Umudike Journal of Engineering and Technology (UJET); Special Edition, August 2019, pp. 1-8; Michael Okpara University Of Agriculture, Umudike, Print ISSN: 2536-7404, Electronic ISSN: 2545-5257; https://ujetmouau.net; doi: https://doi.org/10.33922/j.ujet_si1_2

INFLUENCE OF HEAT TREATMENT ON MICROBIAL QUALITY OF TIGER NUT-SOY MILK BLENDS

[1]Orhevba B.A.*, [2]Bankole O.S. and [3]Paul T.

[1,2]Department of Agricultural and Bioresources Engineering, Federal University of Technology, P.M.B 65, Minna, Niger State
[3]Department of Agricultural and Bioresources Engineering, Michael Okpara University of Agriculture, Umudike, Abia State

*Corresponding Author: bosede.orhevba@futminna.edu.ng

Abstract

The aim of this study was to evaluate the effect of pasteurisation on the microbial property of tiger nut-soy milk blend. Twenty-six different formulations (F_1 – F_{26}) were prepared from tiger nut and soy milk and packaged in plastic bottles for pasteurisation. The process treatments employed were; pasteurisation temperature, pasteurisation duration and mixing duration which varied from 60 - 80^{0}C, 5 - 20 secs and 5 - 15 mins respectively. Microbial analysis was carried out on all the twenty-six samples. The method of total coliform bacteria count was adopted in assessing the microbiological quality of the milk. The results showed that all the counts for the microbiological quality of the twenty-six blends were below the maximum acceptable limit of 1.0 x 10^6 (cfu/ml) for consumption. Formulation eighteen was found to be the safest with total coliform bacteria count of 1.0 x 10^3 (cfu/ml). Pasteurised tiger nut-soy milk is safe for drinking. Microbiological examination showed that the total coliform count decreased with increase in pasteurisation temperature.

Keywords: Tiger nut milk, soya milk, pasteurisation, microbial analysis, coliform

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 (Osagie and Eka, 1998).

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). According to Oladele and Aina (2007), the crude protein content of the nuts ranged between 7.15 and 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*. The total area cultivated when Soybean (*Glycine max*) was first introduced in Nigeria in 1908 was 401,000 hectares, while the current yield is about 1270 kg per hectare (Rehman *et al*, 2007). 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. The qualities of Tiger-nut (*Cyperus esculentus*) in this context stimulate its inclusion in the preparation of beverage so as to provide protein energy-rich drink at affordable price in place of animal protein/fat which is scarce and expensive.

Among the numerous soy food items, soymilk (extract of soybean) had been the first product ever prepared and consumed by human. It does not only provide protein but also a source of carbohydrate, lipid, vitamins and minerals (Chien and Snyder, 1983). It is an alternate of dairy animal milk due to its cheaper high-quality protein. It has also been proved to be a healthy drink and is important especially for people who are allergic to cow milk protein and lactose (Rehman *et al*, 2007).

Tiger nut-soy milk is a blended, processed commodity and is a source of 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 safety of soy milk and tiger nut milk blends in various proportions taking into cognizance the importance of heat treating the milk at different temperatures.

MATERIALS AND METHODS

Materials

The soya beans and tiger nuts used for this research work were purchased from Kure Market, Minna, Niger State, Nigeria. The samples of soya beans and tiger nuts used for this experiment are shown in Plates 1 and 2 respectively.

Plate 1: Sample of the soya beans

Plate 2: Sample of the tiger nuts

Procedure for production of tiger nut milk

Samples of dried tiger nuts were purchased from the market and the bad nuts and other foreign materials were picked out. The tiger nuts were then washed and soaked in water (6L: 1kg) for 18 hours. Soaking of the tiger nuts in water helps to soften it so as to blend with ease. The method of Belewu and Abodunrin (2006) was used. The soaked tiger nuts were milled into slurry. The slurry was then pressed using muslin cloth to extract the milk. The extracted milk was divided into three parts and diluted with 50%, 60% and 70% of water respectively. The flow chart for the production of tiger nut milk is shown in Figure 1. The filtrate was stored in a plastic container for further processing.

