Effects of Temperature and Steeping Time on the Proximate Compositions and Selected Physical Properties of Soybean Flour

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ABSTRACT: A study to investigate the effect of temperature (80, 85, 90, 95, and 100ºC) and steeping time (12, 15, 18, 21, and 24 hours) on the proximate composition (ash, crude fibre, fat , protein, and carbohydrate) values (%) and physical properties (bulk density, packed density (g/ml), angle of repose (degrees), and particle size (µm)) on soybean flour was conducted. Response surface Methodology (RSM) was used for the work. Central Composite Design in Design Expert (version 6.0, 2002, Minneapolis, United States) computer software package was used to design the experiment. Analysis was also done and all results were presented with a three dimensional plot. The results showed that the moisture content of the soybeans flour ranged from 3.26 – 7.35, 3.40 – 4.50 for ash, 3.15 – 4.82 for crude fibre, 31.32 – 35.21 for protein, 19.37 – 22.65 for fat, and 29.66 – 35.19% for carbohydrate. Angle of repose for soybeans flour samples ranged from 33.15 – 38.16o, bulk density varied between 0.30-0.36, packed density varied between 0.52 – 0.57g/ml and particle size distribution varied between 0.44 – .98µm for the different samples. It was observed that longer steeping time and drying temperature resulted to an increase in the protein content and decrease in crude fibre content. Conversely shorter steeping time and lower drying temperature resulted in an increase in the fat content. A nine-point hedonic scale on sensory and acceptability tests showed that sample soaked for 24 hours and dried at 90oC was most preferred in taste and overall acceptability.

KEYWORDS: Soybean flour; packed density; angle of repose; steeping time, temperature.

I. INTRODUCTION

Legumes are said to be one of the earliest plants discovered by man and they have been in existence for thousands of years. Legumes contain some anti-nutritional compounds which include saponins, tannins, lectins, protease inhibitors etc. For the legumes to be useful, the anti-nutrient have to be inactivated before being used for either animal or human nutrition due to their minimal health-protecting ability. Examples of legumes include soybeans, peanuts, lentils, clover, peas etc (Burnham *et al.,* 2004). These legumes are very good sources of abundant nutrients, including carbohydrate, protein, oils, vitamins and minerals. A soybean (Glycine max L. Merril) belongs to a species of legume, which originated in East Asia. It is in the family of leguminosae, sub family Papiliondase, and genus *Glycine L.* Soybean is widely cultivated for its edible bean, which has a wide range of uses. Soybeans originated from China but latter spread to other parts of the world *(Glycine max, 2012)*.

Dry soybeans contain about 5% minerals with other components, 19% oil, 35% carbohydrate of which 17% is dietary fibre and 36% protein (Ajayi *et al.*, 2011). Some of the healthiest foods in the world are processed from soybean products due to its high protein content and several other health benefits. Soybeans cannot be eaten raw on harvest because they contain several anti-nutrients like trypsin inhibitors, protease inhibitors etc. Soybean has to undergo effective thermal inactivation to subdue and reduce these anti-nutrients

thereby improving soy's digestibility. Soybeans are helpful in preventing diarrhoea, constipation and diabetes in the health sector of the economy (Cristina *et al.*, 2011). In some part of the world soybeans products have not been well accepted because of its beany flavour and anti-nutritional factors (Tunde and Souley 2009).

They provide rich nutrition sources to both human being and animals. Soybean has tremendous effect on human health and they constitute essential ingredient in animal feed production. In animal feed processing, dried soybean seeds can be used for producing flour concentrates while the immature pods are either consumed fed as roughage to animals. Soybean seeds can be used to produce soy concentrate, protein isolate and other soy products. They are also used in the production of soy sauce such as soymilk; soy meal, textured soy protein (TSP), soy flour, tofu, tempeh, soy lecithin and soybean oil (Anders, 2013). Typical soybean flour is defatted soy flour, which is obtained from solvent extracted flakes, and it contains less than 1% oil. Another example is the low fat soy flour; which is produced by adding back some oil to the defatted soy flour.

