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Impact of process strictures on the composition and sorption isotherms of Bambara nut flour using RSM

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Abstract

The global concern for the diversification of the uses of plant foods to improve normal and the paretic nutrition for diabetes control has shifted research interest to enhancing the potential sources of beneficial constituents in plant foods. Studies have shown that the fibre and protein contents of Bambara nut can weaken the absorption of sugar, reduce sugar response and increase insulin sensitivity and therefore recommended as a supplement for type II diabetes. Diabetic patients in Nigeria rely on Bambara nut flour as food because of its insulin building ability in the body system, but the challenge is its unavailability all the year round in the market outlets when needed. Presently, there is paucity of information on the storage techniques of Bambara nut flour that can prolong the shelf-life for later usage. Experimental research design method was adopted. Bambara nut was sourced and milled to flour, stored in a room temperature (28-32°C) for 6 months. Particle size distribution using sieve analysis was carried out to separate the flours at particle size levels 20, 40, 60, 80 and 100 mesh numbers (850, 425, 250, 180 and 150µm) as designed in the face central composite design (FCCD) response surface methodology of Design Expert 7.0.0 software. Moisture sorption characteristic of the flour was determined. The effects of particle size and storage duration on the proximate composition of the flour were investigated. The data obtained were analyzed using the Design-Expert software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA). The experimental data generated was fitted to a polynomial regression model for predicting maximum shelf-life. In order to correlate the response variables to the independent variables, multiple regressions were used to fit the coefficient of the polynomial model. The quality of fit of the model was evaluated using analysis of variance (ANOVA). The suitability of the models was compared and evaluated using correlation coefficient (R^2). The results obtained from the study showed that storage duration had effect on the proximate composition of Bambara nut flour. The flour particle size had effect on the proximate composition. The time for the flour to reach equilibrium varied from 6 – 14 days. The results obtained in the study showed the response surface model employed is a good one. The model correlation coefficient (R^2) of the responses was found to be 0.9701, 0.9688 and 0.9138 for the flour ash, protein, and fibre contents, respectively. Levels of significance obtained were 0.02, 0.03 and 0.03 for the flour ash, protein and fibre contents which were high and attested to the fitness of the model in evaluating the responses. Optimum particle size, moisture content and storage time were found to be 150.12µm, 6.32% (wb), and 23.62 weeks**.**

Keywords: Flour, Particle Size, Relative Humidity, Temperature, Shelf Life

1. Introduction

Bambara nut (Vigna subterranea) is an indigenous crop grown widely in the African continent. It is an indigenous African crop that has been cultivated in Africa for years. (Temegne et al., 2018) reported that Bambara nut is now widely cultivated throughout tropical Africa, Indonesia, Malaysia, India, Sri Lanka, Philippines, South Pacific, parts of northern Australia, Papua New Guinea, Central and South America. Nigeria is one of the major producers of the crop and it is locally called Fyegbankpo (Jukun), Okpa (Ibo), Epiroro (Yoruba) and Gurjiya (Hausa). It is known in South Africa as Jugoboon; Nyimo in Shona (Zimbabwe). It is a highly nutritious plant that plays a crucial role in people's diets (Jideani and Mpotokwane, 2009). It is the third most important grain legume after ground nut and cowpea in Nigeria (Lacroix et al., 2003). It is grown in the African continent from Senegal to Kenya, and from the

Sahara to South Africa and Madagascar (Swanevelder, 1998). Beyond Africa, Bambara nut is cultivated in Brazil where it is known as Mandubi d‟Angola‟ as well as in West Java and southern Thailand. Other tropical locations such as Middle East, Syria and Greece could also grow Bambara nut. Small-scale cultivation trials of Bambara nut have been successful in Florida, United States.

The crop believed to has been domesticated in West Africa from its presumed wild ancestor (Fery, 2002; Heller et al., 1997). Odeigah and Osanyinpeju, (1998) reported that the nut has become less important in many parts of Africa because of the expansion of American groundnut (Arachis hypgaea) production. Bambara nut is extensively cultivated in West Africa, Nigeria produced over 100,000 metric tons, follow closely by Niger with 30,000 metric tons and Ghana 20,000 metric tons annually (Asiedu, 1989).

Bambara nut is a low cost crop, used as food, medicine and animal feed. It is grown by families for their own subsistence and for their annual income. Majority of Bambara nut is produced for home consumption although a small amount is grown as a cash crop. Bambara nut is consumed in several ways; it is essentially grown for human consumption. It can be used as an ingredient in cooking, used as baking flour, or eaten as a snack (Goli, 1995). Because of its nutritional value, it can be used to prepare steamed gel (moi moi) for human consumption. The seeds eaten fresh or grilled while immature an also prepared into Bambara milk, which is often preferred to milk prepared from other pulses because of its flavour and colour. In many West African countries, the fresh pods of Bambara nut are boiled with salt and pepper and eaten as snack. In East Africa, the seeds are roasted, pulverized and used in preparing soup. Dry seeds are ground into powder, which is used for bread making or prepared into stiff porridge, a very popular semi-fluid food in some parts of Nigeria. Bambara nut is also used for livestock feeds (Goli, 1995; Heller et al., 1997). It also serves as food for poultry. People live on Bambara nut, an uncertain proposition for other legumes (National Research Centre, 1996). Hence, it could be a tool for attacking Africa‘s malnutrition.

