

## Protein and Fat Content of Tiger Nut-Soy Milk Blends as Influenced by Pasteurization

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### ABSTRACT

The aim of this study was to evaluate the effect of pasteurization on the protein and fat content of tiger nut-soy milk. Twenty six different formulations were prepared from tiger nut and soy milk which were packaged in plastic bottles for pasteurization. Optimal mixture design of Response Surface Methodology (RSM) was used for the experimental design. The process treatments employed were; pasteurization temperature, pasteurization duration and mixing duration which varied between 60 - 80 °C, 5 - 20 secs and 5 - 15 mins respectively. Nutritional analysis was carried out on all the twenty six samples. Protein and fat contents were determined with respect to the proximate analysis. The results showed that formulation twenty four with blend constituents of 37, 5 and 50% of tiger nut milk, soy milk, water and process treatments of 60 °C, 20 secs and 5 mins of pasteurization temperature, pasteurization duration and mixing duration respectively had the highest percentage of fat (3.181%). The highest protein value (5.69%) was found in formulation eleven with blend constituents of 27, 5 and 60% of tiger nut milk, soy milk, water and process treatments of 80 °C, 20 secs and 5 mins of pasteurization temperature, pasteurization duration and mixing duration respectively. The most abundant in moisture content (93.065%) was formulation eighteen with blend constituents of 5, 17 and 70% of tiger nut milk, soy milk, water and process treatments of 60 °C, 5 secs and 5 mins of pasteurization temperature, pasteurization duration and mixing duration respectively. Increase in pasteurization temperature favoured increase in fat content of the blends. However, there was fluctuation in the protein yield pattern.

**Keywords:** *Formulation, Pasteurization, Protein, Tiger nut-Soy milk.*

### 1 INTRODUCTION

Tiger-nut (*Cyperus esculentus* L.) belongs to the division–*Magnoliophyta*, class–*Liliopsida*, order–*cyperales* and family–*Cyperaceae*. It is a cosmopolitan, perennial crop of the same genus as the papyrus plant (Belewu and Belewu, 2007; Adejuyitan, 2011). The tubers which are about the size of peanuts are abundantly produced in Nigeria. It has other names such as ground almond, *zulu* nut, *chufa*, yellow nutgrass, edible rush and rush nut. In Nigeria, Yorubas call it *Imumu*, Hausas *Aya*, the Igbos *Aki* Hausa; it is known as *Ofio* in the Southern part of Nigeria. Since early times (chiefly in West Africa and South Europe), Tiger-nut has been cultivated for its small tuberous rhizomes which are used as hog feed, eaten raw or roasted, or pressed for its juice to produce a beverage (Belewu and Belewu, 2007).

The nuts have excellent nutritional qualities with fat composition similar to Olive oil, they are also rich in mineral content especially phosphorus and potassium but with low sodium content (Martinez, 2003; Belewu and Belewu, 2007). According to Oladele and Aina (2007), the crude protein content of the nuts ranged between 7.15 – 9.7%. *Chuffa*, as it is also called is cultivated in Nigeria primarily because of its rich vegetable milk which is an alternative to cow milk among the rural poor. It is used in the production of yoghurt and *Kunnu* (beverage) to quench thirst in Northern Nigeria (Sowonola *et al.*, 2005).

Tigernut tubers have also been used as alternative to cassava in baking industry (Bosch *et al.*, 2005).

Soybean belongs to the family leguminous, subfamily *papiliondase* and the genus *Glycine* Max. Soybean (*Glycine max* M) with 40% protein and 20% fat content assumes the most predominant position in solving the nutritional imbalances prevailing. It not only provides quality macronutrients but also various other micronutrients, which are otherwise required to fight against malnutrition. Soybean is rich in protein content and can furnish protein supply to bridge up the protein deficiency gap at low-cost than any other crop (Rehman *et al.*, 2007).

Milk has been recognized as an important food for infants and growing children. In developing countries, the cost of dairy milk and their products is prohibitive and this has led to the development of alternative source of milk from plant materials. An inexpensive milk substitute extracted from locally available plant foods like legumes with satisfactory quality and rich in protein could play an important role in protein malnutrition, source of producing acceptable nutritious drink and alleviate problem of short food supply (Wakil and Alao, 2013). Tiger-nut milk (having Spanish name *horchata*) is a refreshing purely natural vegetable drink and or dessert, which is prepared with water, sugar and tiger-nuts. It is a very nutritive, energy drink both for young and old.

