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Optimizationof Particle Size and Storage Duration on the Protein, Fat and Moisture Contents ofVignasubterranea Flour

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ABSTRACT:Objective: To optimized particle size and storage duration on the protein, fat and moisture contents of Vignasubterranea flour. **Methods**: Flour was prepared fromVignasubterranea flour. Particle size distribution using sieve analysis was carried out to separate the flour at a range of 20, 60, 100 mesh numbers (850, 250, 150µm), respectively. The flour samples were stored in low density polythene bag for 6 months at ambient temperature. At monthly intervals, the flours were analyzed for moisture, fat and protein contents**. Findings:**Moisture content was found to be 12.00%, 11.17% and 11.67%; fat content of 7.5 ± 0.001%, 8.1 ± 0.001% and 7.0 ± 0.001%; Protein content 24.94%, 21.01% and 27.13%; for the flour with particle sizes 850, 250 150µm respectively. The results obtained in the study showed that particle size and storage duration had significant impact on the moisture, fat and protein contents of Vigna subterraneanflour.**Novelty:** Flour with particle size of 150µm had the lowest moisture content of 11.17% and would be most effective for shelf-life stability of Vignasubterranea flour stored at ambient condition.

Key words: Optimization, Particle Size, VignaSubterranea Flour, Response Surface Methodology, Storage Duration

I. INTRODUCTION

[1] Vignasubterranea is an indigenous crop grown widely in the African continent. It is the third most important grain legume after ground nut and cowpea in Nigeria (Lacroixet al., 2003; Fery, 2002). Until lately, Vignasubterraneais considered under researched and intermediate crop with creeping stems at ground levels. The Vignasubterranean,or round beans, is cultivated in many African countries where it is known by various names, according to different local languages.

[2] Jideani&Mpotokwane, (2009) reported thatVignasubterranea is a highly nutritious plant that plays a crucial role in people's diets.Nwodo and Obinna(2012) reported that Vignasubterranea is an important food source that can be exploited particularly in the developing countries where there is shortage of animal protein and under nutrition. The black seeded variety has a reputation of being a treatment for impotence in Botswana. Goliet al., 1997; Nti, 2009 also wrote that the dark seeded varieties (black and red) have higher nutrient and mineral contents than the light-seeded ones (cream).

[3] Processing Vignasubterranea into different food forms helps to stabilize shelf-life and improve quality (Inyanget al., 2006; Lebotet al., 2009). Additionally, processing can also increase or decrease the quality attributes of the processed products. One of the postharvest challenges limiting the shelf-life and quality of flour is particle size of the flour. In particular, flour is a hygroscopic product, which could be exposed to high humidity during handling and storage.

[4] Handling and storage of Vignasubterraneaflour must therefore be carefully controlled so that they have the required composition to attain shelf stability over the range of environmental and storage conditions that will experience during storage and consumption.

[5] Studies have shown that, particle size has great effect on the moisture and fat contents, when Vignasubterranea flour is blend with other confectioneries (Nwosu, J.N 2013; Iminabo et al, 2005) but there is limited information with regard to the influence of particle size on the moisture, fat and protein contents of Vignasubterranea flour. This study therefore is aimed atoptimizing particle size and storage duration on the protein, fat and

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moisture contents of Vignasubterranea flourduring handling and storage.

II. MATERIALS AND METHODS

RESEARCH MATERIALS

The materials used for this study were: Vignasubterraneaflour, food grade chemicals,low density polythene bags and water.

SOURCE OFVIGNA SUBTERRANEA

Vignasubterranea was purchased from a local market in Wukari, Taraba state, Nigeria. The seeds were manually cleaned to remove foreign matters, premature and broken ones.

PREPARATION OF EXPERIMENTAL SAMPLES

The Vignasubterranean seeds were milled into flour using a magnetic sieve grinding machine at the Department of Agricultural and Bioresources Engineering, University of Nigeria, Nsukka as described by Ngabea et al, (2016). The flour particle size distribution using sieve analysis was carried out by placing 45g of the sample on a tier of sieves of decreasing aperture to separate the flour at a range of 20, 60 and 100 mesh numbers (850, 250 and 150µm). A pan collector was placed beneath the 150µm sieve. The shaker was operated for 12 minutes each time and the percentage particle retention on each sieve were recorded as described by Ngabea et al, (2019).The samples were stored and monitored for a period of six (6) months under ambient temperature. At monthly intervals, the samples were analyzed for moisture, fat and protein contents as designed (Table 1).