Despite the nutritional and economic importance of Bambara nut, there is no industrial use of the crop in Nigeria and most of the African countries. For the use of Bambara nut in the production of flours, the flour needs to be stored properly prior to utilization in order to maintain the quality, safety and storage stability. Like most agricultural products, Bambara nut flour is hygroscopic and the storage environment could adversely affect its quality. Consequently, the study of determining the Influence of process parameters on the composition and sorption properties of Bambara nut flour using RSM is imperative. Knowledge of the Bambara nut flour process parameter and optimum storage condition is important for the shelf-life prediction and the optimum moisture content level acceptability for the storage of the flour.

2.0 Material and methods

2.1 Study and Experimental Locations

The study was conducted at the Department of Agricultural and Bioresources Engineering, University of Nigeria, Nsukka while the experiments were carried out at the laboratories of the Departments of Food Science and Technology, University of Nigeria Nsukka and Federal University Wukari, respectively.

2.2 Research materials

The materials used for this study were, a brown variety of Bambara nuts- *Vigna subterranea* , food grade chemicals and water.

2.3 Source of Bambara nut

The brown variety of Bambara nut was purchased from a local market in Mu-Baka village, Donga LGA, Taraba State. North-eastern Nigeria. The seeds were manually cleaned to remove foreign matters, immature and broken ones.

2.4 Chemicals and reagents

All the chemicals used were of analytical grade (Distilled water, sulphuric acid, sodium Hydroxide, Selenium tablets, Boric acid, Methyl red, Hydrochloric acid and Refined Vegetable oil). Some were purchased from Nsukka market while others from VEKO Scientific Chemical Shop, Jimeta-Yola.

2.5 Methods

The experiment was in two stages, the first stage was conducted for the determination of Proximate Composition of the stored Bambara nut flour. The second stage involved the determination of Bambara nut flour Moisture Sorption characteristics.

2.5.1 Preparation of experimental samples

The Bambara seeds were milled into flour using a magnetic sieve grinding machine as described by Ngabea et al, (2016). Particle size distribution using sieve analysis was carried out to separate the flour at a range of 20 - 100 mesh numbers (850 - 150µm) as designed in the face central composite design (FCCD) response surface methodology of Design Expert 7.0.0 software.

2.5.2 Treatment and experimental design

The treatments were arranged according to the face centered composite design (FCCD) surface response of Design Expert 7.0.0 software.

2.5.3 Determination of Proximate Composition of Bambara nut flour

The, ash, protein and fibre contents of the Bambara nut flour were determined using the methods of AOAC (2010).

2.5.4 Determination of Ash Content

Five grams of each sample were weighed into a porcelain crucible, respectively. The samples were charred by igniting the material on a hot plate in the fume cupboard. The crucible was then placed in the Vecstar Muffle Furnace and maintained at 550ºC for six hours. It was then cooled in a dessicator and was weighed out.
The percentage ash content was determined as shown in equation 1

$$\% \text{ Ash} = \frac{(Weight\ of\ crusible\ and\ sample)(weight\ of\ empty\ crusible)}{(weigght\ of\ sample)} \times 100 \quad (1)$$

2.5.5 Determination of crude fibre

Defatted sample (2g) was placed in a 500 ml conical flask, 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was then digested for 30 min, thereafter the sodium hydroxide solution was drained out and the sample was then washed with boiling distilled water. Finally, the sample was placed in a dried crucible and oven dried at 110ºC overnight. The sample was allowed to cool in a desiccator and then weighed (W1). The sample was ashed at 550ºC in a muffle furnace for two hours, cooled in a desiccator and then reweighed (W2). Extracted fibre was expressed as percentage of the original undeffated sample and calculated according to the formula:

$$Crude\ Fibre\ (\%) = \frac{Digested\ sample\ (W1) - Ashed\ sample\ (W2)}{Weight\ of\ sample} \times 100 \quad (2)$$

2.5.6 Determination of crude protein content

Kjedahl nitrogen method was used for the determination of crude protein. One gram of the sample from each treatment was introduced into 800 ml digestion flask. 5 selenium tablets were added to the sample as catalyst. Twenty milliliters of concentrated sulphuric acid was added to each sample and fixed to the digestion flask until a clear solution was obtained. The cooling digest was transferred into 100 ml volumetric flask and was made up to mark with distilled water. The distillation apparatus was set up and rinsed for 10 minutes. After boiling, 20 ml of 4% boric acid was pipetted into conical flasks, 5 drops of methyl red was added to each flask as indicator and the digest was diluted with 75ml distilled water. 10ml of the digest was made alkaline with 20ml of 20% NaOH and distilled. The steam exit of the distillatory was closed and the changed in colour of boric acid solution to green was noted. The mixture was distilled for 15 minutes (AOAC, 2010) and boric acid along with distillate was then titrated against 0.1N hydrochloric acid and consequently, the percentage total nitrogen was calculated as indicated below:

$$\% \text{ total of nitrogen} = \frac{Titre \times Normality \times 0.014}{Weigght\ of\ sample} \times 100 \quad (3)$$

$$\% \text{ Crude Protein} = \% \text{ total nitrogen} \times 6.25 \quad (4)$$

Where:
6.25 is a constant (AOAC, 2010)

2.5.7 Determination of Moisture Sorption Isotherm
The adsorption isotherms of the samples were carried out according to AOAC, (2010) standard using static gravimetric method as described by Labuza, (1984). An incubator was used as temperature control chamber. The experimental set up consists of saturated salt of lithium chloride, potassium acetate, MgCl, KCO_2, MgN_2, sodium nitrate, Nacl, ammonium sulphate and barium chloride solutions which created different relative humidity environmental storage conditions with the corresponding water activities of 0.11, 0.21, 0.33, 0.43, 0.50, 0.67, 0.76, 0.86 and 0.90, respectively. The samples were arranged in dessicators. The duplicate flour samples of 3g each were placed on the saturated salt solutions in the dessiators and kept in a cabinet at a controlled temperatures of 20, 30, 40 and 50 ºC, respectively. The weight of the samples was taken after every 24 hours with a digital weighing balance until constant weight is attained. Each of the samples was oven dried at 110 ºC to a constant weight to obtained the equilibrium moisture content (dry basis). Graph of equilibrium moisture content against water activities (relative humidity) was plotted.

