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The Effects of Shelf Life on the Chemical and Microbiological Quality of African Breadfruit-Corn Milk

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ABSTRACT

The objective of this study is to provide alternative milk analogue that will augment the conventional cow milk and assess the chemical and microbial stability under ambient storage conditions. African breadfruit-corn milk was obtained from a blend of African breadfruit and corn milks at 60:40 proportions. This was formulated with emulsifier (0.01% Glycerolmonostearate), stabilizer (0.01% Carrageenan), sweetener (2% Sucrose), milk flavor (to taste), and preservatives (0.01% Sodium benzoate and 0.01% Potassium sorbate). Soymilk was produced and formulated similarly to serve as a control. The two milk samples were stored at ambient conditions for 28 days during which time they were examined for chemical and microbiological changes. The breadfruit-corn milk had better chemical and microbiological stability, which suggests that the shelf life of breadfruit-corn milk can be extended through some industrial processes as in commercial soymilk drinks.

Keywords: African Breadfruit, Corn,Phyto-Milk, Soymilk, Stability.

1. INTRODUCTION

Dairy milk is an excellent source of all nutrients except iron and ascorbate and has been recognized as an important food for infants and growing children (Obizoba and Anyika, 1995; Ukwuru*et al*., 2011). Throughout the world, there are more than six billion consumers of milk and milk products and over 750 million people live within dairy farming households (Hemme and Otte, 2010).

However, Gerosa and Skoet (2012) reported that growth in average per caput energy intake from dairy products in developing regions has been slower than for other livestock products and that regional distribution total dairy consumption was lowest in sub-Saharan Africa in the year 2007. An inexpensive substitute in the form of a milk or beverage made from locally available plant food high in protein, with satisfactory quality could play an important role to reduce protein malnutrition (Ukwuru and Ogbodo, 2011).

Prior to the development of such phyto-milk, which serve as a less expensive substitute for dairy milk, direct milk consumption as a beverage was not common in Nigeria (Iwuoha and Umunnakwe, 1997; Onweluzo and Owo, 2005). The most popular varieties internationally are soymilk, almond milk, rice milk and coconut milk (Esperly, 2008).

Onweluzo and Nwakalor (2009) developed and evaluated vegetable milk from *Treculia Africana*. Nwakalor (2014) investigated the effect of storage on the microbiological quality of formulated African breadfruit beverage. Supavititpatana *et al*. (2010) studied the storage stability of yoghurt developed from sweet corn. Production of soy-corn milk blend has been reported (Omueti and Ajomale, 2005; Kolapo and Oladimeji, 2008).

Among the sources of vegetable milk, soybean has received very high research attention and more efforts are being made to improve the quality of soymilk (Sunyoung*et al*., 2000). Unlike the soybean, works on African breadfruit seed milk has been scanty, and its combination with corn to obtain legume-cereal milk could not be found in literature as at the time of this study.

Researched information on shelf stability of African breadfruit-corn milk could pave the way for its commercial exploitation as another variety of milk analogue to complement soymilk. In addition to providing food safety guide, this will add value to this tree crop and boost the economic fortunes of growers.

2. MATERIALS AND METHOD

Source of Material

The seeds of African breadfruit (*Treculiaafricana* var *africana*) and soybean (*TGX 513-IE*) were purchased from OyeAgu Market Abagana, Njikoka L.G.A, Anamabra State, Nigeria. The green field sweet corn (*Golden cob F1*) was purchased from Songhai, Enugu Initiative, Heneke, Ezeagu L.G.A, Enugu State, Nigeria.

Production of African breadfruit milk

The method described by Onweluzo and Nwakalor (2009) was slightly modified.