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 as presented in Table 1

Table 1 Experimental Design for two (2) numerical factors of central composite design in responsesurface

Run	Block	Time (months)	Particle size (mesh no)	Y_1	Y_2	Y_3
1	B_1	4	20			
2	B_1	6	100			
3	B_1	2	20			
4	B_1	4	60			
5	B_1	4	60			
6	B_1	2	100			
7	B_1	4	60			
8	B_1	4	60			
9	B_1	4	20			
10	B_1	4	60			
11	B_1	2	60			
12	B_1	6	60			
13	B_1	6	20			

Where Y_1, Y_2, Y_3 are the responses for moisture, protein andfat respectively. The responses were determined using the relationship in Equation (1).

Responses (%)

$$= \frac{Y_f - Y_i}{Y_i} 100$$

Where:

Y_i and $Y_f$are initial and final values of proximate composition.

DETERMINATION OF MOISTURE CONTENT OF VIGNA SUBTERRANEA FLOUR

Five grams of each sample were weighed into pre-weighed aluminium drying dish. The sample was dried to a constant weight in an oven at 105ºC for four hours (AOAC, 1990).

The moisture content was determined as follows:

$$\frac{M_1 - M_2}{M_1 - M_0} \times 100 \quad (1)$$

(2)

Where:

M_0 = Weight of aluminium dish

M_1 = Weight of fresh sample + dish

M_2 = Weight of dried sample + dish

DETERMINATION OF FAT CONTENT OF VIGNA SUBTERRANEA FLOUR

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Soxhlet apparatus was used for the fat content determination.5g of the sample was weighed in a thimble and plugged with cotton wool. The thimble was then inserted in a soxhlet apparatus. A formerly weighed clean dried 250 ml flask was filled with 180 ml of petroleum ether of 45 – 65ºC boiling points.The soxhlet apparatus were assembled and allowed to reflux for 6.5 hours, the solvent was recovered and the flask with the extract was dried in the oven at 105ºC for 30 minutes. It was then cooled in the dessicator and weighed. The crude fat was calculated as stated in equation 4 (AOAC, 2010).

$$\% \text{ Fat} = (W3 - W2)/W1 \times 100 \quad (3)$$

Where:
W1 = weight of sample
W2 = weight of empty flask
W3 = weight of flask extracted oil

DETERMINATION OF PROTEIN CONTENT OF VIGNA SUBTERRANEA FLOUR

Kjedahl nitrogen method was used for the determination of the protein. 1.5g of the sample from each treatment was introduced into 900 ml digestion flask. 5 selenium tablets were added to the sample as catalyst. 25ml of concentrated H_2SO_4 was added to each sample until a clear solution was obtained. The cooling digest was poured into 100 ml volumetric flask and was made up to mark with distilled water.

The distillation apparatus were rinsed and arranged for 15 minutes. The apparatus were boiled, 20 ml of 4% boric acid was pipetted into conical flasks, 6 drops of methyl red was added to each flask as indicator and the digest was diluted with 75 ml distilled water. Alkaline was produced from 10 ml of the digest with 20 ml of 20% sodium hydroxide and distilled. The steam exit of the distillatory was closed and the boric acid solution colour changed to green. The mixture was distilled for 15 minutes (AOAC, 1990) and boric acid along with distillate was then titrated against 0.1N Hcl and thus, the percentage total nitrogen was calculated as shown in equations 3 and 4:

$$\% \text{ total of nitrogen} = \frac{\text{Titre} \times \text{Normality} \times 0.014}{\text{Weight of sample}} \times 100 \quad (4)$$

$$\% \text{ Crude Protein} = \% \text{ total nitrogen} \times 6.25 \quad (5)$$

Where:
6.25 is a constant (AOAC, 1990)
6.26

III. RESULTS AND DISCUSSION

The influence of the particle size on the moisture, fat and protein contents of Vignasubterranea flour is presented in Table 2. Particle size and storage duration had significant impact on the moisture, fat and protein contents of Vignasubterranea flour ($p < 0.05$). The result showed that, Vignasubterranea flour stored in different particle sizes has significant effect on the moisture, fat and protein contents.