2.5.8 Determination of equilibrium moisture content
Equilibrium moisture content was determined by calculating the original moisture content and the known change in weight on dry basis (Akubor and Egbekun, 2017). The relation (equation 5) is used in calculating the equilibrium moisture content (EMC) of the samples.

$$\text{EMC} = \left(\frac{Adsorbed\ moisture}{Weight\ of\ sample}\right) \text{X } 100 \quad (5)$$

2.5.9 Determination of water activity
The water activity a_w of the sample was determined from the relative humidity of the air surrounding the sample when the air and the sample are at equilibrium which is equilibrium relative humidity. The equilibrium relative humidity value was divided by 100 to get the water (a_w) value.

2.6 Experimental design and data analysis
A face central composite design (FCCD) of Response Surface Methodology (RSM) was used for the experimental design. The factors or independent variables were storage time and particle size, while the responses were the proximate compositions (ash, protein and fibre contents).
The independent variables and the responses are detailed in Table 4. The outline of experimental design with the coded levels is given on Table 5. Table 1 showed the variation of parameters and the coded factors.

Table 1: Variation of parameter for two (2) numerical factors design in surface response

Numerical Factor		
Variable	Low level (-1)	High level (+1)
Time (weeks)	8	24
Particle size	20 mesh number	100 mesh number

Note that, the low level and high level interval of 8 and 24 for the time (weeks) was obtained from the result of the preliminary investigation conducted to study the changes in proximate composition of Bambara nut flour under ambient storage condition, where the changes were noticed in the 8th week of storage (low level -1) as shown on Table 1

2.6.1 Modeling of the flour shelf-life with respect to particle size and time
The experiment was carried out as designed in Table 2. The data obtained were analyzed using the Design-Expert software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA). The experimental data generated was fitted to a polynomial regression model for predicting maximum shelf-life. In order to correlate the response variables to the independent variables, multiple regressions were used to fit the coefficient of the polynomial model of the response. The quality of fit of the model was evaluated using analysis of variance (ANOVA).

2.6.2 Validation of the regression model

The model developed was examined for Test for significance, lack-of-fit and coefficient of determination (R^2) which was integrated into the analysis of variance (ANOVA) to examine the adequacy of the regression model while response surface and contour plots were designed with the Design-Expert software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA). R^2 was calculated as shown in equation 6 and 7

$$R^2 = \frac{\text{Sum of square residual}}{\text{Model sum of square +sum of square residual}} \quad (6)$$

$$R^2 \text{ adj} = 1 - \frac{n-1}{n-p}\left((1) - R^2\right) \quad (7)$$

2.6.3 Process Optimization

To optimize the response variables, contour and surface plots were plotted using the Design Expert software as described by Floros and Chinnan (1988). A second order polynomial was used to predict the experimental behavior (Equation 8).

$$Y = \beta o + \beta_1 X_1 + \beta_2 X_2 + \beta_1 X^2{}_1 + \beta_2 X^2{}_2 + \beta_{11} X_1 X_2 + \beta_{12} X_1 X_2 + \varepsilon \quad (8)$$

Where,

X_1, and X_2 are the factors: storage time and particle sizes

β is a constant coefficient of linear, interaction and square terms respectively

ε is the random error term.

Pearson correlation analysis (p = 0.05) was performed using the Design-Expert software (Version 7.0.0, Stat-Ease Inc., Minneapolis, USA).

3.0 Results and Discussions

3.1 Effect of particle size and storage duration on the proximate compositions of stored Bambara nut flour

The results of the proximate composition (ash, protein and fibre contents) of the Bambara nut flour are presented on Table 2. The mean effect of the particle size and storage duration on the percentage proximate composition of Bambara nut flour showed that there were substantial differences in the moisture content of the flour sample at the beginning of the storage period and at the end of the storage period in all the three particle sizes. The result showed that the moisture content ranged from 8.67% - 12.01%. At the beginning of the storage period, the moisture content was 10.17, 11.17 and 12.01% for flours with particle sizes 850, 250 and 150µm respectively and the moisture content decreased as the storage period increased. By the end of the storage period in the 6th month, the moisture contents dropped to 8.67, 9.00 and 9.05% for flours with particle sizes 850, 250 and 150µm respectively. This variation could however be due to variation in relative humidity of the environment within the storage period and the respective bulk densities of the flours. The storage commenced in October with average relative humidity of 84.28% and end in March with average relative humidity of 66.82%. This result of the moisture content during storage is in close agreement with earlier findings of Muhammad et al, (2003) on Wheat flour. It was reported that mould growth and insect infestation was higher in the flour with moisture content of 13.5% compare to the wheat flour with moisture content of 9%. It was concluded that the flour with moisture content of 9 and 10% are suitable for storage stability and longer shelf life of wheat flour. The moisture content range corresponds with the recommended moisture content (6-14%) for flour stability by Standard Organization of Nigeria, (2003).