Approximately 1.5 kg of African breadfruit seed was parboiled, air dried and hulled in a hand mill (Corona, Landers YCIA, South Africa) whose teeth has been adjusted to approximately 15 mm, to remove the hull without breaking the seeds. This was subsequently winnowed, sorted to remove extraneous materials. Exactly 1 kg of the seeds was soaked in potable water for 6 h. The soak water was changed every 2 h during the 6 h duration to forestall fermentation. The seeds were repeatedly washed with distilled water before wet-milling in a variable speed blender (SB-736, Sonic, Japan), with intermittent addition of distilled water. The slurry was filtered through double layer linen cloth and the residue was wet-milled twice more to further extract milk, leaving a final seed to water ratio of 1:3 (w/v). The filtrate was boiled for 20 min with continuous stirring and was re-filtered to obtain plain breadfruit milk as in Figure 1.

Production of corn milk

The method described by Supavititpatana *et al*. (2010) was used with slight modification. The green field sweet corn was firstly husked, the silks removed and washed with water. The grains were separated from the cob using knife, cleaned to remove hairs and extraneous materials. Exactly 1 kg of the grains was weighed and soaked in potable water for 6 h; with soak water changed every 2 h as before. This was repeatedly washed with distilled water before wet-milling in a variable speed blender with intermittent addition of distilled water. The slurry was filtered, re-milled and filtered twice to a final grain to water ratio of 1:3 (w/v). The filtrate was boiled for 15 min, re-filtered to obtain plain corn milk as in Figure 2.

Production of soymilk

The soybean seeds were prepared using the soaking, blanching and cold extraction method of Udeozor (2012). Exactly 1 Kg of the seeds were sorted and washed, soaked in excess volume of water for 8 h, decanted and boiled for 30 min. The grains were threshed out and washed repeatedly to obtain clean cotyledon. This was further washed with distilled water and wet-milled in a variable speed blender as before. The slurry was filtered through double layer linen cloth and the residue was re-milled and extracted twice more to a final seed to water ratio of 1:3 (w/v). The filtrate was boiled for 20 min, re-filtered to obtain plain soymilk.

Production of African breadfruit-corn milk

The blending method of Udeozor (2012) was used. Appropriate portions of African breadfruit milk and corn milk taken from previous productions were blended on 60:40 proportions to obtain plain breadfruit-corn milk.

Product formulation

The plain soymilk and African breadfruit milk were formulated with emulsifier (0.02% Glycerylmonostearate), stabilizer (0.02% Carrageenan), sweetener (2% Sucrose), and preservative (0.01% Sodium benzoate; 0.01% Potassium sorbate). This was homogenized in a 5–speed hand mixer (MC–HM 6630 Master Chef and Crown Star, China), pasteurized at 65^{0}C for 15 min, hot-filled into sterile 250 ml screw capped plastic bottles, rapidly cooled and stored at ambient temperatureas in Figure 3.