As a result of its high oil content, the common hammer mill should not be used for grinding, thus a specialized alpine fine impact mill can be used for its production. (National Soybean Research Laboratory, 2012). The objective of this work being to determine the effects of temperature and steeping time on the proximate composition and selected

physical properties of soybean flour. The findings will enhance the processing of soybean flour with minimum beany flavour and better quality to make the product more acceptable.

II MATERIALS AND METHOD

A. Materials

The equipment used for the measurement of mass was a digital weighing balance with accuracy of 0.01 g, model scout Pro SPU 402, China. Drying was done with a digital temperature oven (Wise Ven model WOF 105, Korea.) Two 2.5 litre desiccator was also used for the work. The sieves (U.S. series) used were numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and the pan. Other equipment used was conical flask for density measurement, locally fabricated equipment for angle of repose measurement and container for pack density measurement.

B. Experimental Design

Design expert (version 6.0, 2002, Minneapolis, United States) computer software package was used to design the experiment, analyze and present all results with a three dimensional plots. Response surface methodology under Central composite design (CCD) was used. Five levels of temperature (80,85,90,95,and 100°C) and five levels of steeping time (12,15,18,21,and 24 hours) were substituted into the experimental design interface of the software. Central Composite Rotatable Design comprises mainly of three design points namely factorial points (n_f), axial points (n_a) and central points (n_c).

According to the Central Composite Rotatable Design,(CCRD) the total number of treatment combinations is: $n = 2^k (n_f) + 2(n_a) + (n_c)$ Where; 'k' is the number of independent variables and 'n' is the number of repetition of experiments at the centre point. The total number of design points is $= 2^k + 2k + n_o$ (Fakayode et $al.$, 2016). Therefore, the CCRD involves 13 experiments consisting of 2^2 factorial CCD, with axial points (α = 2) and five replications at the center points. For each independent variable, the levels were chosen with respect to preliminary experiments/ investigations and literature reviews from other researchers.

C. Experimental Procedure

Glycine Max (*soybean*) was purchased from Akpan Andem market in Uyo, Akwa-Ibom State, Nigeria. They were brought into Food Processing Engineering laboratory for the work at University of Uyo .Uyo, Nigeria. The experiment was done at a room temperature of 23°C and the average relative humidity was 76%. Five (5kg) kilograms of the soybeans were bought and manually sorted to remove unwanted materials like broken seeds and stones before processing.

Table1: Experimental Design (Second Order Design in the Two Variables).

Run	Coded Values		Actual Values	
	X_1	X_2	Temp	Time
1	1.000	1.000	95	21
2	0.000	0.000	90	18
3	1.000	-1.000	95	15
4	0.000	0.000	90	18
5	0.000	0.000	90	18
6	-1.000	-1.000	85	15
7	0.000	0.000	90	18
8	-2.000	0.000	80	18
9	0.000	-2.000	90	12
10	0.000	0.000	90	18
11	0.000	2.000	90	24
12	-1.000	1.000	85	21
13	2.000	0.000	100	18

After sorting the soybean seeds, they were divided into thirteen (13) subsamples of 300g each. Each of the subsamples was then subjected to the treatment combination as outlined by the design expert in terms of temperature and steeping time. The samples were then steeped in clean water at a time interval (12, 15, 18, 21, and 24 hours), thereafter it was dried/roasted in digital oven at the designed temperature (80, 85, 90, 95 and 100°C). The major reason for drying is to deactivate anti nutrients and to help remove seed coat .The equilibrium moisture content was also noted. The samples were then milled in laboratory blender for further analyses.