Table 2: Proximate composition of Bambara nut flour Stored for a period of six months

Month	Size (µm)	Ash	Protein	Fibre
1st Month	850	3.33	18.35	2.56
	250	3.00	20.01	2.56
	150	3.00	20.13	2.67
2ndMonth	850	3.00	21.01	3.33
	250	3.00	23.63	2.67
	150	3.00	23.64	3.00
3rd Month	850	3.00	21.64	3.33
	250	3.00	23.01	3.00
	150	3.30	25.01	2.84
4th Month	850	3.00	24.51	3.00
	250	3.00	26.01	2.67
	150	3.30	26.76	3.00
5th Month	850	3.33	27.51	2.92
	250	3.33	28.01	2.65
	150	3.00	28.51	2.85
6th Month	850	3.33	26.63	2.67
	250	3.33	28.01	2.58
	150	3.00	28.38	2.72

Table 2 also showed that, the protein contents of Bambara nut flour increases as the storage period increased. At the beginning of the storage period the protein content was 18.35, 20.13 and 21.01% for the flour with particle sizes 850, 250 and 150µm. At the end of the storage period at the 6th month, the protein content of the flour rose to 25.01, 25.38 and 26.63% for the flours with particle sizes 850, 250 and 150µm respectively. The result showed that the storage duration has effect on the protein content of Bambara nut flour. And there was a significant difference ($P<0.05$) in the protein content of the flour as the storage period increased. The results suggested that particle size was a major factor affecting the quality attributes of Bambara nut flour. The textural properties of the flour could be modified by the variation of the flour particle sizes. The increase in protein content as the storage period progressed is an indication of a good quality attributes and storage stability in all the particles sizes of the flour. Because higher protein content caused flours to have more water absorption capacity and other farinograph parameters were affected largely by protein quality. Flours with higher protein qualities produced stronger dough.

The result of the ash content as presented on Table 2 showed that, at the beginning of the storage, the ash contents were 3.33, 3.00 and 3.00% for the flours with particle sizes 850, 250 and 150µm respectively and at the end of the storage period, the ash content was found to be 3.33, 3.33 and 3.00%. There was no significant difference in the ash contents of Bambara nut flour throughout the storage period from the 1st month to the 6th month. The low ash content of the flour could be due to low organic mineral content. Ash content refers to the amount of ash that would be left over if you were to burn 100g of flour. Higher ash content indicates that the flour contains more of the germ, bran, and outer endosperm. Lower ash content means that the flour is more highly refined. Ash content is a good indicator of bran contamination in food flours. As flour extraction rate is increased, the amount of contamination with non-endosperm increases and the ash content increases. The results therefore showed an indication of storage stability in all the three particle sizes. This is in close agreement with Aidoo *et al.,* (2010) on cowpea flour.

The fibre content of Bambara nut flour ranged from 2.56% – 3.0%. In the 1st month, the four fibre contents were 2.56, 2.56 and 2.67% for the flour with particle sizes 850, 250 and 150µm respectively. At the end of the storage period in the 6th month, the flour fibre contents were 2.67, 2.58 and 2.72%, respectively. The result showed that storage duration does not have much effect on the fibre content of Bambara nut flour. The fibre content slightly increased as the storage period increased in all the particle sizes. The slight increase in fibre content could probably relate to the thickness and hard peel of the seeds which were not eliminated prior to the flour sample preparation.

3.2 Experimental Results of Sorption Isotherms

The experimental results for the equilibrium moisture content of Bambara nut flour at each water activity (a_w) at temperatures 20°C, 30°C, 40°C, 50°C are presented in Table 3.

Table 3: Experimental results for the adsorption sorption isotherms of Bambara nut flour at each water activity (a_w)

Conc. Salts Solutions at different a_w	Temperatures			
	20°C	30°C EMC	40°C	50°C
0.1 Lithium chloride	0.867	0.832	0.867	0.886
0.2 Potassium acetate	0.814	0.811	0.819	0.825
0.3 Magnesium chloride	0.739	0.712	0.741	0.752
0.4 Potassium carbonate	0.674	0.636	0.678	0.691
0.5 Magnesium nitrate	0.549	0.522	0.534	0.549
0.6 Sodium nitrate	0.452	0.416	0.461	0.470
0.7 Sodium chloride	0.364	0.365	0.369	0.371
0.8 Ammonium sulphate	0.261	0.259	0.274	0.282
0.9 Barium chloride	0.172	0.174	0.182	0.191

The results showed that the equilibrium moisture content of all samples increased with water activity at selected temperatures.
Table 3 showed the Equilibrium moisture content data obtained for Bambara nut flour at different water activities and temperatures. The results showed that temperature depended on the behavior of the sorption isotherm; increase in temperature causes the capacity of the sorption to increases also. The decrease in the equilibrium moisture content with increase in water activity makes the flour more susceptible to microbial spoilage. Therefore, Bambara nut flour requires careful handling and proper storage environment to make it stable over long period of time.