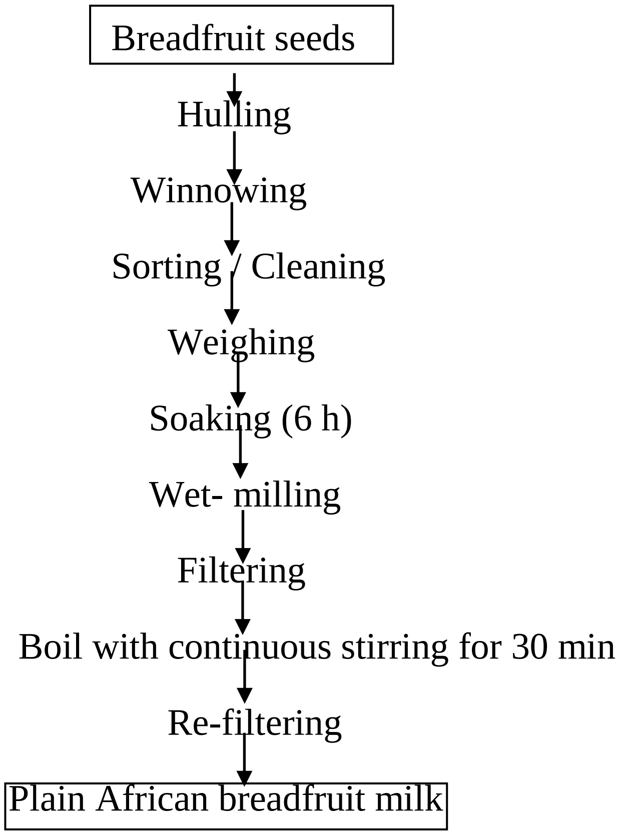

Fig.1: Flow chart for the production of plain African breadfruit milk

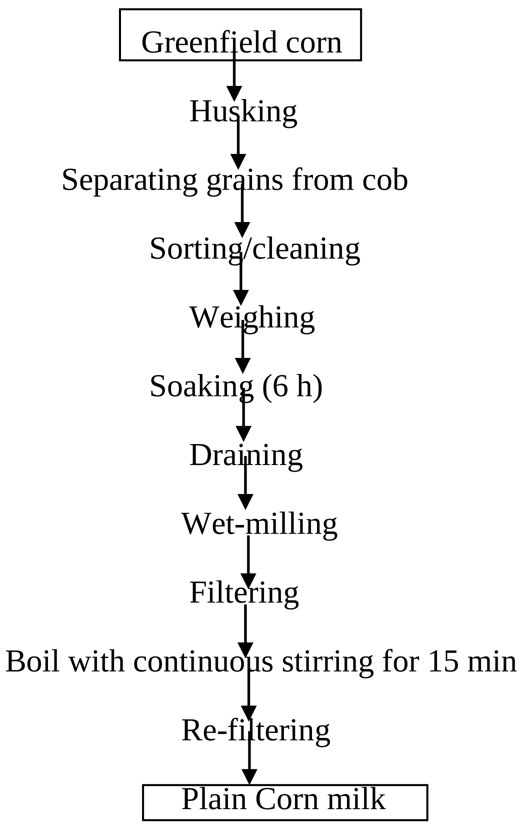

Fig.2: Flow chart for the production of Plain Corn milk

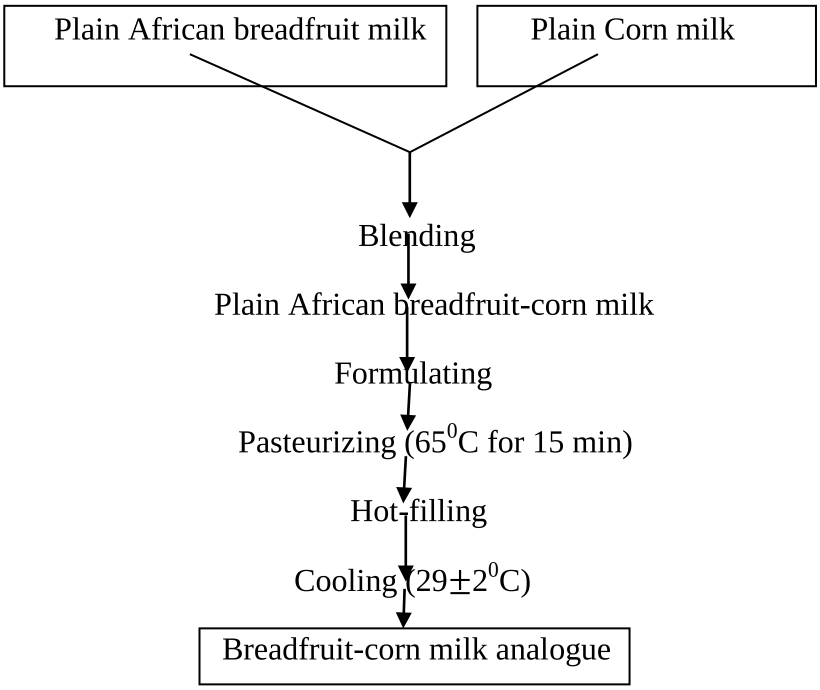

Fig.3: Flow chart for the production of African breadfruit-corn milk

3. CHEMICAL ANALYSIS

Total solids

The total solids of the milk samples were determined according to the method of analysis described by the Association of Official and Analytical Chemists AOAC (1990).

pH value

The pH of samples was measured by electrometric method using Laboratory pH meter Hanna Model HI 991300 (APHA, 1995).