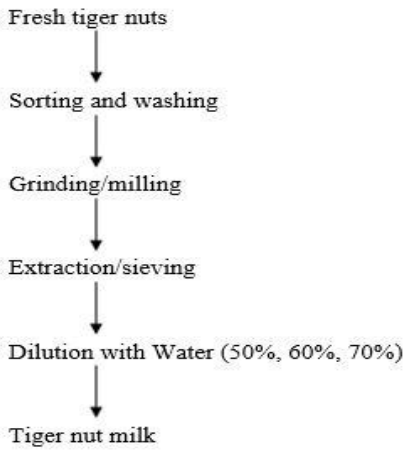

Figure 1: The flow chart for tiger nut milk production

Procedure for Production of Soy Milk

Five kilograms (5Kg) of soya beans was soaked for 18 hours in 15 Litres 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.

Figure 2: The flow chart for soymilk production

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 as shown in Table 1. This was done using a LEXUS food blender operated at speed level one (450rpm) for the duration specified for the various samples. 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 cooled immediately to room temperature (28±2^{0}C). The flow chart for tiger nut-soy milk drink production is shown in Figure 3.

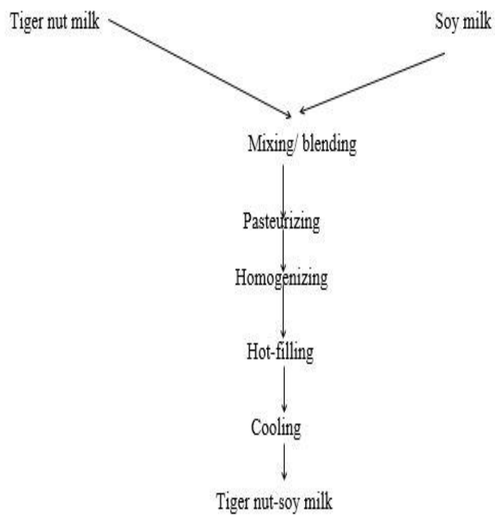

Figure 3**:** The flow chart for tiger nut-soy milk drink production.

Analyses were carried out in duplicates for each sample and results obtained were computed into means. These were subjected to analysis of variance (ANOVA).

Design of the Experiment

Optimal mixture design of Response Surface Methodology (RSM) was used for the experimental design. 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 response was microbiological load (total coliform count).

Table 1: Formulation of Tiger Nut-Soy Milk Blends

FORMULATION	A (%)	B (%)	C (%)	D (^{0}C)	E (Sec.)	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

Where:

A = Tiger nut Milk

B = Soya Milk

C = Water

D = Pasteurization temperature

E = Pasteurization Duration

F = Mixing Duration

A design summary of the various process treatments is presented in Table 2.

Table 2: Summary of the blend constituents and process treatments

Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A Tiger nut Milk	%	Mixture	5.00	37.00	0.000	1.000
B Soya Milk	%	Mixture	5.00	37.00	0.000	1.000
C Water	%	Mixture	50.00	70.00	0.000	0.625
D P/ Temperature	^{0}C	Numeric	60.00	80.00	-1.000	1.000
E P/ Duration	sec	Numeric	5.00	20.00	-1.000	1.000
F M/Duration	min	Numeric	5.00	15.00	-1.000	1.000

Microbial Analysis

The method adopted for the microbial analysis was the total coliform bacteria count which is according to the standard methods for examination of dairy products (Marshal, 1992).

Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analyzed statistically using SPSS 20.0 statistical package to determine the analysis of variance (ANOVA) and the Duncan multiple range test was used to verify the significance of difference between the means.

RESULTS AND DISCUSSION

The twenty-six separate food formulations and their corresponding results for microbiological load is presented in Table 3.