3.2.1 Sorption Isotherms

The adsorption moisture sorption isotherms of Bambara nut flour at 20°C, 30°C, 40°C and 50°C are presented in Figure 1

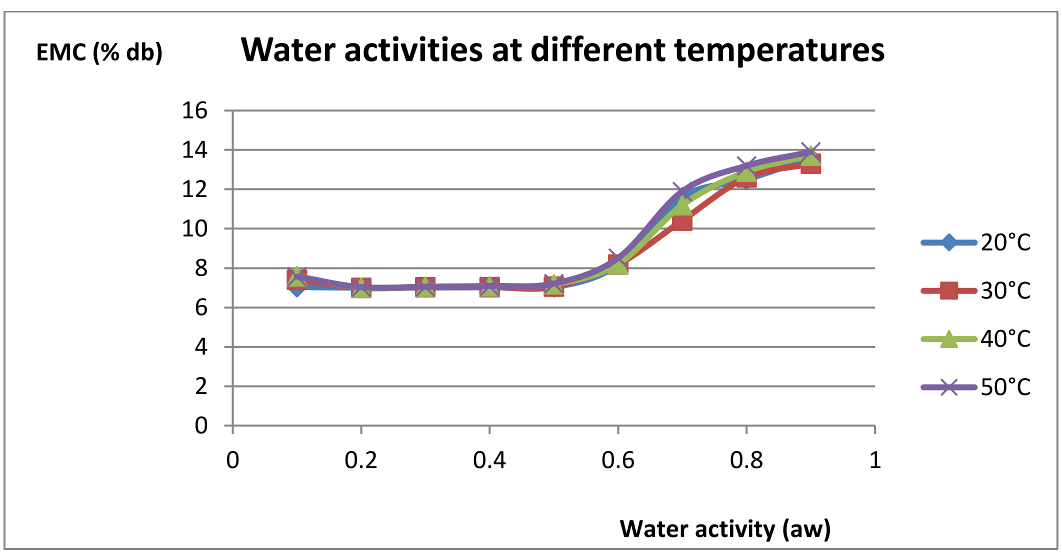

Figure 1: Adsorption Moisture Sorption Isotherms of Bambara nut flour at 20°C, 30°C, 40°C and 50°C

The adsorption moisture sorption characteristics of Bambara nut four at 20°C, 30°C, 40°C and 50°C are shown in Figure 1. Each point on the curve of sorption isotherms showed the average of two replications. The four curves of isotherms exhibited a sigmoid shape (type II) isotherm which is common for many hygroscopic products like the Bambara nut flour as described according to the classification of Brunauer in Aqua Lab University application note (2018). This type of curve is typical of many food materials. At low and intermediate water activities (relative temperature) the equilibrium moisture content of Bambara nut flour of the four temperature study increased slowly at the initial stages (0.1 – 0.6 a_w), and a steep rise with increase in water activity (0.6 – 0.9 a_w) was noticed on the curve. This behavior was reported for African locust bean pulp flour (Akubor, 2017). Similar result was reported for ginger slices (Alkali *et al.*, 2009). This behavior and characteristics of Bambara nut flour could be attributed to physical adsorption of moisture on polymeric molecules at low water activities. The forces involve in physical adsorption of moisture are mainly those of molecular interaction which induced quarapole and dipole attractions. These are the types of forces responsible for non-ideal behavior of gases and vander waal forces of attraction of water molecules to the flour. The time taken for the Bambara nut flour to reach equilibrium varied from 7 - 14 days and was shorter at lower water activities than at higher temperature.

3.2.2 Influence of temperature on moisture sorption isotherm of Bambara nut flour

Figure 1 showed that the equilibrium moisture content of Bambara nut flour at water activities of 0.1 – 0.6 remained stable with increase in temperature. However, at higher water activities (0.7 – 0.9), the equilibrium moisture content increased with increase in temperature. This is similar to the result reported in the literature by Nutama, (2010) on taro flour. This showed that the Bambara nut flour within the water activities of 0.1 - 0.6 became less hygroscopic with increase in temperature. This agrees with the result reported for ginger slices (Alkali et al., 2009). This behavior is compelled with the thermodynamic relationship according to Ilesanmi and Gungula, (2016). $\Delta G - \Delta H = T\Delta S$ where ΔH, $T\Delta S$ and ΔG are changes in the enthalpy, entropy and free energy, respectively. Therefore, increase in temperature represents an unfavorable environmental storage conditions for Bambara nut flour.

3.3 Modeling of the effects of storage time and particle size on the proximate composition of Bambara nut flour

The descriptive statistics, experimental ranges and levels of the independent variables for the experimental design for proximate analysis of Bambara nut flour are summarised in Table 4.

Table 4: Experimental ranges and levels of the independent variables for the proximate analysis of Bambara nut flour

	Storage Time (Weeks)	Particle Size (Mesh)	Ash (%)	Protein (%)	Fibre (%)
1	24.00	20.00	3.33	36.76	2.96
2	8.00	60.00	3.00	20.13	2.33
3	8.00	100.0	3.00	21.01	2.00
4	8.00	20.00	2.67	22.76	2.67
5	16.00	100.00	3.00	19.26	3.00
6	16.00	20.00	3.30	21.01	3.33
7	16.00	60.00	3.30	20.13	2.67
8	24.00	60.00	3.33	29.76	2.66
9	16.00	60.00	3.30	20.13	2.67
10	16.00	60.00	3.30	20.13	2.67
11	24.00	100.0	2.67	33.26	3.00
12	16.00	60.00	3.30	20.13	2.67
13	16.00	60.00	3.30	20.13	2.67

The coefficient of the regression equations for the measured responses, the linear, quadratic and interaction terms of the selected variables are presented in Table 5.