Soy milk (extract of soybean) does not only provide protein but also a source of carbohydrate, lipid, vitamins and minerals (Bahareh, (2009)). It is an alternate of dairy animal milk due to its cheaper high-quality protein and has been proved to be a healthy drink. It is important especially for people who are allergic to cow milk protein and lactose (Rehman *et al*, 2007). Tigernut-Soy Milk is a blended, processed commodity and is a source of high quality energy, protein, minerals, and vitamins; combining the nutritional benefits of both.

Modern pasteurization is the application of adequate heat to a product for a period of time for the purpose of destroying pathogenic microorganisms, yet leaving the product acceptable from sensory and nutritional stand point (Lewis and Heppell, 2000).

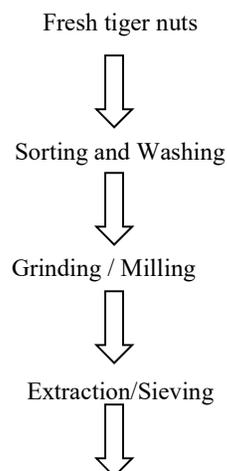
Therefore, this study sought to evaluate the effect of pasteurization on the protein and lipid content of tiger nut-soy milk blends.

## 2 METHODOLOGY

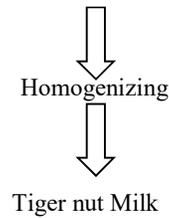
Mature soya beans and fresh tiger nuts used for this research work were purchased from Kure Market, Minna, Niger State, Nigeria. The samples were sorted by removing foreign materials and bad nuts and seeds which may affect the taste and keeping quality of the drink.

### 2.1 Procedure for production of tiger nut milk

Five kilograms of tiger nuts were washed and soaked in water (6L: 1kg) for 18 hours (Awonorin and Udeozor, 2014). Soaking of the tiger nuts in water helps to soften it so as to blend with ease. The soaked tiger nuts were milled into slurry. The slurry was then pressed using muslin cloth to extract the milk and the milk extracted was divided into three parts and diluted with 50%, 60% and 70% of water respectively (Belewu and Abodunrin, 2008). The flow chart for production of tiger nut milk is shown in Figure 1. The filtrate was stored in a plastic container for further processing.



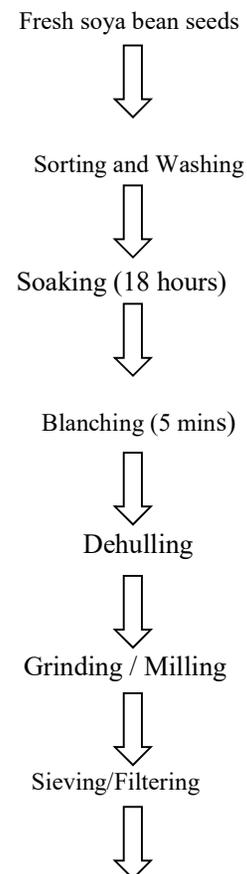
Dilution with Water (50%, 60%, 70%)



**Fig. 1: Flow chart for tiger nut milk production**

### 2.2 Procedure for Production of Soy Milk

Five kilograms (5Kg) of soya beans was soaked for 18 hours in 15 Litters of portable water to give a bean-water ratio of 1:3. The soaked beans were drained, rinsed with portable water and dehulled. Afterwards, the dehulled beans were milled. The resulting slurry was filtered through a muslin cloth and the extract (milk) obtained boiled for 2 hours after which it was divided into three parts and diluted with water at 50%, 60% and 70% respectively. The flow chart for soymilk production is shown in Figure 2.