The output parameters from the work were investigated as follows:

1.) Equilibrium Moisture Content:

Moisture content was determined by taking about 5 g of sample into petri dish of known weight. It was then dried in the oven at about 105°C for 24 hours. The samples were cooled in a desiccator and reweighed. The moisture content was calculated as shown in Eqn (1).

$$\% \, MC = \frac{Change \, in \, Weight}{Initial \, Weight \, of \, Food \, Sample} \times 100 \qquad (1)$$

2.) Protein Content

The proximate composition of the samples were carried out by the methods described by (AOAC 2012) while the Protein Content was determined by means of a micro-Kjedhal method which comprises distillation, titration and wet digestion. In the method, 3 g of sample were weighed into a boiling tube containing 25 ml concentrated *tetraoxosulphate-VI* acid ($H_2SO_{4 \, aq}$) and a catalytic agent which contains 5 g *potassium tetraoxosulphate-VI* (K_2SO_4), 0.15 g each of *copper tetraoxosulphate-VI* ($CuSO_4$) and titanium oxide (TiO_2). The tube was heated at low temperature to initiate digestion after which it was diluted with 10ml of 40% *sodium hydroxide* (*NaOH*), 5 ml *disodium trioxosulphate-IV* ($Na_2S_2O_3$) and 100

ml distilled water. Anti-bumping agent was then added, and the sample diluted with 10 ml of boric acid (H_3BO_3).

The ammonia (NH_4) content in the distillate was determined by titrating with 0.1 N standard hydrochloric acid (HCl) using a 25 ml burette. A blank was also prepared without the sample. The amount of crude protein was obtained by multiplying the protein value thus obtained by a conversion factor, and the result was expressed in percentage as shown in Eqn (2).

$$\% \, CP = \frac{Actual \, Titre \, Value - Titre \, of \, the \, Blank}{Weight \, of \, the \, Sample} \times CF \times 100 \quad (2)$$

3.) Fat Content

10 g of the sample were enveloped in a filter paper and weighed by means of a chemical balance, and placed in a well-cleaned extraction thimble, oven-dried. It was then cooled in the desiccator and re-weighed. Then 25 ml of petroleum ether solvent was taken in a flask and used extracting the fat content, after which the solvent was evaporated by oven-drying. This was then cooled in a desiccator and weighed. The percentage fat content was computed based on Eqn (3).

$$\% \, Total \, FC = \frac{Weight \, of \, Fat \, Extracted}{Weight \, of \, Food \, Sample} \times 100 \quad (3)$$

4.) Crude Fibre

In determining the crude fibre content, about 5 g of each sample was weighed into a 500 ml titration flask and 100 ml of trichloroacetic acid (TCA) digestion reagent added. This was then boiled and refluxed for exactly 40 minutes after boiling. The flask was then removed from the heater and allowed to cool for a about 5 minutes before filtering by means of a Whatman paper (15.0 cm No. 4). The residue was then transferred to a porcelain dish after washing with hot water stirring briefly with a spatula. The sample was then dried for 24 hours in an oven set at 105°C. After drying, the sample was transferred into a desiccator, weighed and its weight recorded as W_1. It was then charbroiled for 6 hours in a muffle furnace at 500°C, allowed to cool and the reweighed (this weight was W_2). Percentage crude fibre was calculated as in Eqn (4).

$$\% \, Crude \, Fibre = \frac{W_1 - W_2}{W_o} \times 100 \quad (4)$$

where W_o = Dry weight of food sample, and W_1, W_2 are as recorded.

5.) Ash Content

About 5 g of each sample were taken into crucibles in two replications. The samples were then burnt at 550°C in a muffle furnace till a light grey ash was seen while the weight remained constant. The samples were cooled in the desiccators in order to prevent re-absorption of moisture and weighed to get the ash content. Percent ash content was computed from Eqn (5) after the method described.

$$\% \, Ash \, Content = 100 - (PC + FC + MC + CF) \quad (5)$$

where PC, FC, MC and CF are percent protein, fat, moisture and crude fibre contents respectively. Nutritional qualities

were determined in terms of fat, protein, crude fibre, ash, and carbohydrate for each treatment combination.

