Influence of Processing Techniques and Packaging Materials on Anti-Nutritional Properties of Soybean Flour

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ABSTRACT
This study investigated the anti-nutritional properties of soya bean flour produced from steeping and gelatinization techniques and stored in different packaging materials. The freshly harvested soya bean seeds used for the study were cleaned and soaked for five different durations (6, 9, 12, 15, and 18 hours); cooked at five different cooking times (20, 25, 30, 35 and 40 mins); packaged in five different materials (paper bag, low density polythene bag, composite bag, high density polythene bag and plastic) and stored for five different periods (20, 40, 60, 80 and 100 days). A Central Composite Orthogonal Design was used. The samples of flour were then subjected to anti-nutritional analysis. All experiments were carried out in triplicates. Data obtained was analyzed using Design Expert 9.0; the Analysis of Variance (ANOVA) was conducted and empirical models developed. Statistical analysis shows that steeping duration had significant effect ($P \leq 0.05$) on the cyanide, tannin and phytic acid contents of the flour. The gelatinization duration, storage duration and packaging materials had no significant effect ($P \leq 0.05$) on the cyanide content, tannin content, saponin content and oxalate content of the flour samples.

Keywords: *Cyanide content, oxalate content, soya beans, steeping, tannin content.*

1 INTRODUCTION

Soybean (*Glycine max*) is a legume that originated from Eastern Asia (www.fao.org), it was introduced into Nigeria in 1908 (Omotayo *et al.,* 2007). According to Ajayi *et al*., (2011), soya bean is being acknowledged as a viable and essentially nutritious crop with numerous health benefits. The crop has also been identified as a major raw material for many domestic and industrial products. Soybean has a great demand due to its versatility; it has found many applications in the formulation of both human and animal foods, pharmaceutical and confectionery industries and other industrial uses (Omotayo *et al.,* 2007). It is utilized in many forms like bean, meal, cake and oil. The industrial demand and domestic supply level for soybean was estimated to be about 634,000 metric tons and 386,864 metric tons respectively (Omotayo *et al.,* 2007). According to Soetan and Oyewole (2009), soybeans contain anti-nutritional factors such as tannin which must be inactivated by heat during processing before usage.

Soybean flours can be obtained by grinding full-fat dehulled soybeans or defatted flakes made from dehulled soybeans; they are then allowed to pass through a 100-mesh standard screen (www.fao.org). Steeping and gelatinization techniques are used in the processing of soybean to flour so as to minimise the anti-nutritional properties (Bibianalgbabul and Sule, 2013).

Packaging has become part of our daily lives. Packaged products are found all over the world. The global packaging market is currently valued at $597billion and is estimated to reach $820billion by 2016 (Silayoi and Speece, 2004). Consumers are increasingly demanding higher quality packaging for products which has in turn increased the role of packaging in the sale of goods and services (Institute of Packaging Ghana, IOPG, 2014). Packages come in such forms as packaged foods, canned drinks, and bottled water.

W Packaging helps consumers know the contents of products, and serve as instruction guides. Manufacturing and expiry dates, warning symbols, net weight, country of origin, recyclable symbol, company's address and nutritional facts are all very essential facts that, through packaging are provided to the consumers. Best packaging material for soybean flour, gives aesthetic delight and satisfaction, and also tactile pleasure (Soroka, 2002).

The objective of storage is to preserve the properties of the flour present after it has been processed, thereby making it possible to obtain and market sub-products with satisfactory quality. Vitality of the product can be preserved and the grinding quality and nutritive properties of the food can be maintained (Brooker *et al*., 1992).

Most soya products are made out of soya flour, thus there is a high demand for the flour in commercial quantity. The knowledge of a suitable processing technique and packaging material for soybean flour will go a long way to meeting this demand.

The use of other types of flour from locally available root, tubers, legumes and cereal crops in production will reduce the cost of wheat importation in Nigeria. This will help save the dwindling foreign reserves. The losses incurred in soybean can be reduced by processing it into flour. This will also increase the earning of farmers of this crop. The use of better packaging materials for soybean flour will reduce losses during transportation and storage.