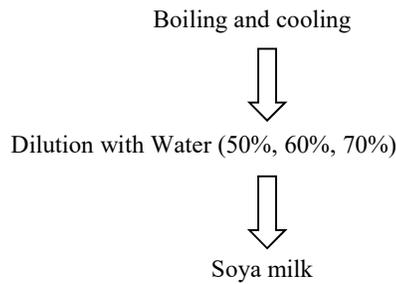


Fig. 2: Flow chart for soya milk production

### 2.3 Procedure for Preparation of Tiger Nut-Soy Milk Drink

Tiger nut milk and soya milk were combined in various proportions to obtain the final products (Awonorin and Udeozor, 2014). This was done using a LEXUS (S/No: SM 31070359) food blender operated at speed level one for the duration specified for the various samples (Awonorin and Udeozor, 2014). The resulting homogenized blends were packaged in plastic bottles. They were then pasteurized at the indicated temperatures and durations as specified for each of the samples in Table 1 using a water bath. After heating, they were allowed to slowly cool to room temperature ( $28\pm 2^{\circ}\text{C}$ ). The flow chart for tiger nut-soy milk drink production is shown in Figure 3.

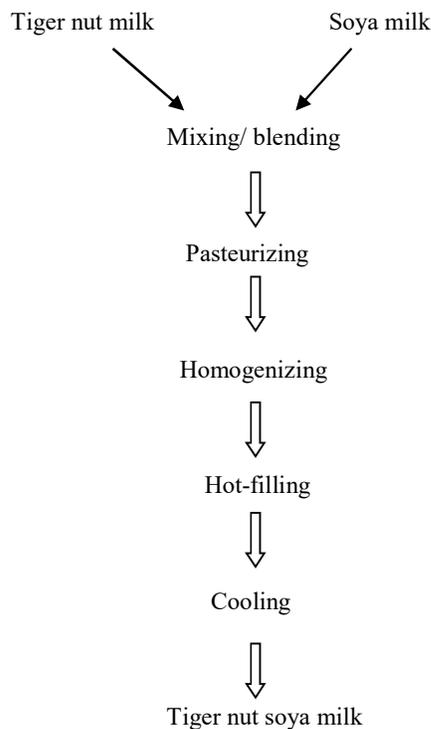


Fig. 3: Flow chart for tiger nut-soy milk production.

### 2.4 Design of the Experiment

Optimal mixture design of Response Surface Methodology (RSM) was used for the experimental design (Design-Expert Version 8). This generated twenty six experimental runs (Table 1). The independent variables were tiger nut milk, soy milk and water. The factors of treatments include pasteurization temperature, pasteurization duration and (constituent) mixing duration. While the responses were protein and fat. A design summary of the various process treatments are presented in Table 2.

#### 1: Formulation of Tiger Nut-Soy Milk Blends

FORMULATION	A (%)	B (%)	C (%)	D ( $^{\circ}\text{C}$ )	E (Secs)	F (Mins)
1	21	21	50	60	5	5
2	37	5	50	80	20	15
3	5	37	50	60	20	15
4	5	37	50	80	5	5
5	5	17	70	80	20	15
6	5	37	50	60	20	5
7	5	37	50	80	20	5
8	16	16	60	60	5	15
9	17	5	70	60	20	15
10	37	5	50	80	5	5
11	27	5	60	80	20	5
12	37	5	50	60	5	15
13	16	16	60	60	5	15
14	5	37	50	60	5	5
15	17	5	70	80	5	15
16	37	5	50	70	12.5	10
17	37	5	50	60	20	5
18	5	17	70	60	5	5
19	17	5	70	60	20	15
20	21	21	50	60	5	5

21	5	37	50	80	5	15
22	5	27	60	70	12.5	10
23	11	11	70	80	20	5
24	37	5	50	60	20	5
25	37	5	50	80	5	5
26	17	5	70	75	8.75	7.5

**Table 2: Summary of the blend constituents and process treatments**

Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	%	Mixture	5.00	37.00	0.000	1.000
B	%	Mixture	5.00	37.00	0.000	1.000
C	%	Mixture	50.00	70.00	0.000	0.625
D	°C	Numeric	60.00	80.00	-1.000	1.000
E	Sec	Numeric	5.00	20.00	-1.000	1.000
F	Min	Numeric	5.00	15.00	-1.000	1.000

Where:

- A: Tiger nut Milk
- B: Soya Milk
- C: Water
- D: Pasteurizing Temperature
- E: Pasteurizing Duration
- F: Mixing Duration

### 2.5 Proximate Analysis

Protein and lipid content of the samples was determined according to the method described by the Association of Official Analytical Chemists (AOAC, 2000).

### 2.6 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analyzed statistically using Design-Expert Version 8 statistical package to determine the analysis of variance (ANOVA) and the Duncan multiple range test was used to separate the means.