Physical properties studied were determined as follows

1.) Bulk Density

The method described by Mpotokwane *et. al.* (2008) and (Powder Bulk Solids 2016), was used to determine bulk density with slight modification. A measuring cylinder (100ml was filled with flour to mark (100ml), and the content weighed. The tapped or packed bulk density was also obtained following the same procedure but tapping for 50times prior to weighing. Bulk density was calculated as a ratio of bulk weight and volume of container (g/ml)

10.) Angle of Repose

Angle of Repose of the soybean powder was found by method described by Andrew, 2017. The soy flour was poured onto a flat wooden surface to build a pile from the top. This resulted in a pile with relatively circular base .The height (h) and the diameter (b) of the pile were measured. Angle of repose (angle β) was evaluated using the formula below;

$$Tan \, \beta = h \, /(\, b/2) \quad (6)$$

$$\beta = tan^{-1} \, h \, / \, (b/2) \quad (7)$$

where;
h = height of the flour
b/2 = radius of the flour (determined by dividing the diameter by two Ogunjimi *et al.,* 2002 as reported by Adebowale *et al.,* 2011.

2.) Particle Size:

Sieve analysis was done by a method described by Sonaye and Baxi (2012). The Finest Modulus, FM is obtained with eqn (8) while average particle size is obtained using eqn (9).

$$FM = \sum (\% \, mass \, retained \, x \, A.N) \, / \, 100 \quad (8)$$

$$Average \, particle \, size = \{0.004(2)^{FM} inch\} \quad (9)$$

where;

AN = assigned number of the sieves
FM = finest modulus
1 inch = 25.4 mm

3.) Data Analysis

The experimental design was based on two factors at five levels each. The two independent variables were drying temperature (80,85,90,95,and 100^{0}C) and steeping time (12,15,18,21,and 24 hours) coded as -2, -1, 0, 1 ,2 respectively for both drying temperature and steeping time as shown on Table 1 .After generating the data from the experiment, they were fed into the software package for analysis. Plotting of a three dimensional graphs and other analysis for the soybean flour were done with the help of the software package.

III. RESULTS AND DISCUSSION

A.) Presentation of Results

The experimental data obtained from the work is shown in Table 2. On the table are the results obtained from the research work on ash, crude fibre, fat, moisture content, protein and carbohydrate content for the various experimental conditions.

Table 2: Proximate Analysis of Soybeans Flour at Various Processing Conditions

Temp (ºC)	Time (hr)	Ash (%)	CF (%)	Fat (%)	MC (%)	PTN (%)	CHO (%)
95.00	21.00	3.64	3.46	19.79	6.64	35.21	30.19
90.00	18.00	4.00	4.12	21.36	5.75	33.60	31.17
95.00	15.00	4.35	4.36	21.92	4.48	32.62	33.23
90.00	18.00	4.25	4.00	21.34	5.35	33.50	31.56
90.00	18.00	4.13	4.13	21.37	5.55	33.75	31.07
85.00	15.00	4.28	4.38	22.35	5.73	32.19	32.17
90.00	18.00	4.17	4.08	21.36	5.43	33.62	31.34
80.00	18.00	3.40	4.12	21.81	6.65	33.31	30.38
90.00	12.00	4.25	4.82	22.65	3.26	31.32	35.19
90.00	18.00	4.21	4.04	21.32	5.38	33.57	31.48
90.00	24.00	4.50	3.15	19.37	7.35	35.11	30.52
85.00	21.00	3.60	3.49	20.65	6.75	34.94	29.66
100.00	18.00	4.33	4.12	21.00	5.30	33.93	31.66

