193.64^{0}C, at time of 39.57min and 386.17g feed quantity. The HCN output was below the lethal dose for humans. The haemagglutinin content and activity were severely reduced at temperature time combination of above 180^{0}c and 40mins.

Introduction

African breadfruits (*Teculia africana*) is an important food crop of the family Moraceace family. African breadfruit bears fruit heads with oval seeds. The seeds when extracted from fruit heads are used for the formulation of highly nutritious porridge, meaning diets, extruded products, biscuits (Nwabueze *et al.,* 2007) or eaten as roasted seed snacks. The nutritional compositions of African bread fruits indicate 17-22% protein, 9-11% fat, 50-63% carbohydrate, 2-4% crude fibre with varying amounts of mineral elements and vitamins (Nwokocha and Ugbomoiko, 2008). As consistent with most plant foods African breadfruit seeds contain anti-nutrients such as phytate, Tannins, haemagglutins, oxalates, alkaloids, HCN etc. These nutritional important anti-nutritional factors hinder the utilization of African breadfruit seeds as food due to their interference in nutrient digestion and bioavailability in human body. A food grade flour should be nutritionally wholesome with acceptable functional properties needed to add value to formulated foods (Ihekeronye and Ngoddy, 1985). The aim of processing African bread fruit seeds into flour for use in other food systems is to improve nutrients and reduce or eliminate completely anti-nutrients in order to promote efficient utilization of the nutrients and enhancement of its functional properties. Irrespective of some beneficial effects of anti-nutrients as anti-oxidants and anti-toxicity element (Wise and Gilbert 1981), their ability to chelete or complex vitamins and minerals are of critical nutritional importance. Parboiling and Roasting are the most commonly used heat processing methods of African breadfruits seeds. These methods improve nutrients and digestibility with important concerns for total nutrient density of product etc. (Nwabueze *et al.*, 2007). Optimum retention of nutrients including minerals and vitamins in processed foods is vital for good health and proper functioning of the metabolic pathways in humans. One way of achieving maximum detoxification is through modulation of process variables.

Roasting is a dry heat cooking of African breadfruits seeds. It employs varying ranges of temperature and time depending on the mass of African breadfruits being roasted. This study used Central composite Rotable Design and Response surface method as an important process modulation optimization tool for process and product quality to determine the optimum process variables (roasting temperature, time and feed quality) combination and minimization of anti –nutrient of food grade flour of roasted African bread fruits seeds.

Roasted African breadfruit seeds flour is an important input in food formulation systems for its nutritional and functional properties. The result of this study would invariably guide locally processors to produce nutritionally acceptable flour from roasted African breadfruit seed.

Materials and Methods

Freshly harvested African breadfruits (*Teculia africana*) seeds were purchased from Umuahia main market. The seeds were screened for contaminants and air dried under shade at ambient (28 $\pm$ 4^{0}C) temperature, roasted according to experimental design.

The experimental design used Central Composite Rotable design (8 factorial, 6 axial and 6 rotations at the centre) with a minimum of 15 experimental runs. The experimental variables (Range and levels) and central composite Rotable design are described in Tables 1 and 2.

Table 1: Range and Levels of Experimental Variables.

		a	b	c	d	e
Independent variables	Code	-1682	-1	O	1	1.682
Roasting temperature RT (^{0}C)	X_1	126.36	140	160	180	193.64
Roasting Time RM (min)	X_2	31.59	35	40	45	48.41
Feed quantity fQ (g)	X_3	331.80	400	500	600	`668.20

Table 2 : Experimental Layout.

X1 (RT)	x2 (RM)	x3 (FQ)	Combinations	Replication	Experimental
$\pm$1	$\pm$	$\pm$1	8	1	8
$\pm$ 1.682	0	0	2	1	2
0	$\pm$1.682	0	2	1	2
0	0	$\pm$1.682	2	1	2
0	0	0	1	6	6

The roasted samples were dehulled, milled into flour, sieved (200µm mesh) appropriately labelled in cellophane bags and stored in plastic containers for anti-nutrients assay.

Determination of Anti-Nutrients of samples: Phytate was determined by the anion exchange method. HCN of the samples were determined by colorimetric method. Oxalate and Alkaloids were analyzes were carried out using the methods described by Onwuka (2005). Tannin contents were determined using the Folin – Denis Spectrophotometric methods (Person, 1976).

Determination and Activity of Haemagglutinin in Samples: The method of Onwuka (2005) was used to isolate haemagglutinin from the flour samples. About 0.5g of sample was dispersed in 10ml normal saline solution (buffered at pH 6.4 with 0.1m phosphate buffer solution).and allowed to stand at room temperature for 30 minutes, then centrifuged at 1500rpm to obtain haemagglutinin extract.