Table 3: Mean Values of the Coliform Count of the Tiger Nut-Soy Milk Blends

Formulation	Bacteria (CFU/ml)
1.	1.5×10^3
2.	1.5×10^3
3.	2.1×10^3
4.	1.7×10^3
5.	2.2×10^3
6.	2.2×10^3
7.	2.0×10^3
8.	1.7×10^3
9.	2.6×10^3
10.	2.0×10^3
11.	1.2×10^3
12.	2.0×10^3
13.	2.1×10^3
14.	1.6×10^3
15.	2.8×10^3
16.	1.4×10^3
17.	1.6×10^3
18.	1.0×10^3
19.	2.2×10^3
20.	2.0×10^3
21.	1.6×10^3
22.	1.8×10^3
23.	2.1×10^3
24.	1.8×10^3
25.	2.6×10^3
26.	1.2×10^3

Microbiological Load of the Tiger nut-Soy Milk Blends

The bacterial load of the samples ranged from 1.0×10^3 CFU/ml to 2.8×10^3 CFU/ml (Table 3). This result indicates that according to the current guidelines for microbiological quality of milk and dairy products (FAO/WHO, 2002), all the pasteurized milk samples are satisfactory (Montville and Matthews, 2005). This is an indication that the pasteurization temperatures employed in this study were adequate. Furthermore, Ihekoronye and Ngoddy (1995) reported that milk samples that have 5.0×10^3 CFU/ml of bacteria is classified as good for consumption, 1.0×10^4 to 4.0×10^4 CFU/ml is considered fairly good, 2.0×10^6 is passable, while over 2.0×10^6 is bad for consumption. Therefore, the higher the microbiological load, the more unsafe and greater the sample's susceptibility to spoilage (Brooks et al; 2003)

The analysis of variance (ANOVA) for the response surface combined linear x linear model of the coliform bacteria load is shown in Table 4. The model expression developed that relates the coliform yield and the six reaction parameters (A, B, C, D, E, F) may not be considered suitable because its p-value of 0.0778 is greater than 0.05. The model F-value of 2.25 implies the model may be considered fairly significant. The model fit was also checked with the correlation factor R^2, which equals to 63.85%.

Most of the factors from the ANOVA analysis had p-values greater than 0.05 which may not be considered significant except the interaction between water and mixing duration (CF) with a p-value of 0.0048 which is highly significant. Therefore it suffices to say that the only statistically significant factors of the model that influences the level of bacteria load in the samples is CF.

Table 4: ANOVA for Combined Linear x Linear Model of the Coliform Bacteria Load.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	3.141E+006	11	2.855E+005	2.25	0.0778
Linear Mixture	1.044E+005	2	52179.79	0.41	0.6709
AD	4692.59	1	4692.59	0.037	0.8503
AE	4.862E+005	1	4.862E+005	3.83	0.0707
AF	23834.44	1	23834.44	0.19	0.6715
BD	52.23	1	52.23	4.112E-004	0.9841
BE	3.674E+005	1	3.674E+005	2.89	0.1111
BF	21231.46	1	21231.46	0.17	0.6888
CD	29819.68	1	29819.68	0.23	0.6355
CE	1.065E+005	1	1.065E+005	0.84	0.3752
CF	1.423E+006	1	1.423E+006	11.20	0.0048
Residual	1.778E+006	14	1.270E+005		
Lack of Fit	1.293E+006	9	1.437E+005	1.48	0.3469
Pure Error	4.850E+005	5	97000.00		
Cor Total	4.919E+006	25			
Std. Dev.	356.39		R-Squared	0.6385	
Mean	1865.38		Adj R-Squared	0.3544	
C.V.	19.11		Pred R-Squared	-0.6253	
PRESS	7.995E+006		Adeq Precision	6.642	

The value of the determination coefficient, R^2 (0.6385) indicates that the sample variation of 63.85% is attributed to independent variables and 26.15% of the total variations is not explained by the model. The value of the Coefficient of Variation CV% (19.11) 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 5 shows the regression coefficient estimates of coliform bacteria load.

Table 5: Regression Coefficients Estimates of the Coliform Bacteria Load.

Component	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High
A-Tiger nut Milk	1795.57	1	135.44	1505.08	2086.06
B-Soya Milk	1805.74	1	142.76	1499.55	2111.93
C-Water	1878.24	1	226.42	1392.61	2363.87
AD	26.84	1	139.62	-272.61	326.28
AE	-273.16	1	139.62	-572.61	26.28
AF	-63.87	1	147.45	-380.13	252.38
BD	-2.95	1	145.27	-314.52	308.63
BE	247.05	1	145.27	-64.52	558.63
BF	-59.36	1	145.19	-370.75	252.04
CD	115.73	1	238.85	-396.56	628.02
CE	223.11	1	243.61	-299.38	745.59
CF	836.91	1	250.04	300.63	1373.20