CF = Crude Fibre, MC = Moisture Content, PTN = Protein, CHO = Carbohydrate

B) Discussion of Results

1.) Effect of Processing Factors on Ash Content

From the results, the sample soaked for 24 hours and dried at 90ºC had the maximum ash value of (4.50%) and the sample soaked for 18 hours and dried at 80ºC had the least ash value (3.40%). The maximum ash content as shown in Fig. 1 was the sample soaked for 24 hours and dried at 100ºC. The
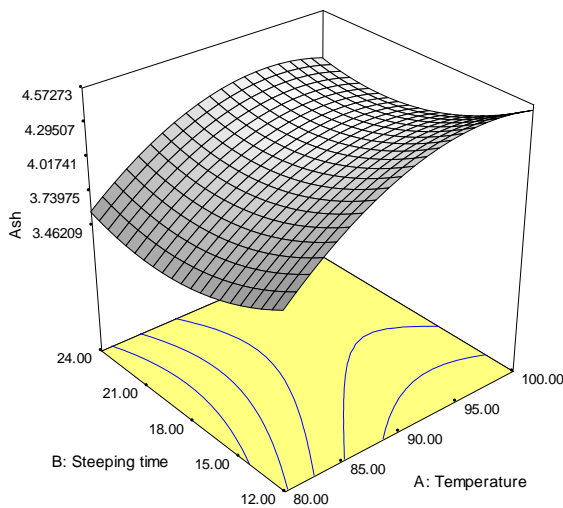

Fig. 1: Effect of drying temperature and steeping time on ash content.

results showed that the samples were affected by an interaction between the temperature and time. The result also indicated that the ash content increased at higher steeping time and lower drying temperature for some samples and vice versa. This result is due to the removal of moisture, which gave rise to an increase in the concentration of nutrients (Yusufe et *al.,* 2017).

2.) Effect of Processing Factors on Crude Fibre Content

The sample with the highest fibre value was the sample soaked for 12 hours and dried at 90ºC (4.82%) while the sample with the least fibre value was the sample soaked for 24 hours and dried at 90ºC (3.15%) as shown in Fig 2. There was significant difference in the fibre content of the different samples due to the drying time and steeping time. The higher the drying temperature and steeping time, the lower the crude fibre content, Onifade *et al.,* (2013). Decrease in the fibre content of the fermented samples are in line with studies by El-Adawy *et al.,*(2000), the decrease can be attributed to the degradation of fibre content by the fermenting microbes (Taghdir *et al.,*2016)
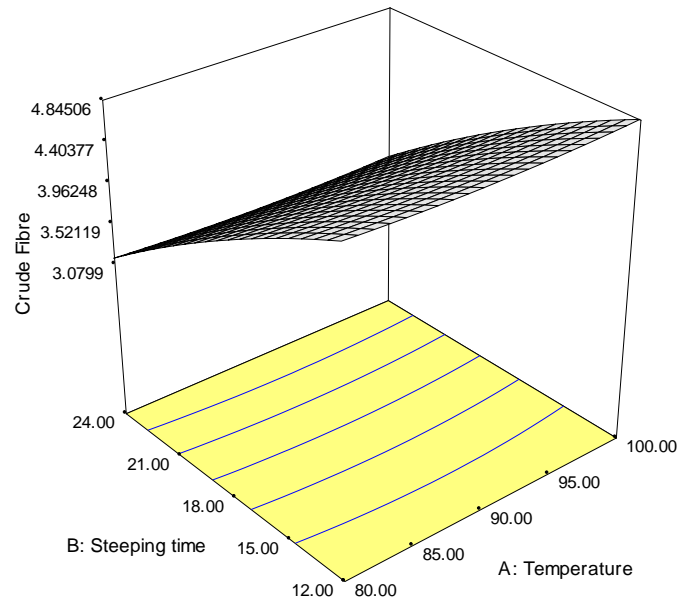

Fig. 2: Effect of drying temperature and steeping time on crude fibre content.