This research finds relevance in the establishment of the best packaging materials and storage conditions for soybean flour that will serve as baseline for confectionary industries.

2 METHODOLOGY

2.1 MATERIALS

Freshly harvested soybean (*Glycine max*) seeds that were used in producing the flour were obtained from a farmer in Lapai, Niger State, Nigeria. The chemicals and reagents that were used were obtained from the Food Science Laboratory, School of Agricultural Technology, Federal University of Technology Minna, Niger State, Nigeria.

2.2 METHODS

2.2.2 SAMPLE PREPARATION

The flour was prepared according to the method described by Bibianalgbabul and Sule (2013). The grains were manually cleaned by separating all foreign materials, such as sticks, dirt, husk, chaff and other extraneous materials from the soybeans. The soybeans were washed in clean water and soaked for 6 hrs. This method was repeated for soaking times of 9, 12, 15 and 18 hrs. The soaked soybeans were poured in boiling water, and boiled for 20, 25, 30, 35 and 40 mins. The soybeans were washed and the coats removed and separated from the beans in cold water. The beans were dried at 65^0C, grinded into flour, sieved, packaged and stored for 100 days. The experimental design employed in this study is the Central Composite Orthogonal Design giving twenty-five runs as shown in Table 1.

Table 1: Central Composite Orthogonal Design

Run	Sample	X_1(mins)	X_2(hrs)	X_3(days)	X_4(materials)
1	A	-1(9)	-1(25)	-1(40)	-1(LDPE)
2	B	+1(15)	-1(25)	-1(40)	-1(LDPE)
3	C	-1(9)	+1(35)	-1(40)	-1(LDPE)
4	D	+1(15)	+1(35)	-1(40)	-1(LDPE)
5	E	-1(9)	-1(25)	+1(80)	-1(LDPE)
6	F	+1(15)	-1(25)	+1(80)	-1(LDPE)
7	G	-1(9)	+1(35)	+1(80)	-1(LDPE)
8	H	+1(15)	+1(35)	+1(80)	-1(LDPE)
9	I	-1(9)	-1(25)	-1(40)	+1(HDPE)
10	J	+1(15)	-1(25)	-1(40)	+1(HDPE)
11	K	-1(9)	+1(35)	-1(40)	+1(HDPE)
12	L	+1(15)	+1(35)	-1(40)	+1(HDPE)
13	M	-1(9)	-1(25)	+1(80)	+1(HDPE)
14	N	+1(15)	-1(25)	+1(80)	+1(HDPE)
15	O	-1(9)	+1(35)	+1(80)	+1(HDPE)
16	P	+1(15)	+1(35)	+1(80)	+1(HDPE)
17	Q	-α(6)	0(30)	0(60)	0(Composite)
18	R	+α(18)	0(30)	0(60)	0(Composite)
19	S	0(12)	-α(20)	0(60)	0(Composite)
20	T	0(12)	+α(40)	0(60)	0(Composite)
21	U	0(12)	0(30)	-α(20)	0(Composite)
22	V	0(12)	0(30)	+α(100)	0(Composite)
23	W	0(12)	0(30)	0(60)	-α(Paper)
24	X	0(12)	0(30)	0(60)	+α(Plastic)
25	Y	0(12)	0(30)	0(60)	0(Composite)

X_1= Steeping duration, X_2= Gelatinization duration, X_3= Storage duration, X_4= Packaging materials

PACKAGING

Electronic weighing balance was used to measure 200 grams of the flour and packaged in paper bag, low density polythene bag, composite (mixed), high density polythene bag and plastic as shown in Plate I.

Paper bag

Low density polythene bag

Composite

High density polythene bag

Plastic
Plate I: Soybeans flour in different packaging materials.