## 3 RESULTS AND DISCUSSION

The twenty six separate food formulations and their corresponding results for the protein and fat content are presented in Table 3. A summary of the statistically analyzed results is also presented in Table 4.

**Table 3: Mean Values of Protein and Fat Content of Tiger nut-Soy Milk Blends**

Formulation	Protein	Fat
1.	5.25	2.34
2.	3.5	2.77
3.	3.33	1.90
4.	4.73	2.10
5.	4.38	3.17
6.	3.51	3.11
7.	5.16	2.19
8.	3.28	1.64
9.	3.94	2.96
10.	3.5	2.70
11.	5.69	2.02
12.	4.38	3.17
13.	4.38	1.87
14.	3.94	2.01
15.	3.5	3.11
16.	4.41	3.13
17.	4.38	3.14
18.	4.59	1.38
19.	4.38	2.21
20.	5.25	2.33
21.	3.5	1.65
22.	5.47	1.62
23.	4.38	3.12
24.	3.5	3.18
25.	3.33	2.86

26. 5.25 1.56

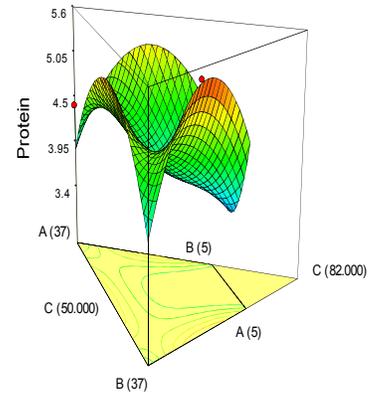
**Table 4: Summary of the statistically analyzed results of the responses obtained.**

Response	Name	Unit	Min	Max	Mean	Std. Dev.
Y1	Protein	%	3.28	5.69	4.27	0.73
Y2	Fat	%	1.39	3.18	4.27	0.73

Design-Expert® Software

Protein  
5.69  
3.28  
X1 = A: Tigernut Milk  
X2 = B: Soya Milk  
X3 = D: Water

Actual Factors  
D: Pasteurization Temperature = 70.00  
E: Pasteurization Duration = 12.50  
F: Mixing Duration = 10.00



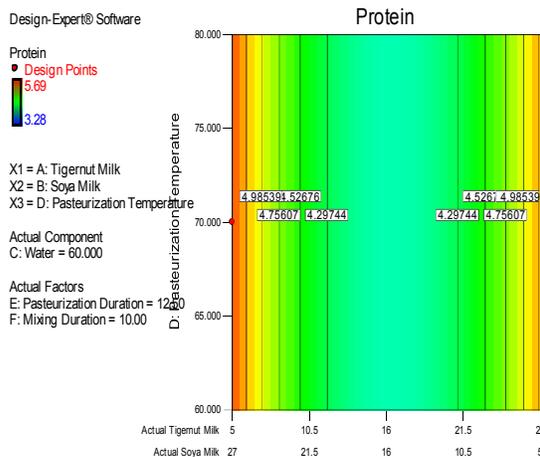
### 3.1 Effect of Pasteurization on the Protein Content of Tiger Nut-Soy Milk Bends.

The protein content of the samples ranged from 3.28 to 5.89% (Table 3). There were fluctuations in the protein content of the tiger nut-soy milk blends. The contour mix-process plot (Figure 4) gives a clear view of the protein yield in relations to the various process parameters, while the relationship between protein yield and the blend constituents is shown in Figure 5.

**Fig. 5: 3 D Surface Plots Showing Protein Yield with Respect to the Effect of Pasteurization**

The fluctuation in the protein yield pattern may be attributed to the destructive effect of heat process involved in pasteurization on the amino acids as well as the tannin-protein complex which have contrast effects (Imafidon *et al.*, 1997). Where amino acids are destroyed there is a consequent reduction in the total Nitrogen which leads to decrease in the protein content of the resulting pasteurized milk (Oluwaniyi *et al.*, 2009), whereas, a rise in the amount of protein may be credited to the destruction of tannin-protein complex (Belewu and Belewu, 2007).

Generally, the range of protein content of the various formulations after pasteurization shows there are no serious adverse effect on the protein content as a result of pasteurizing the milk.