Table 2: Effect of particle size on the moisture, fat and protein contents of Vignasubterranea flour Stored for a period of six months

Month	Particle Size (µm)	Moisture	Protein	Fat
	850	10.17	18.35	5.67
1st Month	**250**	11.17	20.01	5.69
	150	12.01	20.13	5.71
	850	9.67	21.01	5.71
2ndMonth	**250**	10.03	23.63	5.73
	150	11.33	23.64	5.74
	850	9.21	21.64	5.79
3rd Month	**250**	9.40	23.01	5.80
	150	9.53	25.01	6.01
	850	8.99	24.51	6.72
4th Month	**250**	9.00	26.01	6.73
	150	9.67	26.76	6.79
	850	8.84	27.51	7.19

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5th Month	**250**	8.92	28.01	7.60
	150	8.98	28.51	7.73
	850	8.67	26.63	7.80
6th Month	**250**	9.00	28.01	7.83
	150	9.05	28.38	7.97

EFFECT OF PARTICLE SIZE AND STORAGE DURATION ON THE MOISTURE CONTENT OF STORED VIGGNA SUBTERRANEAN FLOUR

The results of the moisture content of the Vignasubterranea flour is presented on Table 2. The mean effect of the particle size and storage duration on the percentage moisture content of Vignasubterranea 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.

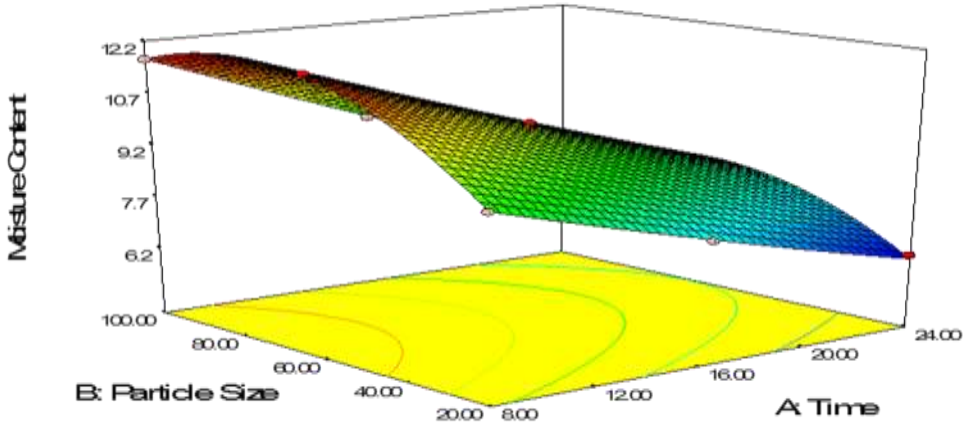

Fig. 1: 3D Surface plot of the effect of particle size and storage time on the moisture content of Vignasubterranea flour