2.2.3 Storage

The packaged soybean flour was stored for a period of 100 days. The anti-nutritional properties were determined every 20 days so as to assess the effect of the packaging materials on them during storage.

2.2.4 Analysis of Anti-nutritional properties

The anti-nutritional contents determined are cyanide, tannin, saponin, oxalate and phytic acid. The Cyanide content was determined by the alkaline picrate method according to the method used by Onwuka, (2005).

Tannin was determined according to Association of Official Analytical Chemist, AOAC (2002). Saponin was determined according to the method described by Kim and Wampler, (2009).

Oxalate in the sample was determined by the permanganate titrimetric method as described by Liener, (1994). The phytic acid content was determined using a modified indirect colorimetric method reported by Wheeler and Ferrel (1971).

2.3 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analysed using Design Expert 9.0; Analysis of Variance (ANOVA) was conducted and empirical models were developed.

3 RESULTS AND DISCUSSION

3.1 Results

The mean of the replicated results obtained for the anti-nutritional properties are presented in Table 2.

Table 2: Anti-Nutritional Content of Soybean Flour

Run	Sample	Cyanide (g/100g)	Tannin (g/100g)	Saponin (g/100g)	Oxalate (g/100g)	Phytate (g/100g)
1	A	0.0554	0.8309	0.5496	0.0119	0.9429
2	B	0.0335	0.6938	0.5062	0.0119	0.9803
3	C	0.0526	0.8300	0.5570	0.0119	0.9429
4	D	0.0335	0.7143	0.5062	0.0119	0.9803
5	E	0.0554	0.8309	0.5496	0.0119	0.9429
6	F	0.0335	0.6938	0.5062	0.0119	0.9808
7	G	0.0526	0.8300	0.5570	0.0119	0.9429
8	H	0.0333	0.7143	05062	0.0119	0.9808
9	I	0.0554	0.8309	0.5496	0.0119	0.9429
10	J	0.0335	0.6938	0.5062	0.0119	0.9803
11	K	0.0526	0.8300	0.5570	0.0119	0.9429
12	L	0.0333	0.7143	0.5062	0.0119	0.9803
13	M	0.0554	0.8309	0.5496	0.0119	0.9429
14	N	0.0335	0.6938	0.5062	0.0119	0.9803
15	O	0.0526	0.8300	0.5570	0.0119	0.9429
16	P	0.0333	0.7143	0.5062	0.0119	0.9803
17	Q	0.0824	0.8329	0.6253	0.0119	0.9832
18	R	0.0333	0.8056	0.5451	0.0103	0.9738
19	S	0.0529	0.6234	0.5210	0.0127	0.9554
20	T	0.0463	0.6490	0.5316	0.0128	0.9614
21	U	0.0463	0.6144	0.5154	0.0129	0.9554
22	V	0.0463	0.6144	0.5154	0.0129	0.9554
23	W	0.0463	0.6144	0.5154	0.0129	0.9554
24	X	0.0463	0.6144	0.5154	0.0129	0.9554
25	Y	0.0463	0.6144	0.5154	0.0129	0.9554

X_1 = Steeping duration, X_2 = Gelatinization duration, X_3 = Storage duration,
X_4 = Packaging Material

3.2 DISCUSSION

3.2.1 The impact of processing parameters on the anti-nutritional properties of soybean flour

3.2.1.1 Cyanide Content

The ANOVA result (Table 3) indicated that the linear model is significant. The linear term of the model indicated that an increase in steeping and gelatinization duration reduces the cyanide content of the flour.

Statistical analysis shows that steeping duration had significant effect ($P \leq 0.05$) on the cyanide content while the gelatinization duration, storage duration and packaging materials had no significant effect on the cyanide properties of soybean flour as their values were higher than 0.05. Only the interaction between the steeping duration and gelatinization duration had positive effect on the cyanide content as shown in the model equation (1). The 3D plots showing the effect of variables on cyanide content is shown in Figures 1 and 2.