Table 5: Regression coefficients of predicted quadratic model for proximate composition of Bambara nut flour

		Dependent	
Coefficients	**Ash**	**Protein**	**Fibre**
Intercept	3.31	19.65	2.72
A	0.11	5.98	0.27
B	-0.11	-1.17	-0.16
AB	-0.25	-0.44	0.18
A^2	-0.18	6.50	-0.34
B^2	-0.20	1.69	0.33
R^2	0.9701	0.9688	0.9138
Adj. R^2	0.9488	0.9465	0.8522
C.V (%)	1.77	5.76	4.61
Adeq. Precision	18.900	17.203	12.962
Mean	3.14	23.43	2.71
Std. Dev.	0.056	1.35	0.13

A = Storage time, B = Particle size

The results of the proximate composition of the flour showed that the linear (A,B), interaction (AB) parameters and square (A^2, B^2) terms were all significant at $p<0.05$ as shown in Table 5.

3.3.1 Fitting of the quadratic model

The quadratic model fittings are shown in Table 5. The analysis of variance (ANOVA) showed that the model was significant ($p<0.05$) for the predicted flour moisture, ash, protein, fat, fibre and carbohydrate contents. The correlation coefficient (R^2) 0.9701, 0.9688 and 0.9138 for the flour ash, protein and fibre contents, respectively were obtained. The R-squared value is an indication of the level of responses that can be explained by a particular model. These results showed that 97.01%, 96.88% and 91.38% of the responses could be explained by the model. Levels of significance obtained were 0.02, 0.03 and 0.03 for the flour ash, protein and fibre contents, respectively. These levels were high and attested to the fitness of the model in evaluating the responses. The results obtained in the study showed that the model employed is a good one and could be used for the prediction of the flour maximum shelf life, particle size and storage time in respect to the proximate compositions of Bambara nut flour for the processing, handling and storage of the flour.

Using the experimental data in Table 4, second degree polynomial equation model for the flour ash, protein and fibre contents, respectively were regressed and the final equations in term of coded factors for the linear, interaction and square terms, respectively are shown in equations 9–11.

$$\text{Ash content} = +3.31 + 0.11A - 0.11B - 0.25AB - 0.18A^2 - 0.2B^2 \quad (9)$$

$$\text{Protein content} = +19.65 + 5.98A - 1.17B - 0.44AB + 6.50A^2 + 1.69B^2 \quad (10)$$

$$\text{Fibre content} = +2.72 + 0.27A - 0.16B + 0.18AB - 0.34A^2 + 0.33B^2 \quad (11)$$

3.3.2 Numerical optimization of particle size and storage duration on the proximate composition of Bambara nut flour

The graphical representation of 3 dimensional surface and contour plots of response surface in Figures 2 - 7 show the relationships between the dependent and independent variables of the proximate compositions for handling and storage of Bambara nut flour.

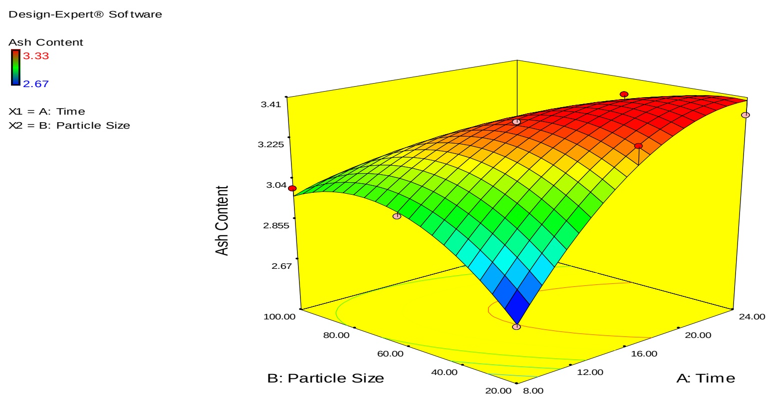

Figure 2: 3D surface plot of the effects of particle size and storage time on the Ash content of Bambara nut flour

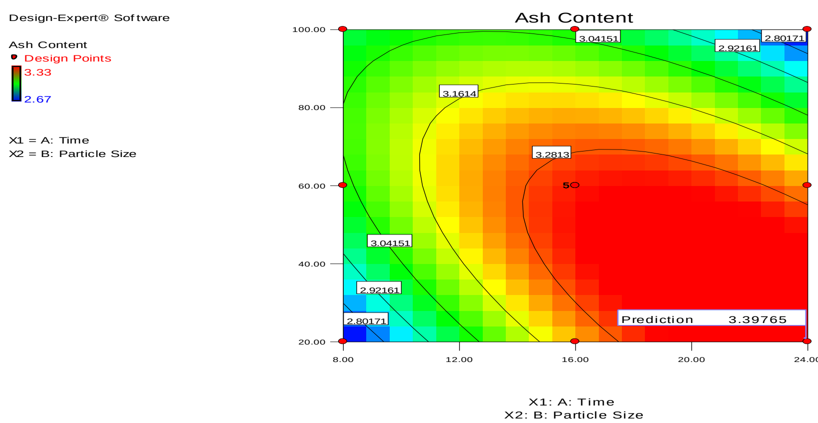

Figure 3: Contour plot of the effects of particle size and storage time on the Ash content of Bambara nut flour

Figures 2 and 3 showed the three dimensional plots of the effects of particle size and storage duration on the ash content of Bambara nut flour. The ash content has the optimizer values of 3.28%, 3.16%, 3.04%, 2.92% and 2.80% with the maximum response ash content value of 3.39%. However, there were no significant differences on the ash content. The ash content slightly decreased as the storage period progressed. The reduction could be as result of biochemical activities of microorganisms in the flour. This is in perfect agreement with the result of Awoyale *et al*, (2013).