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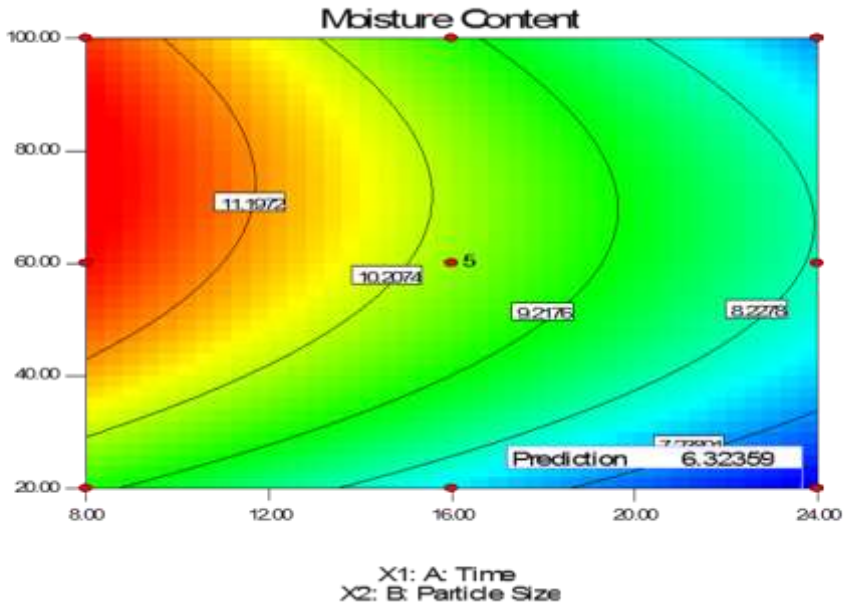

Fig. 2: Contour plot of the effect of particle size and storage time on the moisture content of Vigna subterranean flour

Figures 1 showed 3 dimensional surface plots of moisture content and figure 2 contour plots. The function has the optimizer values of moisture contents of 11.1972%, 10.2074%, 9.2176%, 8.2278% and 7.2971% with the maximum response moisture content value of 6.32%. The values are the set of alternatives as defined by Aris, (1964) as getting the best out of sets of alternatives. The predicted value of 6.32% signified the recommended moisture content in storing Bambara nut flour. The optimal particle size, moisture content and storage duration was estimated to be 150.12µm, 6.32% (wb) and 23.62 weeks, respectively. However, the optimized result from the moisture content is significantly influenced by particle size and storage time for Vigna subterranean flour handling and storage. The predicted result of the moisture content range corresponds andis in agreement with the recommended moisture content (6 – 14%) for storing food flour and its shelf stability by Standard Organization of Nigeria (SON, 2003).

EFFECT OF PARTICLE SIZE AND STORAGE DURATION ON THE FAT CONTENT OF STORED VIGGNA SUBTERRANEAN FLOUR

The fat content of Vignasubterranea flour as presented on Table 2 showed that there was a slight increase in all the particle sizes of the flour samples from the beginning of the storage period in the first three months of storage. From the 1st month of storage to 4th month, the fat content was found to be 5.67, 5.69 and 5.71% for 850, 250 and 150µm flour particles sizes, respectively. As the storage period increased between 3rd month and the 6th month, the fat content increased from 5.79, 5.80 and 6.01% to 7.80, 7.83 and 7.97% for the flour with particle sizes 850, 250 and 150µm respectively. These differences could be likely attributable to several factors, such as different growing conditions, season, and maturity of the seeds. This result is in agreement with Adebowaleet al., (2002) on the characterization of the physicochemical properties of Bambara nut. The fat content value in Vignasubterranea flour gradually increased with storage time and particle size. However, the fat content value did not exceed the 5.67 – 7.9% limit which was reported by Adebowaleet al., (2002) to be the minimum limit for odour to be acceptable. The fat content level of the flour during 14 days storage at elevated condition (50°C) was about 5.8%, which implies that Vignasubterranea flour will maintain a good quality and storage stability at a considerably long period of storage since elevated condition hastens rancidity. Fat content is extremely important parameter in flour storage. As the flour storage progressed, the fat content deteriorates through microbial attack and enzyme activity which causes the development of oxidation of unsaturated fatty acids. This results in the development of bad odours, caking of the flour and deterioration.