Table 3: ANOVA of the Cyanide Properties of Soybean Flour

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	0.002911	10	0.000291	9.801546	< 0.0001
X_1-Steeping Duration	0.002878	1	0.002878	96.90154	< 0.0001
X_2-Gel. Duration	2.6E-05	1	2.6E-05	0.87692	0.3649
X_3-Storage Duration	1.67E-09	1	1.67E-09	5.61E-05	0.9941
X_4-Package Material	1.67E-09	1	1.67E-09	5.61E-05	0.9941
$X_1 X_2$	7.02E-06	1	7.02E-06	0.236474	0.6343
$X_1 X_3$	2.5E-09	1	2.5E-09	8.42E-05	0.9928
$X_1 X_4$	2.5E-09	1	2.5E-09	8.42E-05	0.9928
$X_2 X_3$	2.5E-09	1	2.5E-09	8.42E-05	0.9928
$X_2 X_4$	2.5E-09	1	2.5E-09	8.42E-05	0.9928
$X_3 X_4$	2.5E-09	1	2.5E-09	8.42E-05	0.9928
Residual	0.000416	14	2.97E-05		
Cor Total	0.003326	24			

* Values of "Prob > F" less than 0.05 indicate model terms are significant
X_1 = Steeping duration, X_2 = Gelatinization duration, X_3 = Storage duration,

Final equation in terms of coded factors

$$Cyanide = 0.045832 - 0.0219X_1 - 0.00208X_2 - 1.7E-05X_3 - 1.7E-05X_4 + 0.002651X_1X_2 - 5E-05X_1X_3 - 5E-05X_1X_4 - 5E-05X_2X_3 - 5E-05X_2X_4 - 0.00005X_3X_4 \quad (1)$$

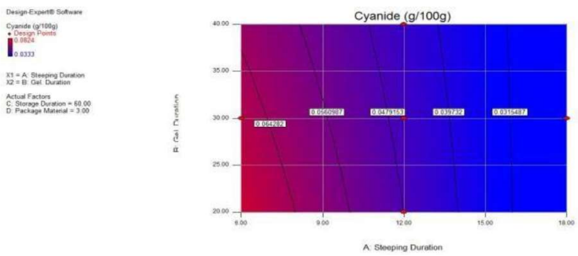

Fig. 1: Effect of Steeping Duration and Gelatinization Duration on the Cyanide Content of Soybean Flour.

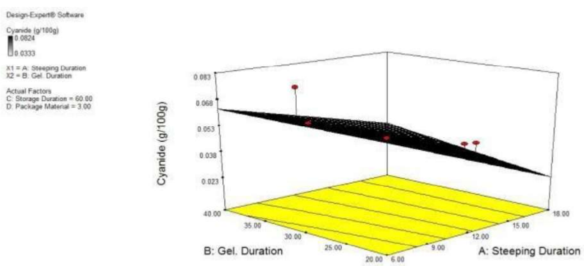

Fig. 2: Three-Dimensional Surface Plot of Cyanide Content Mode

3.2.1.2 Tannin Content

The ANOVA result (Table 4) showed that linear model is not significant. However, steeping duration is seen to have significant effect on tannin content of the flour while the gelatinization duration, storage duration and packaging materials had no significant effect on the tannin content of the flour as presented in Table 4. Only the interaction between the steeping duration and gelatinization duration had positive effect on the tannin content as shown in the model equation (2). The plot of the tannin content of the flour is shown in Figures 3 and 4.