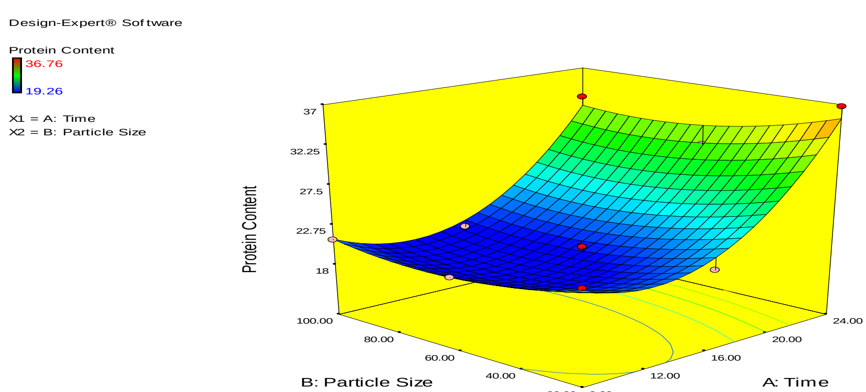

Figure 4: 3D surface plot of the effects of particle size and storage time on the Protein content of Bambara nut flour

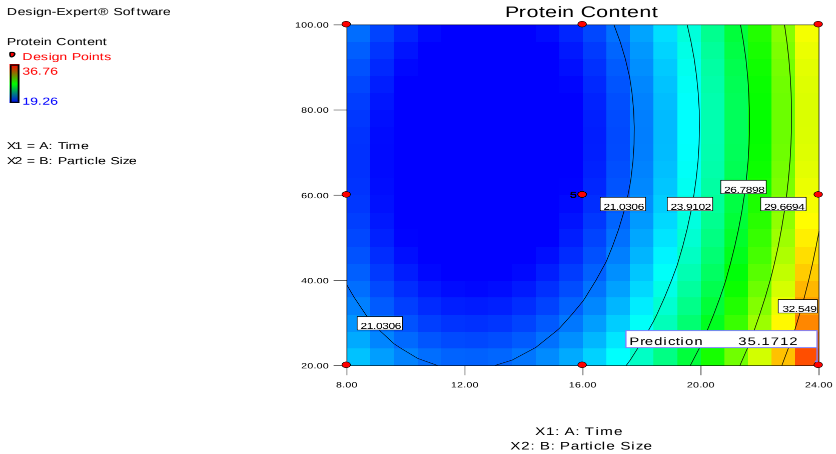

Figure 5: Contour plot of the effects of particle size and storage time on the Protein content of Bambara nut flour

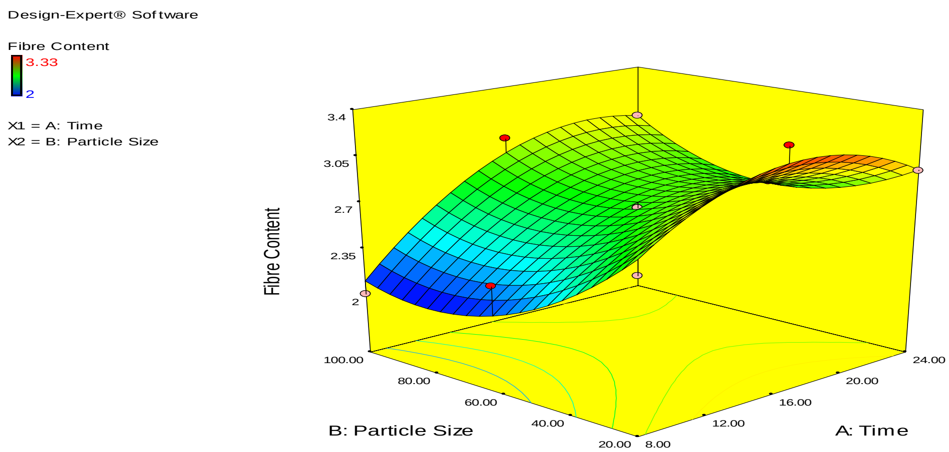

Figure 6: 3D surface plot of the effects of particle size and storage time on the Fibre content of Bambara nut flour

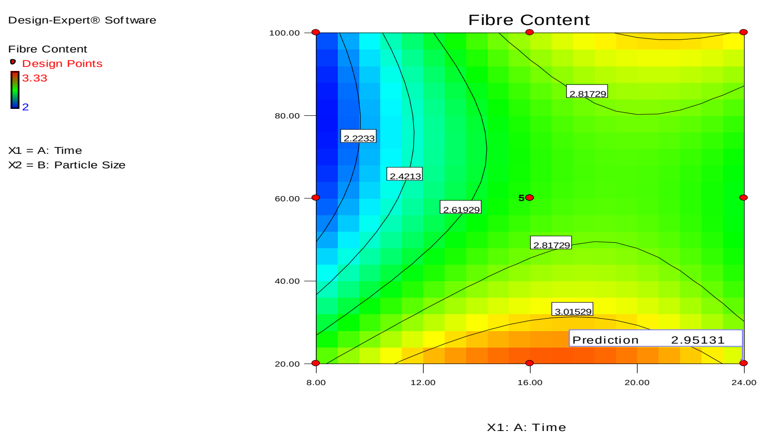

Figure 7: Contour plot of the effects of particle size and storage time on the Fibre content of Bambara nut flour