Trysin treated rabbit blood was used to estimate haemagglutinin activity. About 3ml of the rabbit blood was treated with 50µg/ml trypsin (N-Benzoyl-L trypsin ethyl ester 98% purity) for one hour at room, then centrifuged to obtain blood cells. Samples of one millilitre of treated blood cells were mixed with 0.1ml haemagglutinin extract, allowed to stand for 4 hours at room temperature and absorbance taken at 620nm in a spectrophometer.

Extent of agglutination was observed using a microscope.
Haemagglutinin Hn (Hu/100g) = (b-a)f
b = Absorbance of test sample, a = absorbance of control, f = experimental factor (extract/test sample x 100ml).

Statistical assay: Data generated from experimental runs was analyzed using polynomial regression. The study was based on the hypothesis that response (y) is functionally related to roasting temperature, roasting time and feed quality by the equation.

$$Y = \beta_0 + \sum \beta 1\, xi + \sum \beta_{11}\, x_{12} + \sum \beta ji\, Xi\, Xj\ \mathcal{E} \quad \ldots\ldots\ldots\ldots\ldots..(1)$$

Where
Y = response; β_0 = intercept, Xiji = independent variables,
Optimization (minimize) of anti-nutrients was calculated using Minitab statistical software version 15 of Minitab Inc. USA.

Results and Discussion

The anti-nutrient values of roasted African breadfruit seed flour are shown on Table 3.

Table 3: Anti-nutrient Contents of Roasted African Breadfruit Seed Flour.

S/No Expt. Runs	Roasting temperature RT(^{0}C)	Roasting time RM(min)	Feed quantity FQ(g)	Phytate (mg/100 g)	Total phenols (mg/100 g)	Tannin (mg/100 g)	Hydrogen cyanide (mg/100 g)	Alkaloid (mg/100 g)	Oxalate (mg/100 g)
1	140	35	400	0.50	0.80	0.16	0.088	0.44	7.75
2	140	35	600	0.50	0.80	1.10	0.080	0.45	7.80
3	140	45	400	0.47	0.77	0.09	0.044	0.41	6.90
4	140	45	600	0.44	0.77	0.07	0.042	0.40	6.90
5	180	35	400	0.51	0.66	0.10	0.088	0.36	6.73
6	180	35	600	0.48	0.63	0.15	0.085	0.38	6.70
7	180	45	400	0.47	0.60	0.09	0.044	0.40	6.60
8	180	45	600	0.46	0.61	0.07	0.040	0.40	6.05
9	126.36	40	500	0.48	0.90	0.50	0.061	0.47	8.06
10	193.64	40	500	0.48	0.57	0.08	0.041	0.33	6.25
11	160	31.59	500	0.55	0.81	0.10	0.148	0.45	7.4
12	160	48.41	500	0.16	0.57	0.08	0.039	0.40	6.50
13	160	40	331.80	0.41	0.58	0.07	0.043	0.43	7.2
14	160	40	668.20	0.45	0.73	0.09	0.048	0.38	7.0
15	160	40	500	0.47	0.60	0.09	0.041	0.43	7.3
16	160	40	500	0.47	0.60	0.10	0.044	0.43	7.0
17	160	40	500	0.44	0.60	0.10	0.041	0.42	7.0
18	160	40	500	0.44	0.60	0.10	0.040	0.41	7.1
19	160	40	500	0.41	0.59	0.10	0.040	0.41	7.0
20	160	40	500	0.47	0.59	0.10	0.040	0.40	7.0
Control				**2.30**	**1.80**	**1.017**	**1.26**	0.61	**12.10**

Inactivation of anti-nutrients by roasting showed heat liability of most anti-nutrients associated with African breadfruit seeds. The reductive effects of process variable combinations were significant (P <0.05). These reductions promote nutrient availability and wider application of African breadfruits in food systems. The decrease observed in anti-nutrient values reflects the application and duration of heat during processing. Detoxification of phenols/Tannins is desirable as these anti-nutrients are known to impact poor colour on food due to enzymic browning. HCN content of flour was below the human toxicity (lethal dose 30-210 mg HCN) level and points to its usefulness in infant food formulations since infant lack the enzyme needed to detoxify HCN (Adeparusi; 2000). Similarly the detoxifications of Alkaloid and Oxalates to insignificant values are important as they contribute to bitter taste and esophageal irrigation to foods.

Though roasted African breadfruit seeds may not offer the beneficial health benefits of phytate (Wise and Gilbert, 1981; Zhou and Erdman, 1995). The presence of phytate impairs cytoplasmic function and growth by binding manganese and Zinc (Coulibaly *et al.*, 2011). The absence of phytate from diet correspondingly increased zinc and magnesium absorption by 20% and 60% respectively (Zhou and Erdman, 1995).