Table 4: ANOVA of the Tannin Content of Soybean Flour

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	0.048488	10	0.004849	0.486603	0.8722
X_1-Steeping Duration	0.04733	1	0.04733	4.749844	0.0469
X_2-Gel. Duration	0.0007	1	0.0007	0.070232	0.7949
X_3-Storage Duration	0	1	0	0	1.0000
X_4-Package Material	0	1	0	0	1.0000
X_1X_2	0.000458	1	0.000458	0.045959	0.8333
X_1X_3	0	1	0	0	1.0000
X_1X_4	0	1	0	0	1.0000
X_2X_3	0	1	0	0	1.0000
X_2X_4	0	1	0	0	1.0000
X_3X_4	0	1	0	0	1.0000
Residual	0.139505	14	0.009965		
Cor Total	0.187993	24			

* Values of "Prob > F" less than 0.05 indicate model terms are significant X_1 = Steeping duration, X_2 = Gelatinization duration, X_3 = Storage duration, X_4= Packaging Material

Final equation in terms of coded factors

$$Tannin = 0.730356 - 0.08882X_1 + 0.0214X_1X_2 \quad (2)$$

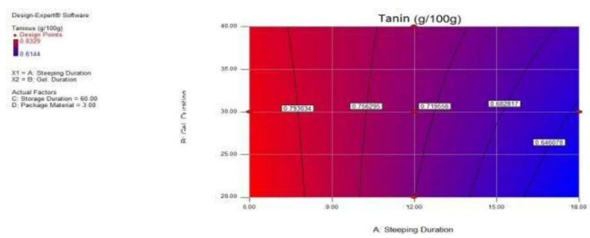

Fig 3: Effect of Steeping Duration and Gelatinization Duration on the Tannin Content of Soybeans Flour.

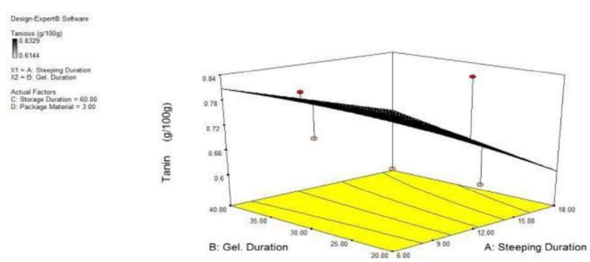

Fig. 4: Three-Dimensional Surface Plot of Tannin Content Model

3.2.1.3 Saponin Content

The statistical analysis shows that there was no significant effect (P≤0.05) on the saponin content of the flour samples. The model (linear) also had no significant influence on the saponin content of the flour as its above 0.05 (Table 5). Steeping duration, gelatinization duration and storage duration all had positive effect on the saponin content of the flour. The interactions between the steeping duration and gelatinization duration; steeping duration and storage duration; gelatinization duration and storage duration also had positive effect on the saponin content as shown in the model equation (3). The 3D plots showing the effect of variables on saponin content are shown in Figures 5 and 6.

Table 5: The ANOVA of the Saponin Properties of Soybean Flour

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	13876583	10	1387658	1.812724	0.1499
X_1-Steeping Duration	1067220	1	1067220	1.39413	0.2574
X_2-Gel. Duration	1067468	1	1067468	1.394454	0.2573
X_3-Storage Duration	1067447	1	1067447	1.394426	0.2573
X_4-Package Material	1067447	1	1067447	1.394426	0.2573
$X_1 X_2$	1601151	1	1601151	2.091614	0.1701
$X_1 X_3$	1601170	1	1601170	2.091639	0.1701
$X_1 X_4$	1601170	1	1601170	2.091639	0.1701
$X_2 X_3$	1601170	1	1601170	2.091639	0.1701
$X_2 X_4$	1601170	1	1601170	2.091639	0.1701
$X_3 X_4$	1601170	1	1601170	2.091639	0.1701
Residual	10717137	14	765509.8		
Cor Total	24593719	24			

* Values of "Prob > F" less than 0.05 indicate model terms are significant X_1 = Steeping duration, X_2 = Gelatinization duration, X_3 = Storage duration, X_4= Packaging Material

Final equation in terms of coded factors

$$Saponin = 202.9908 + 421.7464X_1 + 421.7954X_2 + 421.7912X_3 - 421.791X_4 + 1265.366X_1X_2 + 1265.373X_1X_3 - 1265.37X_1X_4 + 1265.373X_2X_3 - 1265.37X_2X_4 - 1265.37X_3X_4 \quad (3)$$