3.) Effect of Processing Factors on Protein Content

The protein values varied for each of the different samples. The sample that was soaked in water for 21 hours and dried at 95ºC had the highest protein content (35.21%) and the sample with the least protein content was the sample soaked for 12 hours and dried at 90ºC. There were some differences in the protein values of the different samples of soybean flour. Longer soaking hours resulted in increase in the protein content of the soybean flour. The reason being that, during fermentation hydrolysis of protein enzymes occur thereby releasing free amino acids that brought about new protein

synthesis as shown graphically microbes Fig. 3 (Taghdir *et al.,*2016)
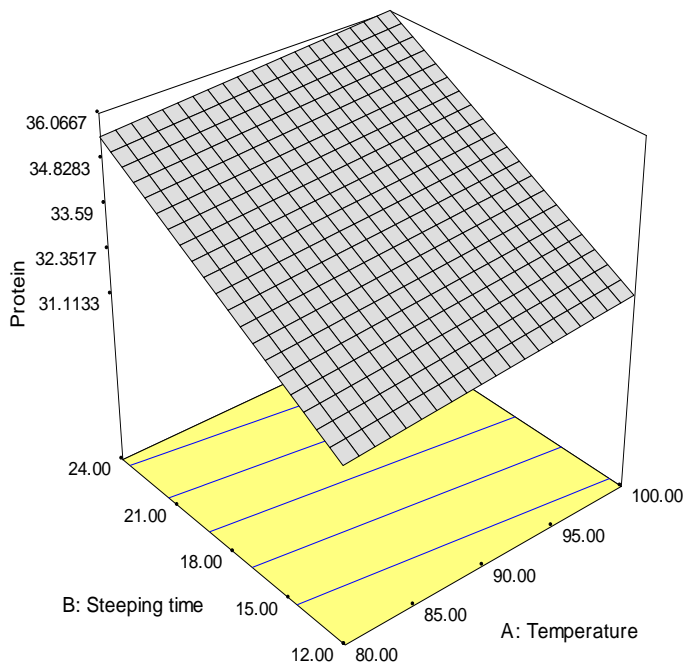

Fig. 3: Effect of drying temperature and steeping time on protein content.

4.) Effect of Processing Factors on Fat Content

The maximum fat content was the sample soaked for 12 hours and dried at 90°C with a fat value of (22.65%) while the sample soaked for 24 hours and dried at 90°C had the least fat value (19.37%). It has been shown that the percentage of fat content decreases with increasing soaking time and increasing drying temperature and this results in good keeping quality due to less rancidity of the flour as shown in Fig,4. From the results, the sample soaked for 24 hours and dried at 90°C had the least fat
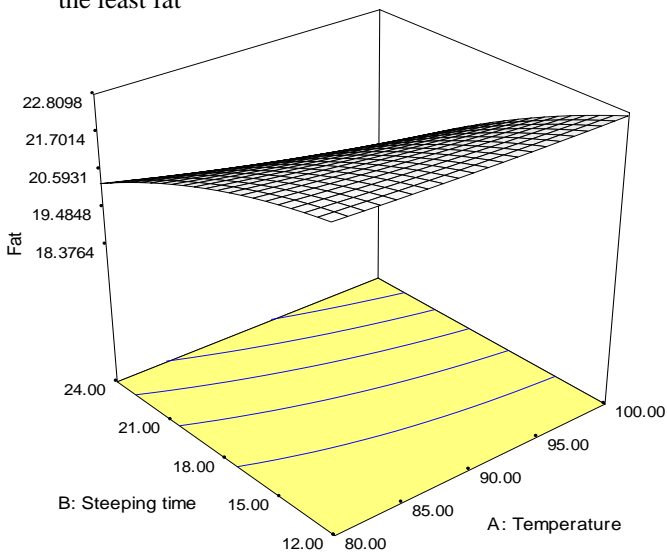

Fig. 4: Effect of drying temperature and steeping time on fat content.

content, which might be due to the metabolic activities of microorganisms and due to high temperature denaturing the fat (Obiakor, and Nwanekezi, 2008).