Figures 4 showed three dimensional surface plots of protein content and figure 5 contour plots. The protein content has the optimizer values 21.03%, 23.91%, 26.78%, 29.63% and 32.54% with the maximum response protein content value of 35.17%. The optimal particle size, protein content and storage duration was estimated to be 150.12µm, 35.17% (wb) and 23.62 weeks, respectively. However, the optimized result from the protein content is significantly influenced by particle size and storage time for Bambara nut flour handling and storage. The protein content of the flour increased with decrease in particle size. The increase on the protein content of the flour with storage might be due to the decrease in the moisture content of the flour. This response finding is in agreement with Adebowale *et al.*, (2002) who reported that liquid retention is an index of the ability of proteins to absorb and retain oil/water which is in turn influences the texture and mouth feel characteristics of foods and food products.

The 3 dimensional plots in figures 6 and 7 showed the effects of particle size and storage duration on the fibre content of Bambara nut flour. The fibre contents of the flour ranged from 2.00 – 3.33%. It was not significantly affected by the storage duration and particle size. The fibre content was slightly increased as the storage period progressed due to the decrease in moisture content of the flour. This is in agreement with result reported by Mpotokwane et al., (2008) for wheat flour during storage.

Due to the insulin building ability of Bambara nut in human body system, diabetic patients rely on this viable crop as food but the challenge is its unavailability in market outlets all the year round for immediate consumption. Also there is paucity of information on the storage techniques of the Bambara nut flour that can prolong the shelf-life for later usage. The processing and storage of Bambara nut flour and the impacts of storage conditions become very vital for a study like this. The hypothesis of this study was that Bambara nut flour stored at different storage conditions will show variations in the proximate composition and physicochemical properties of the flour. The effect of the particle size on the shelf-life and the product quality were also looked at. By the study, the shelf-life of Bambara nut flour could be prolonged under best environmental storage conditions. The research into obtaining optimum storage conditions and maximum shelf life of Bambara nut flour will bring about the desired information for mass production of the flour in the market for consumers' to easily access the product when needed.

4.0. Conclusion

Determination of the optimum storage conditions of Bambara nut flour has been successfully undertaken. Storage duration and particle size of the flour have significant effect on the proximate composition. The relationship between equilibrium moisture content and water activity showed that the adsorption of moisture was stable at low water activity and inclined with increase in water activity (relative humidity) of the storage environment. The time for the flour to reach equilibrium varied from 6 – 14 days. The results obtained in the study showed that the response surface model employed is a good one and could be used for the prediction of the responses (proximate composition) from the production and storage of Bambara nut flour. The correlation coefficient (R^2) of the responses was found to be 0.9983, 0.9701, 0.9688, 0.9862, 0.9138 and 0.9531 for the flour moisture, ash, protein, fat, fibre and carbohydrate contents, respectively. Levels of significance obtained were 0.02, 0.01, and 0.03 for the flour ash, protein and fibre contents, respectively which were high and attested to the fitness of the model in evaluating the responses. Optimum particle size and storage times were found to be 150.12µm, 6.32% (wb), and 23.62 weeks.

The following conclusions were therefore reached based on the results of the study.

1. Shelf life of Bambara nut flour depends on the moisture content, temperature and relative humidity of the storage environment. The result revealed the temperature dependence of the sorptive behavior with increase in temperature, the moisture adsorption capacity of the flour also increased.
2. Storage duration and particle size of the flour have significant effects on the proximate composition.
3. The equilibrium moisture content of Bambara nut flour at the four temperatures studied increased slowly at low water activities of 0.1 – 0.6 but increased rapidly at high water activity of 0.6 – 0.9. The equilibrium moisture content of the flour increased with increase in temperature at high water activity.
4. Response surface methodology was successfully used to optimize the process condition for the storage of Bambara nut flour. The central composite design of response surface methodology was found to be effective to determine the best particle size, moisture content and storage duration for Bambara nut flour handling and storage.
5. The optimal storage conditions of the flour parameters can therefore be used for the storage and optimum shelf life determination of Bambara nut flour.

5.0 Recommendations

1. To prevent Bambara nut flour from absorbing moisture as well as odors and flavors from other foods in storage. Refrigerating Bambara nut flour should be avoided, because some packaging materials are porous and the flour may absorb moisture and odors.
2. The optimal storage conditions of 6.32% (wb) moisture content and 150.12μm particle size is recommended for the shelf stability of Bambara nut flour.
3. It is recommended that for future studies, effect of other compositional and environmental storage conditions such as PH, microbial load, colour, sensory attributes and foam stability should be investigated and the result compared with principal component analysis.

6.0 Limitation

The study was limited to the brown variety of Bambara nut. The flour were stored and monitored for a period of six months under ambient temperature. Proximate composition and moisture absorption characteristics of the flour were determined. The experimental data generated was fitted to a polynomial regression model for predicting maximum shelf-life. Optimum shelf-life, particle size and water activity (relative humidity) for the handling and storage of Bambara nut flour were evaluated.

7.0 Contribution to knowledge

1. This study has generated the following results: new knowledge has been generated on the possibility of determining suitable packaging material, optimum storage conditions and maximum shelf life of Bambara nut flour.
2. Information on the time of storage of the flour without degradation was developed as well as possible ways to obtain qualitative Bambara nut flour.
3. The Bambara nut flour will be available if needed, and this is a great relief for diabetics.

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