The regression analysis from Table 5 produced the following coded equation.

$$Y6: Bacteria\ Load = +1795.57A + 1805.74B + 1878.24C + 26.84AD - 273.16AE - 63.87AF - 2.95BD + 247.05BE - 59.36BF + 115.73CD + 223.11CE + 836.91CF \quad (1)$$

The model equation (1) shows that all the positive coefficient terms such as A, B, C, AD, BE, CD, CE and CF indicate synergetic or favourable effect on the bacteria yield, while the negative coeffcient of the model terms such as AE, AF, BD and BF indicate an antagonistic effect on the bacteria (Betiku *et al.,* 2014).

The linear effect of A, B and C are the general determining factors of bacteria yield as they have the larger coefficients. While the interaction effect of CF is the secondary factor of the response. The water concentration (factor C) has the highest coefficient among the three independent variables. This implies that the yield of bacteria from tigernut-soy milk blends relies greatly on this factor. The contour plots (Fig. 4) and the 3 D surphace plot (Fig. 5) also showed that the bacterial yield increases with increase in water content.

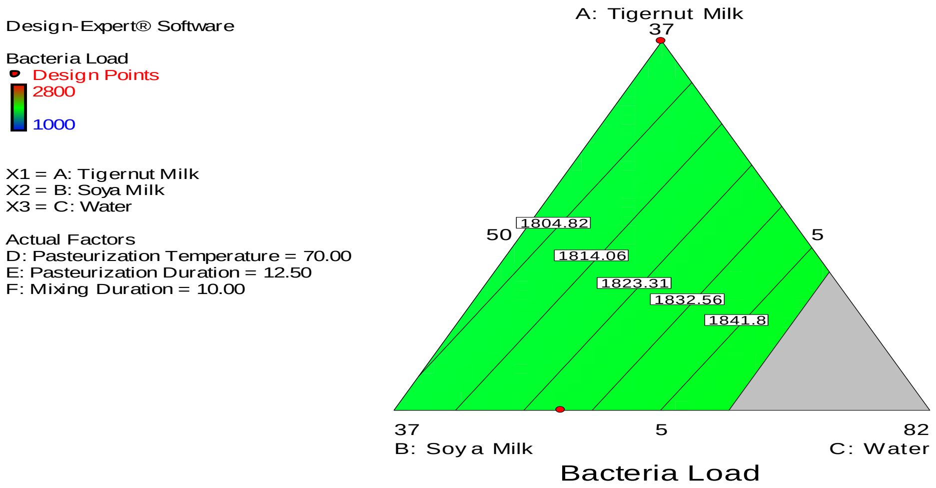

Fig. 4: Contour Plots Showing the Relationship between Bacterial Load and the Various Blend Components

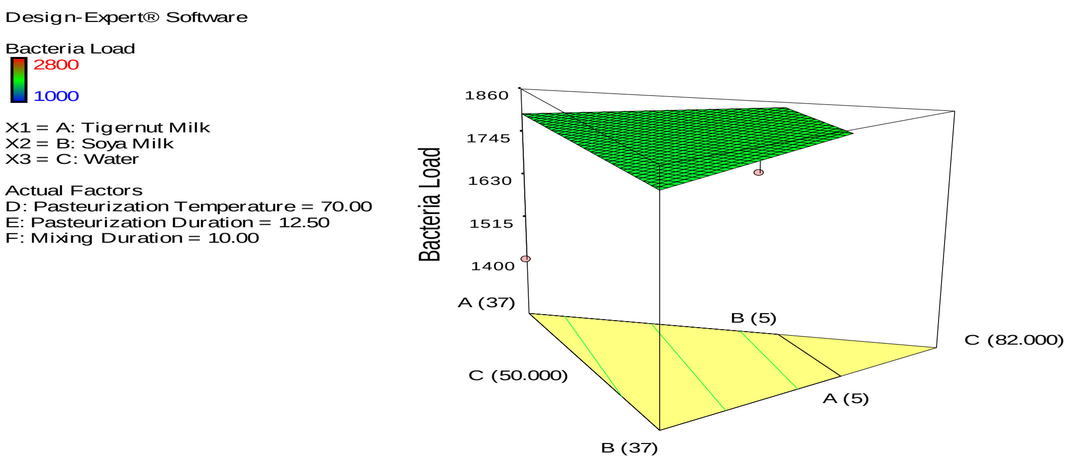

Fig. 5: 3D Surface Plot Showing the Relationship between Bacterial Load and the Blend Components.