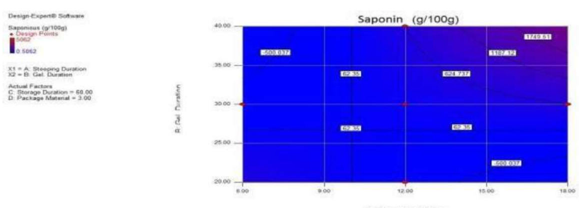

Fig. 5: Effect of Steeping Duration and Gelatinization Duration on the Saponin Content of Soybeans Flour.

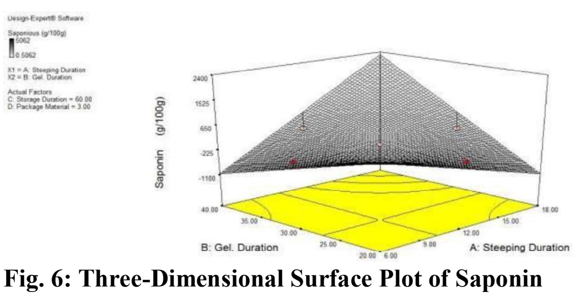

Fig. 6: Three-Dimensional Surface Plot of Saponin Content Model

3.2.1.4 Oxalate Content

Statistical analysis showed that the processing parameters have no significant effect (P≤0.05) on the oxalate content of soybean flour samples; the developed model was also found to be statistically insignificant as shown in Table 6. The 3D plots showing the effect of variables on oxalate content of soybean flour is shown in Figures 7and 8.

Table 6: The ANOVA of the Oxalate Properties of Soybean Flour

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob>F
Model	4.28E-07	10	4.28E-08	0.079518	0.9998
X_1-Steeping Duration	4.27E-07	1	4.27E-07	0.792086	0.3885
X_2-Gel. Duration	1.67E-09	1	1.67E-09	0.003094	0.9564
X_3-Storage Duration	0	1	0	0	1.0000
X_4-Package Material	0	1	0	0	1.0000
$X_1 X_2$	0	1	0	0	1.0000
$X_1 X_3$	0	1	0	0	1.0000
$X_1 X_4$	0	1	0	0	1.0000
$X_2 X_3$	0	1	0	0	1.0000
$X_2 X_4$	0	1	0	0	1.0000
$X_3 X_4$	0	1	0	0	1.0000
Residual	7.54E-06	14	5.39E-07		
Cor Total	7.97E-06	24			

* Values of "Prob > F" less than 0.05 indicate model terms are significant
X_1 = Steeping duration, X_2 = Gelatinization duration, X_3 = Storage duration, X_4= Packaging Material

Final equation in terms of coded factors

$$Oxalate = 0.012104 - 0.00027X_1 + 1.6E - 05X_2$$

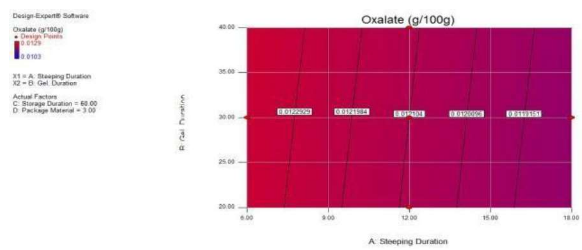

Fig. 7: Effect of Steeping Duration and Gelatinization Duration on the Oxalate Content of Soybeans Flour.

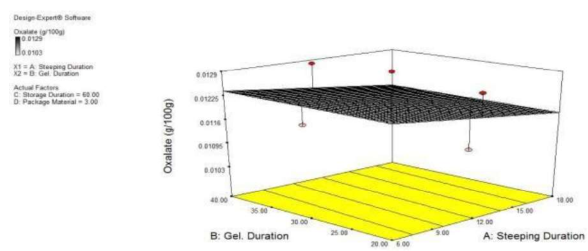

Fig. 8: Three-Dimensional Surface Plot of Oxalate Content Model

3.2.1.5 Phytic Acid Content

The linear model was insignificant in the ANOVA result but has a significant effect (P≤0.05) on the steeping duration as presented in Table 7. The steeping duration, gelatinization duration and interaction between the steeping duration and storage duration all had positive effect on the phytic content of the flour as shown in the model equation (5). The 3D plots showing the effect of variables on phytic acid is shown in Figures 9 and 10.