Titratable acidity

Titratable acidity was determined using the method described by Adeiye *et al*. (2013). Approximately 20 g of the milk sample was weighed into 250 ml conical flask and diluted with twice its volume of distilled water. Approximately 2 ml of phenolphthaleine indicator was added to the mixture and this was titrated with 0.1N sodium hydroxide (NaOH) to a persistent pink colour. The titratable acidity was reported as % lactic acid by weight using 1 ml of 0.1N NaOH = 0.0090 g lactic acid (AOAC 1990).

Viscosity

Approximately 30 ml of sample was filled into a 50 ml beaker. Viscosity was measured using Oswald type viscometer.

MICROBIOLOGICAL ANALYSIS

Total viable count and mould count were carried out according to the method of Ogbulie*et al.* (1998) for serial dilution, inoculation and plate count.

Total viable count

The plates, test tubes and pipettes used were previously sterilized at 160^{0}C for 1 h in an electric oven. 1 ml of sample was transferred aseptically into the first test tube marked 10^{-1} from which subsequent serial dilution were made up to the last test tube marked 10^{-6}. The total viable count of the samples were carried out by inoculating 0.1 ml from 10^{-3} to 10^{-5} dilutions in duplicates for each, using sterile nutrient agar. The plates were incubated at 37^{0}C for 48 h after which colonies formed were counted and expressed in colony forming units per milliliter (cfu/ml).

Mould count

Serial dilution was carried out as before. The mould count was carried out using already sterilized Sabourand dextrose agar by inoculating 0.1 ml from 10^3 and 10^{-4} dilutions in duplicates for each sample. The plates were incubated at 25^0C for 3 to 5 days after which the colony counts per milliliter (cfu/ml) were recorded.

Statistical analysis

Data collected were subjected to analysis using SPSS Version 17. T-Test was employed and the differences between mean values were evaluated at $p<0.05$ using Paired Samples Test.

4. RESULTS AND DISCUSION

CHANGES IN CHEMICAL QUALITY

The initial total solids content of breadfruit-corn milk of 14.13% was significantly ($p<0.05$) higher than the 10.40% of soymilk (control) as can be seen in Fig. 1. The 14.13% of the breadfruit-corn milk was higher than 9.22 to 11.50% reported by Onweluzo and Nwakalor (2009) for breadfruit milk. The higher total solids of breadfruit-corn milk may be attributed to the corn milk that blended with the African breadfruit milk. Nelson *et al*. (1976) reported high levels of suspension particles in corn milk which resulted in high total solids. Elsamani*et al*. (2014) reported that higher sweet corn ratio will increase total solids of beverage. This agrees with Olakunle (2012) who reported higher total solids in soy-corn yoghurt at different substitution levels of corn and soybean than in 100% soy yoghurt, which was attributed to higher levels of suspended particles in soy-corn milk than in soy milk. However the total solids of the milk samples were below the minimum standard (28%) for sweetened dairy milk (FAO/WHO, 2002a). Farinde*et al*. (2008) suggested that the total solids of soymilk could be improved by adding soybean flour to the soymilk. Increase in total solids can also be achieved by concentrating the milk extract or reducing the volume of water used for extraction. Increasing the total solids increases the nutritive value of the product thereby improving the keeping quality (Odu*et al*., 2012). The total solids of the breadfruit-corn milk depreciated during the first five days before stabilizing over a period of two weeks and finally dropped to about 5.66%. The soymilk was relatively stable up to the second week of storage when it decreased for nearly one week before stabilizing. The difference in stability profile may be attributed to biochemical and microbiological changes in relation to the initial pH of breadfruit-corn milk of 4.68, which was lower than the 6.23 of soymilk as in Fig. 2. The pH of the soymilk correlated with the 6.25 value reported by Onweluzo and Nwakalor (2009), while the pH of the breadfruit-corn milk was lower than the 5.37 to 5.84 of breadfruit milk reported by these researchers. The presence of high level of acetic amino acids in corn based beverage (Hosney, 1994) might have contributed to the lower pH of African breadfruit-corn milk. The pH of both milks reduced during storage which was more rapid in the soymilk during the first two weeks of storage. The more acidic pH of breadfruit-corn milk is desirable because it could discourage the growth of pathogens that may cause gastrointestinal problems (Onweluzo and Nwakalor, 2009). Gaman and Sherington (1977) reported gradual decrease in pH under reduced temperature which could be indicative of the beverage relative stability.