**Fig. 4: Contour Mix-Process Plots Showing the Protein Yield in Relation to Pasteurization**

The analysis of the variance (ANOVA) for the response surface combined special cubic x mean model of the protein content of tiger nut-soy milk bends is shown in Table 5. The model expression developed related the protein yield and the six reaction parameters considered (A, B, C, D, E, F); it has a p-value of 0.0526 greater than 0.05, which suggests there might be a more appropriate model for this experiment. The model F-value of 2.59 implies that the model is fairly significant. The model fit was also checked with the correlation factor  $R^2$ , which equals 44.99%.

The significant factors from the ANOVA analysis were: the interactions between tigernut milk and soy milk (AB) with a p-value of 0.0186 which is less than 0.05; the interaction of tigernut milk and water (AC) with p-value of 0.0261<0.05; the interaction between soy milk and water (BC) with p-value of 0.0189<0.05 as well as the intractions among the three componets (tigernut milk, soy

milk and water) (ABC) with a p-value of 0.0056 which is less than 0.05. The other factors of the model were not statistically significant.

**Table 5: ANOVA for Combined Special Cubic x**

**Mean Model of the Protein Yield.**

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F
Model	6.32	6	1.05	2.59	0.0526
Linear Mixt	0.57	2	0.29	0.71	0.5059
AB	2.69	1	2.69	6.63	0.0186
AC	2.37	1	2.37	5.82	0.0261
BC	2.68	1	2.68	6.58	0.0189
ABC	3.96	1	3.96	9.75	0.0056
Residual	7.72	19	0.41		
Lack of Fit	6.62	14	0.47	2.14	0.2052
Pure Error	1.10	5	0.22		
Cor Total	14.04	25			

Std. Dev.	0.64	R-Squared	0.4499
Mean	4.27	Adj R-Squared	0.2762
C.V. %	14.95	Squared	0.0004
PRESS	14.04	Pred R-Squared	5.656
		Squared Adeq Precision	

The value of the determination coefficient  $R^2$  (0.4499) as shown in Table 5 indicates that the sample variation of 44.99% is attributed to independent variables and 55.01% of the total variations is not explained by the model. The value of the Coefficient of Variation CV% (14.95) gives the precision and reliability of the experiment carried out where a lower value of CV indicates a better precision and reliability of the experiments carried out. Table 6 shows regression coefficients of protein yield.

**Table 6: Regression Coefficients Estimates of the Protein Yield.**

Compo Nent	Coeff Est	df	Standard Error	95% CI Low	95% CI High
A	3.88	1	0.24	3.37	4.38
B	4.03	1	0.26	3.48	4.57
C	0.56	1	1.77	-3.14	4.26
AB	4.97	1	1.93	0.93	9.01
AC	11.15	1	4.62	1.48	20.82
BC	11.64	1	4.54	2.14	21.14
ABC	-50.29	1	16.11	-84.00	-16.58

Where:  
A: Tigernut Milk  
B: Soya Milk  
C: Water

The regression analysis of the data on Table 6 produced the following coded equation:

$$Y2: \text{Protein Content} = +3.88A + 4.03B + 0.56C + 4.97AB + 11.15AC + 11.64BC - 50.29ABC \quad 1$$

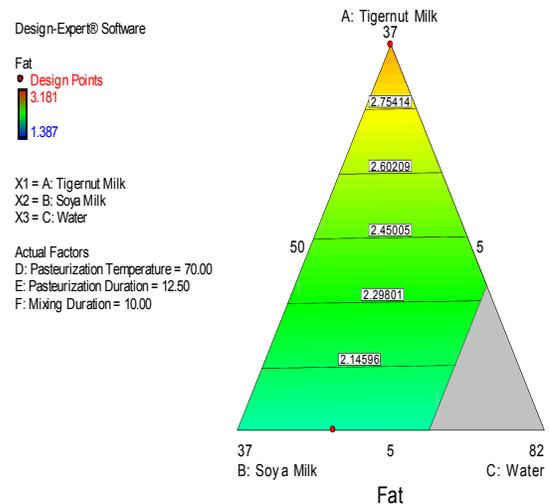
The model equation (eqn 1) shows that all the positive coefficient terms such as A, B, AB, AC and BC indicate synergetic or favourable effect on the protein yield, while the negative coefficient of the model terms ABC indicate an antagonistic effect on the protein yield (Betiku *et al.*, 2014).