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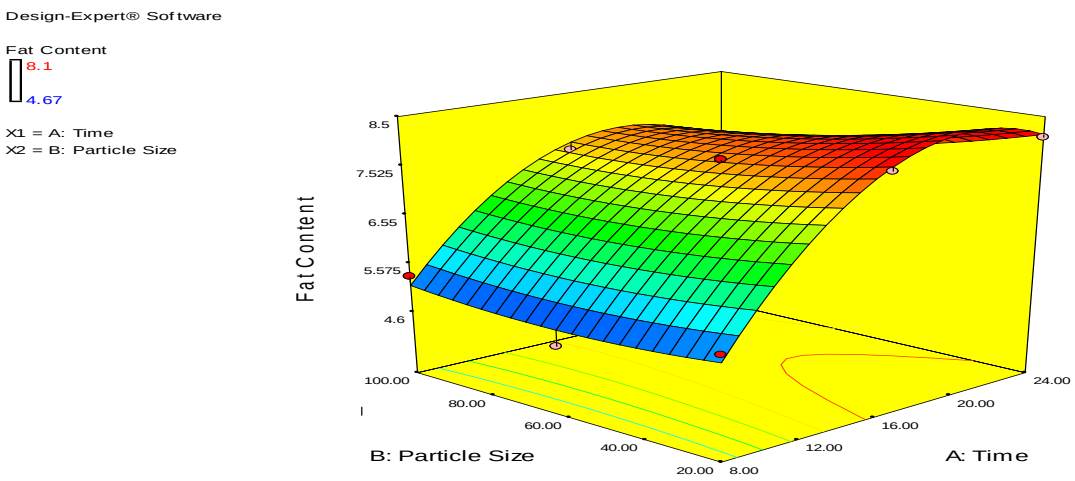

Fig. 3: 3D surface plot of the effects of particle size and storage time on the fat content of Bambara nut flour

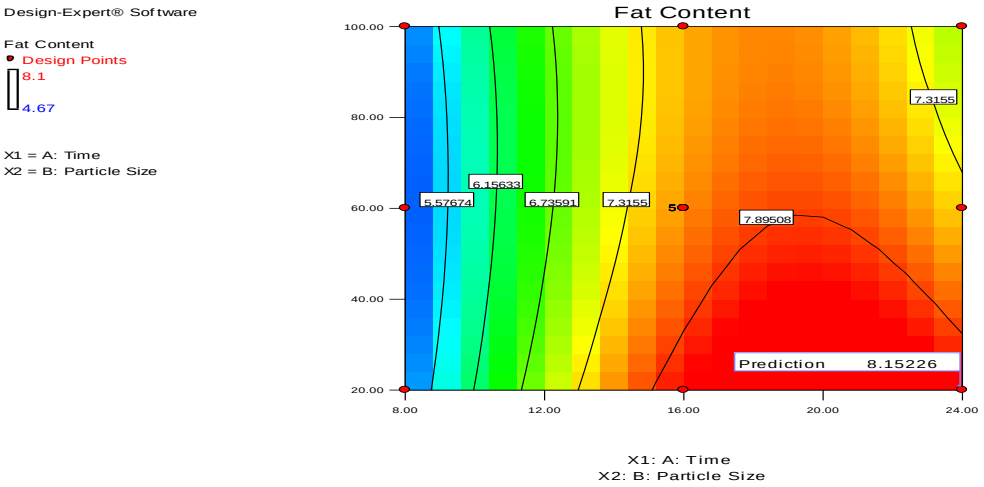

Fig. 4: Contour plot of the effects of particle size and storage time on the fat content of Bambara nut flour

Figures 3 and 4 showed the effects of particle size and storage time on the fat content of Vignasubterranea flour. There was no significant difference (P>0.05) in the fat content of the flour within the first three months of storage. At all the particle sizes (850, 250 and 150µm) the fat content ranged between 5.0 – 6.3%. This is in agreement with result reported by Mpotokwane et al., (2008) for wheat flour during storage.

Effect of particle size and storage duration on the protein content of stored Vignasubterranea flour

Table 2 also showed that, the protein contents of Vignasubterranea 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 Vignasubterranea 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 ofVignasubterranea 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

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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.