5.) Effect of Processing Factors on Moisture Content

As shown in Fig.5 sample soaked in water for 24 hours and dried at 90°C had the highest moisture value (7.35%) while the sample soaked for 12 hours and dried at 90°C had the lowest moisture content value (3.26%). Accordingly samples that were soaked for 15 hours and dried at 85°C, soaked for 15 hours and dried at 95°C, had moisture content values of (5.73% and 4.48%) respectively. The samples soaked for 18 hours and dried at 80°C and 100°C, had moisture content values of (6.65 and 5.30%) respectively. The samples soaked for 21 hours and dried at 85°C and 95°C, had moisture values of (6.75 and 6.64%) respectively.
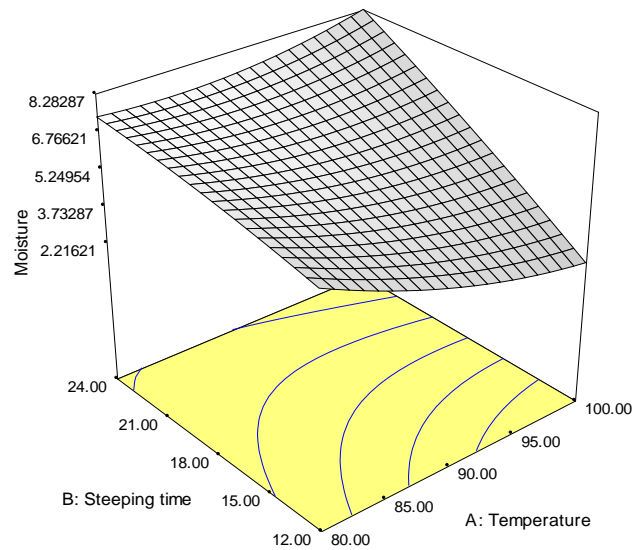

Fig. 5: Effect of drying temperature and steeping time on moisture content.

6.) Effect of Processing Factors on Carbohydrate Content

From Fig.6, the sample soaked for 21 hours and dried at 85°C had the least carbohydrate content while the sample soaked for 12 hours and dried at 90°C had the highest carbohydrate content. The decrease in the carbohydrate content of the sample soaked for 21 hours and dried at 85°C might be due to high utilization of energy by micro flora during fermentation or due to the significant increase in the protein content (Obiakor, and Nwanekezi, 2008).
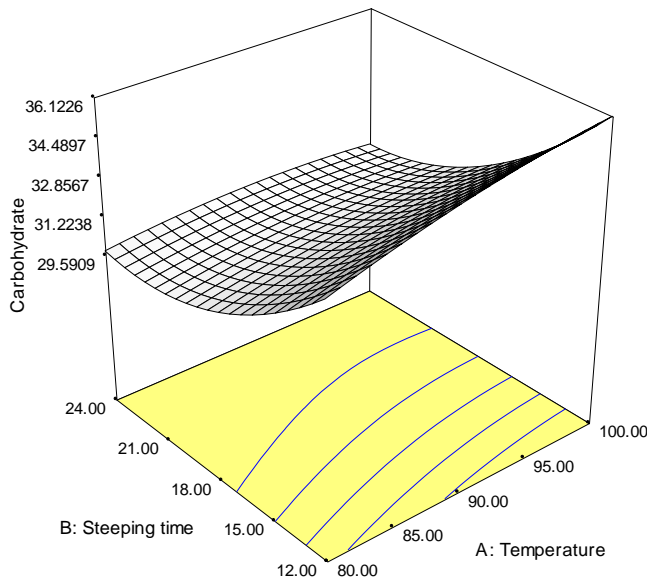
Fig.6: Effect of Drying Temperature and Steeping Time on Carbohydrate Content

7.) Effects of Temperature and Steeping time on Physical Properties of Soybean Flour

The experimental values obtained for of bulk density, packed density, angle of repose and particle size distribution of soybeans flour as influence by temperature and steeping time at various processing conditions is as presented in Table 3.

Table 3: Values of bulk density, packed density, angle of repose and particle size distribution of Soybeans Flour at Various Processing Conditions.