The interaction effect of ABC is the general determining factor of protein yield as it has the largest coefficient. The linear effect of A and B, interaction effect of AC and BC are secondary factors of the response.

**3.2 Effect of Pasteurization on the Fat Content of Tiger Nut-Soy Milk Bends.**

The fat content of the samples ranged from 1.38 to 3.18% (Table 3). The fat value increased with increasing tiger nut milk and decreasing soy milk in the blends when water was kept constant (Fig. 6).

Thus, higher percentage of tiger nut milk in Tiger nut-Soy milk would increase the fat content more than having a larger portion of soy milk in the blend as seen in Figures 7 and 8. Furthermore, Figures 7 and 8 showed that fat content of the various formulations increased with increase in pasteurization temperature.



**Fig. 6: Contour Plot Showing Fat Yield as Determined by Pasteurization.**

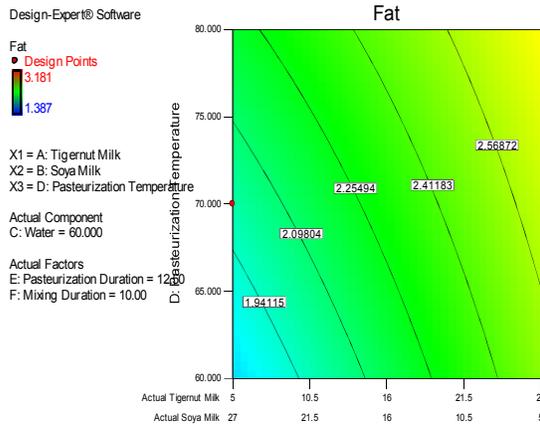


Fig. 7: Contour Mix-Process Plots Showing the Effect of Pasteurization Temperature on Fat Yield

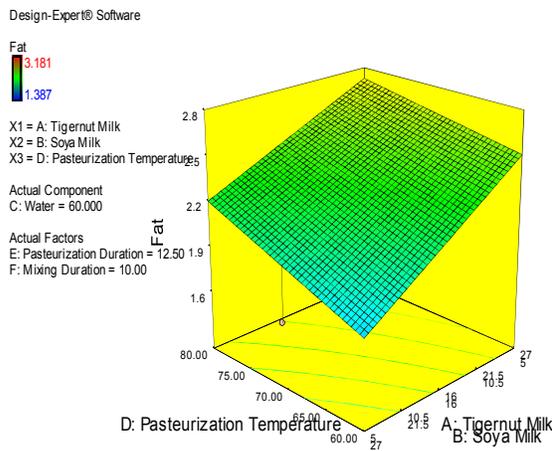


Fig. 8: 3 D Surface Mix-Process Plots Showing the Effect of Pasteurization Temperature on the Yield of Fat with Respect to the Milk Composition.

The analysis of variance (ANOVA) for the response surface combined linear x linear model of fat content of the blends is shown in Table 7. The model expression developed that relates the fat yield and the six reaction parameters (A, B, C, D, E, F) is considered suitable because its p-value of 0.0166 is less than 0.05. The model F-value of 3.43 implies the model is significant. The model fit was also checked with the correlation factor  $R^2$ , which equals to 72.94%.

The only significant factor from the ANOVA analysis is the interaction between water and pasteurization temperature (CD) with a p-value of 0.0194

which is less than 0.05. The other factors of the model are not statistically significant.

Table 7: ANOVA for Combined Linear x Linear Model of the Fat Yield.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob>F
Model	7.00	11	0.64	3.43	0.0166
Linear	2.60	2	1.30	7.01	0.0078
	0.24	1	0.24	1.30	0.2728
AD	5.236E-005	1	5.236E-005	2.823E-004	0.9868
AE	0.005	1	0.047	0.25	0.6223
AF	0.047	1	0.067E-003	0.25	0.8378
BD	8.067E-003	1	0.47	2.56	0.1322
BE	0.003	1	0.47	2.56	0.0770
BF	0.47	1	0.68	3.64	0.0194
CD	0.68	1	1.29	6.97	0.1204
CE	1.29	1	0.51	2.74	0.1204
CF	0.51	14	0.51	2.73	
Residual	0.51	9	0.19		0.0735
Lack of Fit	2.60	5	0.25	3.92	
Pure Error	2.27		0.065		
Cor Total	9.60	25			
Std. Dev	0.43			R-Squared	0.7294
Mean	2.44			Adj R-Squared	0.5167
C.V. %	17.67			Squared	-0.2470
PRESS	11.97			Pred R-Squared	7.711
				Adeq Precision	