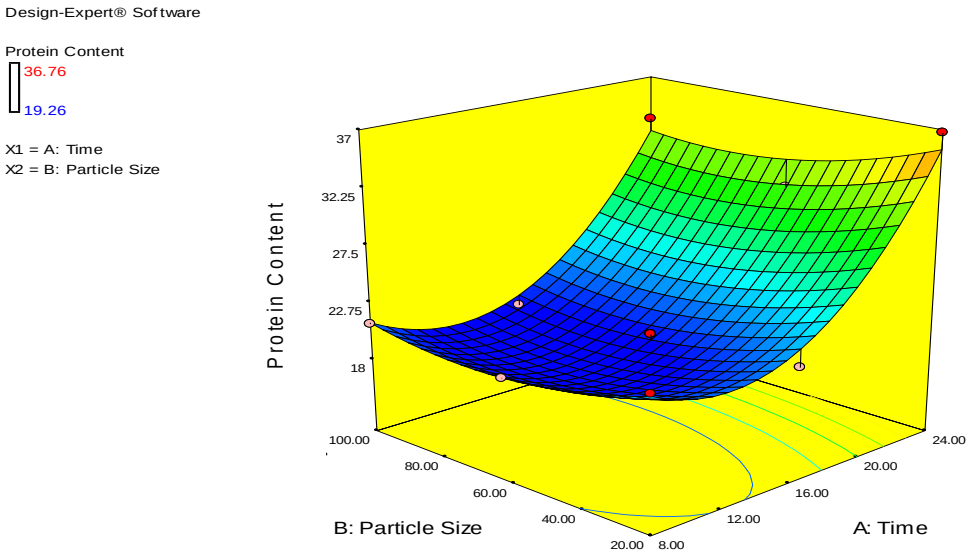

Figure 5: 3D surface plot of the effects of particle size and storage time on the Protein content of Bambara nut flour

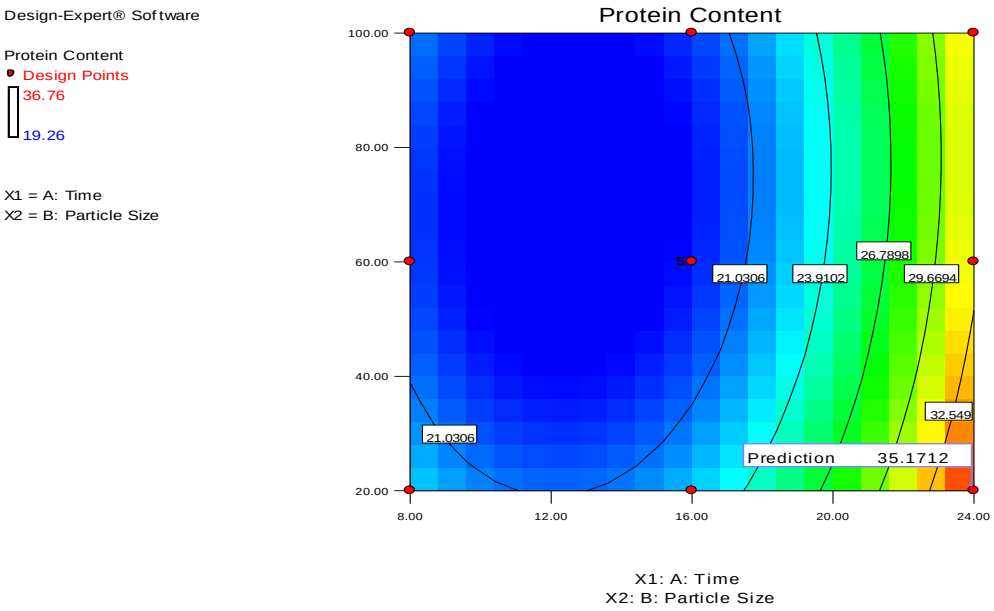

Fig. 6: Contour plot of the effects of particle size and storage time on the Protein content of Bambara nut flour

Figures 5showed 3 dimensional surface plots of protein content and figure 6 contourplots. 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 Vignasubterranea 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 Adebowaleet 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.

IV. CONCLUSION

The effect of particle size and storage duration on the moisture, fat and protein contents ofVignasubterraneaflour has been successfully undertaken. The results obtained in the study showed that particle size and storage duration had significant impact on the moisture, fat and protein contentsof Vignasubterraneaflour. Flour with particle size of 150µmhad the lowest moisture content of 9.5% and would be most effective for shelf-life stability ofVignasubterranea flour stored at ambient condition.

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