Temp (ºC)	Time (hr)	BD (g/ml)		PD (g/ml)		AR (degrees)		PS (μm)
		Mean (g/ml)	SD	Mean (g/ml)	SD	Mean (⁰C)	SD	
95.00	21.00	0.33	0.02	0.55	0.00	36.60	0.83	0.67
90.00	18.00	0.33	0.12	0.55	0.02	38.16	0.61	0.63
95.00	15.00	0.35	0.02	0.55	0.04	35.43	0.73	0.79
90.00	18.00	0.33	0.01	0.55	0.02	38.16	0.61	0.63
90.00	18.00	0.33	0.01	0.55	0.02	38.16	0.61	0.63
85.00	15.00	0.31	0.02	0.51	0.03	36.83	0.20	0.98
90.00	18.00	0.33	0.01	0.55	0.02	38.16	0.61	0.64
80.00	18.00	0.30	0.05	0.56	0.01	34.71	1.02	0.44
90.00	12.00	0.37	0.01	0.52	0.03	34.70	1.30	0.37
90.00	18.00	0.33	0.01	0.55	0.02	38.16	0.61	0.63
90.00	24.00	0.34	0.02	0.57	0.03	33.15	0.03	0.75
85.00	21.00	0.34	0.01	0.55	0.01	37.02	0.97	0.61
100.00	18.00	0.36	0.03	0.57	0.02	34.79	0.97	0.56

BD = Bulk Density, PD = Packed Density, AR = Angle of Repose,
PS = Particle Size, SD = Standard Deviation

From the results in Table 3, the sample soaked for 24 hours and dried at 90ºC had an angle of repose value of 33.15º. Other samples with equivalent able of repose include the sample

soaked for 12 hours and dried at 90ºC, the sample soaked for 15 hours and dried at 95ºC, the sample soaked for 18 hours and dried at 80ºC and the sample soaked for 18 hours and dried at 100ºC. Other samples with the angle of repose include the sample soaked for 15 hours and dried at 85ºC, soaked for 18 hours and dried at 90ºC, soaked for 21 hours and dried at 85ºC, soaked for 21 hours and dried at 95ºC. None of the soybean flour samples had poor or very poor flow properties, which agreed with the findings of Cristina *et al.*, 2011. From the results of some physical properties of soybeans flour at various processing conditions, angle of repose values ranged from 33.15 – 38.16º, 0.30 – 0.36g/ml for bulk density, 0.51 – 0.57g/ml for packed density, and 0.56 – 0.98 μ m for particle size distribution.

IV. CONCLUSION

The results from the work as observed from the three dimensional plots from RSM and other evaluated statistical parameters clearly showed that the proximate analysis and physical properties (bulk density, packed density, angle of repose, particle size analysis) of soybeans flour varied according to the steeping time and drying temperature. Ash content ranged from (3.40 – 4.50%), carbohydrate content ranged from (29.66 – 35.19%), Fat content ranged from (19.37 – 22.66%), fibre content ranged from (3.15 – 4.82%), moisture content ranged from (3.26 – 7.35%) and protein content ranged from (31.32 – 35.21%) for the different samples of soybeans flour. Results showed that longer steeping time and drying temperature resulted in increase in the protein content of soybeans flour.

Considering ash content, longer steeping time and lower drying temperature resulted in an increase in ash value and vice versa. For crude fibre, longer steeping time and higher drying temperature resulted in a decrease in crude fibre value. Soaking for longer hours showed significant decrease in the carbohydrate content of the sample and fat content reduced significantly due to longer soaking hours and higher drying temperatures. Soaking for longer hours reduced fat value for better shelf life, reduced the beany flavour and improved the taste of the soybean flour.

From sensory evaluation of the different samples of soybeans flour, the sample soaked for 24 hours and dried at 90ºC had the overall best value in terms of taste and overall acceptability. From this study it can be said that adequate knowledge of processes to protect nutrients and reduce anti nutrients would increase food value of soybean flour.

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