The estimated regression co-efficient are shown in Table 4.

Table 4: Estimated regression co-coefficients of Anti-nutrients.

	Total phenol	Tannin	HCN	Alkaloid	Oxalate
β_0	7.4142	6.2501	1.6812	1.3129	10.3609
β_1	-0.0485	-0.0953	-0.0025	-0.0036	-0.0533
β_2	-0.1185	-0.134	-0.0632	-0.0308	0.0443
β_3	-0.0223	0.0190	-0.0002	0.0006	0.0131
β_{11}	0.0012	0.0021	0.0001	-0.0012	0.0001
β_{22}	0.0014	0.0005	0.0070	0.0012	-0.0028
β_{33}	0.0011	0.0011	0.0011	-0.0012	-0.0001
β_{12}	-0.0022	0.0013	-0.0011	0.0002	0.0012
β_{13}	-0.0011	-0.0011	0.0011	0.0120	-0.0001
β_{23}	0.0011	-0.0003	0.011	-0.0012	-0.0011
R^2 (%)	90.78	79.13	95.95	80.91	95.16
Adj R^2	82.49	66.34	92.31	63.74	90.80
Lof	NS	NS	NS	NS	NS

Where R^2 = co-efficient of determination, Adj R^2 = adjusted R^2

Lof = lack of fit and NS = NS = Not significant.

Regression analyses showed co-efficient of determination R^2 of 90.78%, 79.13%, 95.95% and 95.16% for Total phenol Tannin, HCN Alkaloid and oxalate, respectively. Except for phytate regression co-efficient including values of R^2 and Non- significant lack of fit between models indicated adequacy of models to account for the variations in data.

The pattern of change in Total phenol contents reflected the significant ($P<0.05$) linear and quadratic effects of roasting temperature and roasting time on total phenol (Tp)contents of roasted seed sample.

The non- linear polynomial equation for total phenol after removing the non-significant terms can be written as

$$Tp = 7.4141-0.04285\ RT - 0.1185RM + 0.0012\ RT^2 + 0.0014\ RM^2 \quad \ldots\ldots\ldots\ldots (2)$$

$$R^2 = 90.78\%.$$

The tannin contents compared with values reported for parboiled seeds of African breadfruits (Nwabueze, *et al.*, 2007). Roasting temperature was observed to be more critical than roasting time. Roasting temperature and feed quality showed significant ($P< 0.05$) effect on tannin while interaction effects between Roasting temperature and time, roasting time and feed quantity were significant and reflected on tannin contents of the roasted seed flour.

The polynomial equation for effects of independent variables on Tannin (Tn) contents can be written as.

$$Tn = 6.2501 - 0.953\ RT - 0.0190FQ + 0.0022\ RTRM - 0.0011\ RMF \quad \ldots\ldots\ldots\ldots(3)$$

$$(R^2 = 79.13\%)$$

HCN exhibited drastic reduction in values, especially for those samples subjected to high process temperature (above 180^0c). Roasting time only among the independent variables influenced significantly HCN content. It seemed probable that in order to achieve better detoxification of HCN duration of heat application is more important than the quantum of heat delivered to the processing product.

The non-linear polynomial equation for detoxification of HCN after removing the non-significant terms can be written as.

$$HCN = 1.68124 - 0.0632\ RM + 0.0070\ RM^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

$$(R^2 = 92.31\%)$$

Alkaloids (Ak) contents were significantly reduced. The observed significant roasting time during the roasting of seeds of African breadfruits. The equation describing the relationship can be written as

$$Ak = 1.3129 + 0.0002\ RT\ RM \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

$$(R^2 = 80.91\%)$$

Oxalate (Ox) contents similarly reflected only the significant interactive effects of Roasting temperature and roasting time as described by the equation.

$$Qx = 10.3609 + 0.0012\ RT\ RM \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(6)$$

$$(R^2 = 95.16\%)$$

Optimization of minimization of anti-nutrients: Minitab statistical software was used to estimate optimum minimization of anti-nutrients of flour of roasted seeds of African bread fruits. The input variables (Temperature, Time and Feed quantity) was optimally found to 193.64^{0}C, 39.57 min and 386.17g respectively. The observed optimally minimized values of anti-nutrients were 0.61mg/100g, 0.24mg/100g, 0.05mg/100g, 0.01mg/100g, 0.44mg/100g and 0.52mg/100g for Total phenol, Tannin, HCN, Alkaloids, Oxalate and phytate respectively (Table 5). The difference between predicted and experimental values was marginal for total phenol, HCN and phytate. Higher minimizations were predicted for Alkaloids and oxalates as shown by this study.