The value of the determination coefficient  $R^2$ , (0.7294) indicates that the sample variation of 72.94% is attributed to independent variables and 27.06% of the total variations is not explained by the model. The value of the Coefficient of Variation CV% (17.67) gives the precision and reliability of the experiment carried out where a lower value of CV% indicates a better precision and reliability of the experiments carried out. Table 8 shows regression coefficients estimates of fat.

Table 8 : Regression Coefficients Estimates of the Fat Yield.

Compo	Coeff Est	df	Standard Error	95% CI Low	95% CI High
A	2.91	1	0.16	2.56	3.26
B	2.00	1	0.17	1.63	2.37
C	1.99	1	0.27	1.40	2.58
AD	-0.19	1	0.17	-0.55	0.17
AE	-2.835E-003	1	0.17	-0.36	0.36
AF	0.003	1	0.18	-0.29	0.47
BD	0.090	1	0.18	-0.41	0.34
BE	-0.037	1	0.18	-	0.66
BF	0.28	1	0.18	0.096	0.041
CD	-0.33	1	0.29	-0.71	1.38
CE	0.76	1	0.29	0.14	1.12
CF	0.49	1	0.30	-0.14	1.15
	0.50			-0.15	

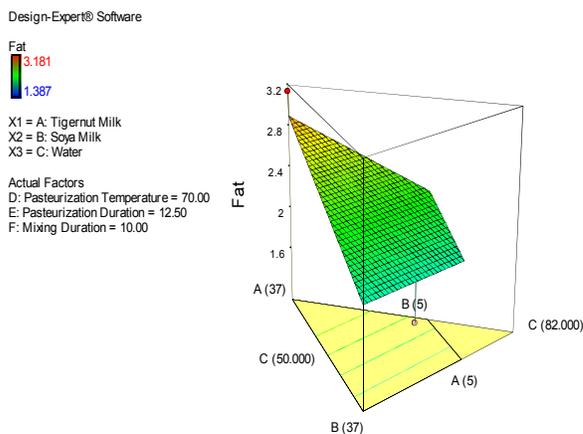
Where:  
A: Tigernut Milk  
B: Soya Milk  
C: Water

The regression analysis of data on Table 8 produced the following coded equation:

$$Y3: \text{Fat Content} = +2.91A + 2.00B + 1.99C - 0.19AD - 2.835(E - 003)AE + 0.090AF - 0.037BD + 0.28BE - 0.33BF + 0.76CD + 0.49CE + 0.50CF \quad (2)$$

The model equation (eqn 2) shows that all the positive coefficient terms such as A, B, C, AF, BE, CD, CE and CF indicate synergetic or favourable effect on the fat yield, while the negative coefficient of the model terms such as AD, AE, and BD indicate antagonistic effect on the fat yield (Betiku *et al.*, 2014).

The linear effect of A and B are the general determining factors of fat yield as they have the larger coefficients. The linear effect of C and the interaction effect of CD are secondary factors of the response. Furthermore, the tigernut milk concentration (factor A) had the highest coefficient among the three independent variables. This implies that the yield of fat from tigernut-soy milk blends relies greatly on this factor as may be clearly visualised in Figure 9.



**Fig. 9: 3 D Surface Plots Showing the Relationship between the Various Constituents and Fat Yield**

#### 4 CONCLUSION

Pasteurized Tiger nut-Soy milk blend is rich in protein and fat composition. When water was kept constant, the higher the percentage of tiger nut milk in tiger nut-soy milk, the more the fat content. There was no clear cut direction as to how protein value could be increased or reduced in the tiger nut-soy milk blends as the yield of protein fluctuated between increasing and decreasing contents of the blends.

Increase in pasteurization temperature favoured increase in fat content of the blends. However, there was fluctuation in the protein yield pattern which was attributed to the destructive effect of heat process involved in pasteurization on the amino acids as well as the tannin-protein complex which had contrast effects.

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