Table 7: The ANOVA of the Phytic Properties of Soybean Flour

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	0.003306	10	0.000331	1.458515	0.2518
X_1-Steeping Duration	0.003299	1	0.003299	14.55748	0.0019
X_2-Gel. Duration	6E-06	1	6E-06	0.026473	0.8731
X_3-Storage Duration	4.17E-08	1	4.17E-08	0.000184	0.9894
X_4-Package Material	4.17E-08	1	4.17E-08	0.000184	0.9894
$X_1 X_2$	0	1	0	0	1.0000
$X_1 X_3$	6.25E-08	1	6.25E-08	0.000276	0.9870
$X_1 X_4$	6.25E-08	1	6.25E-08	0.000276	0.9870
$X_2 X_3$	0	1	0	0	1.0000
$X_2 X_4$	0	1	0	0	1.0000
$X_3 X_4$	6.25E-08	1	6.25E-08	0.000276	0.9870
Residual	0.003173	14	0.000227		
Cor Total	0.006479	24			

* Values of "Prob > F" less than 0.05 indicate model terms are significant
X_1 = Steeping duration, X_2 = Gelatinization duration, X_3 = Storage duration,
X_4 = Packaging Material

Final equation in terms of coded factors

$$Phytic = 0.961496 + 0.02345X_1 + 0.001X_2 + 8.33E-05X_3 - 8.3E-05X_4 + 0.00025X_1X_3 - 0.00025X_1X_4 - 0.00025X_3X_4$$

5

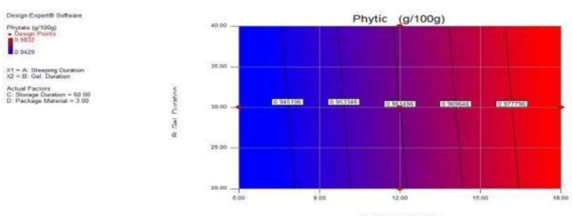

Fig. 9: Effect of Steeping Duration and Gelatinization Duration on the Phytic Content of Soybeans Flour.

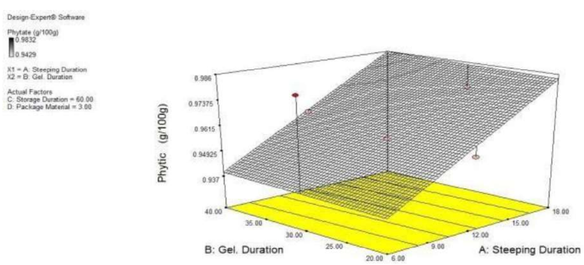

Fig. 10: Three-Dimensional Surface Plot of Phytic Content Model

4 CONCLUSIONS

In evaluating the impact of processing parameters and packaging materials on the anti-nutritional properties of soybean flour, the following conclusions were drawn:

Statistical analysis shows that steeping duration had significant effect (P≤0.05) on the cyanide content while the gelatinization duration, storage duration and packaging materials had no significant effect on the cyanide properties of soybean flour.

Steeping duration is seen to have significant effect on tannin content of the flour while the gelatinization duration, storage duration and packaging materials had no significant effect on the tannin content. Processing parameters and packaging materials had no significant effect (P≤0.05) on the saponin content of the flour samples.

The processing parameters and packaging materials have no significant effect (P≤0.05) on the oxalate content of soybean flour samples; the developed model was also found to be statistically insignificant. Only the steeping duration had significant effect (P≤0.05) on the phytic acid content of the flour.

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