Table 5: Optimum Minimization of Anti-nutrients

Input variables	optimum setting
Roasting temperature (^{0}C)	193.64
Roasting Time (min)	39.57
Feed quality (g)	386.17
Anti-nutrients	
(Responses)	Optimum values
Total phenol	0.61
Tannin	0.24
HCN	0.05
Alkaloid	0.01
Oxalate	0.44
Phytate	0.52

Haemagglutinin Activity: The haemagglutinin (Hn) content and activity of sample are depicted in Table 6 which ranged from 9.65Hu/100g to 120Hu/100g (raw seed flour) with strong to mild activity.

Roasting had less reductive effect on African breadfruit seeds. However, results compared favourably with values reported by Uguw and Oranye (2006), Nwabueze *et al.,* (2007) for parboiled African breadfruits seed and Iwe (2003) for soybean meal.

Roasting temperature and time at lower range had no significant effect ($p<0.05$) on haemagglutinin.

Haemagglutinin has health and nutritional importance traced to its anti-nutritional factor in diet and its ability to coagulate red blood cells.

Haemagglutinin have specific affinity for certain sugar molecules when present in most animal cell membranes and may attach to those receptor groups and affect nutrient availability. Iwe (2003) reported about 25-50% growth inhibition of albino rats feed on soybean meal.

Table 6: Haemagglutinin Content and Activity of Samples

	Variables		Haemagglutinin **Hu/100g**	Haemagglutination activity
RT ^{0}C	**RM Min**	**FQ g**		
<140	35	500	72.18	**xxx**
160	40	500	9.65	**x**
>180	40	500	ND	NS

xxx strong haemagglutination x grainy clumps NS not seen

Conclusion

The process parameters of this study demonstrated a good performance. The Central composite Rotable Design (CCRD) regression analysis and Response surface method (RSM) were effective in locating the optimum condition of detoxification of Anti-nutritional factors of roasted African breadfruit seed flour. RSM can effectively optimize anti-nutrients. At an optimum condition of Roasting temperature at 193.64^{0}C, for a time of 39.57min with feed quality 386.17g minimum values of anti-nutritional factors were Total phenol 0.61mg/100g, Tannin 0.24mg/100g, HCM 0.05mg/100g . Alkaloid 0.01 mg/100g, Oxalate 0.44mg/100g and Phytate 0.52mg/100g. Roast processing has the capacity to detoxify haemagglutinin in African breadfruit seeds.
These predicted values represent minimization of anti-nutrients of flour of roasted African breadfruit seeds produced using CCRD/RSM modulation of variables. The HCN Content was below the lethal dose for man.

References

Adeparusi, E.O. (2000). Effects of Processing on some minerals anti-nutrients and nutritional Composition of African Yam Bean. *J. Sust. Agric. Environ* 3(1) 101-108.

Coulibaly, A. Konakou, B and Chen, J. (2011). Phytic acid in cereal grains; structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects in nutritional quality. *Am.J. Plant fertilization Tech*. 1(1):1-22.

Ihekoronye, A.I. and Ngoddy, P.O. (1985). Integrated Food Science and technology for the Tropics. Macmillan Pub. Ltd London. 36-48 pp

Iwe, M.O. (2003). The science and technology of soybean. Rogoiut Com. Services Ltd., Enugu, Nigeria. 73-101 pp

Nwabueze, T.U. Iwe, M.O and Akobundu, E.N.T. (2007). Unit Operations and analysis for African breadfruit based spaghetti type product at extreme process combination .*J Food Tech* 5 (1): 42-45.

Nwokocha, L.M and Ugbomoiko, J.O. (2008). Effects of Parboiling on the composition and physiochemical properties of *Treculia africana* seed flours. *Pakistan J. Nutr*. 7(2): 317-20

Onwuka, G. I. (2005) *Food Analysis and Instrumentation*: *Theory and Practice*. Napthali Prints. Lagos. 133-139

Person. D (1976). *The Chemical Analysis of Food.* 7th Ed. Church Hill Livingston Edinburg. 6-17 pp.

Uguw, F.M., and Oranye, N.A. (2006). Effects of some processing methods on the toxic component of African breadfruit (*Treculia africana*). *African Journal of Biotechnology,* 5(22): 2329-2333.

Wise, A and Gilbert, D.J. (1981). Binding of Cadmuim and lead to the calcium – Phytate Complex Invitro. *Toxicol let.* 9:45-50

Zhou, J.R and Erdman, J.W. (1995) Phytic acid in health and disease *Crit. Rev. Food Sci. Nutri* 35: 495-508