CONCLUSION

The following conclusions were made from this study: Pasteurized Tiger nut-Soy milk blend is safe for drinking.

Microbiological examination showed that the total coliform count of pasteurized tiger nut-soy milk decreased with increase in the temperature employed during pasteurization.

REFERENCES

Adejuyitan, J. A. (2011). Tiger nut processing: its food uses and health benefits. Food Technology, 6 (3), 197-201.

Belewu, M.A. and K.Y. Belewu, (2007). *Comparative Physico- Evaluation of Tiger-nut, Soybean and Coconut Milk Sources.* Int. J. Agric. Biol. 9:785-787.

Belewu, M. A and Abodunrin, O. A. (2006). Preparation of Kunnu from unexploited rich food source: Tiger nut (Cyperus esculentus). *World Journal of Dairy and Food Sciences*, 2006; 1: 19-21.

Betiku, O., Omilakin, O.R., Ajala, S.O., Okeleye, A.A., Taiwo, A.E. and Solomon, B.O (2014). Mathematical Modelling and Processs Parameter Optimization Studies by Artificial Neural Network and Response Surface Methodology: a Case of Non-Edible Neem (*Azadiracta Indica*) Seed Oil Biodiesel Synthesis. Energy 72, 266-273.

Bosch, L., Alegria A and Farre, R. (2005) Determination of Tigernut and Orgeat Amino Acid Contents. *Food Science Technology International*. 11: 33-40.

Brooks, A. A, Asamudo, N. U and Udoukpo, F. C. (2003). Microbiological and Physico-chemical Analysis of Soymilk and Soyflour sold in Uyo metropolis, Nigeria. Global Journal of Pure and Applied Sciences; 9(4):457-464).

Chien, J.T. and H.E. Snyder, (1983). Detection and control of soymilk astringency, J. Food Sci., 48: 438-440.

FAO/WHO (2002) Milk and Milk Products. Joint FAO/WHO Food Standards Programme. CODEX Alimentarius Commossion, 42.

Ihekoronye, A. I. and Ngoddy, P. O. (1985). Integrated Food Science and Technology for the Tropics. Macmillan Publishers Ltd, London. Pp. 283-294

Lewis, M. and Heppell, N., (2000). Continuous Thermal Processing of Foods; pasteurization and UHT Sterilization. ASPEN Publishers, Gaithersburg, MD.

Marshal R. T. (1992). Standard Methods for the Examination of Dairy Products, 16th edn. Washington, DC: American Public Health Association.

Martinez, V. (2003). Scientific analysis of effects of tiger nut on heart diseases and related aspects In: Tiger Nut and Health. Pp 1-2.

Montville, T. J., and K. R. Matthews (2005): "Food microbiology an introduction", p.30. American Society for Microbiology Press

Oladele, A.K. and Aina, J.O. (2007). Chemical Composition and Functional Properties of Flour Produced from Two Varieties of Tiger nut (Cyperus esculentus), *African Journal of Biotechnology*, 6 (21): 2473-2476.

Osagie, A.U. and S.A. Eka, (1998). Lipid from plant source, structure and distribution. Proc.1st Agric Conf. on Biochemistry of lipids; 103:21-26.

Rehman, S., S. Hussain, H. Nawaz, M.M. Ahmad, N. Huma and W.A. Virk, (2007). *Preparation and quality evaluation of lathyrus sativus L-bovine milk blend, Pak.* J. Nutr., 6: 134-137

Sowonola, O.A., Tunde-Makinde T.Y. and Adedeji F. (2005) Nutritional and Sensory Qualities of Soymilk-Kunnu Blends. *African Journal of Food Agriculture Nutrition and Development*.

Wakil, S. M. and Alao, O. K. (2013). Supplementation of legume-fortified millet-based weaning blends with *Moringa oleifera. Advances in Food Sciences*_35 (4): 166-176.