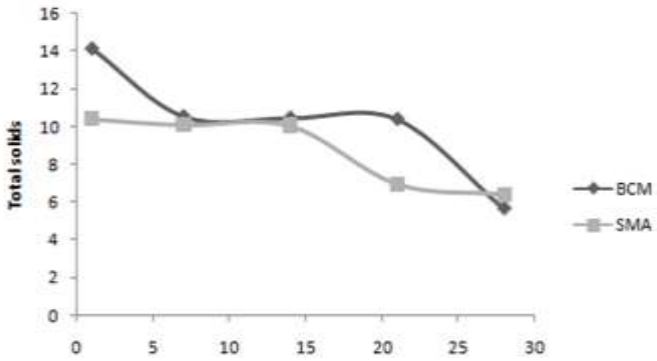

Fig. 1: Changes in total solids of milk samples during storage. BCM=Breadfruit-corn milk, SMA=Soymilk

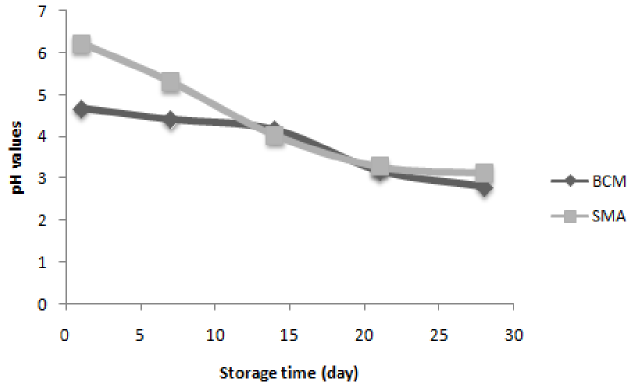

Fig. 2: Changes in pH of milk samples during storage BCM=Breadfruit-corn milk, SMA=Soymilk

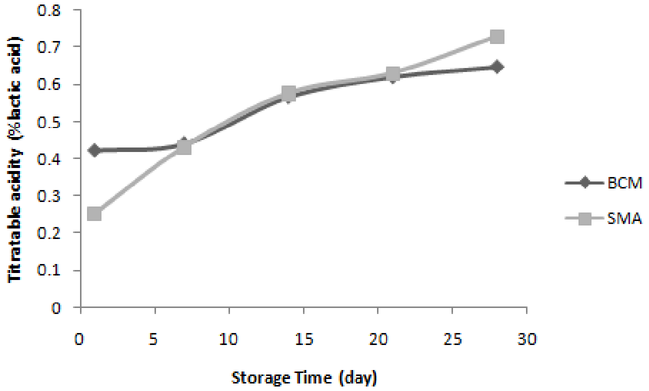

Fig. 3: Changes in titratable acidity of milk samples during storage. BCM=Breadfruit-corn milk, SMA=Soymilk

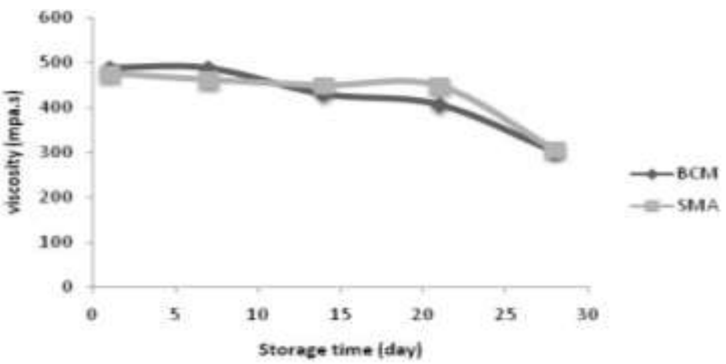

Fig. 4: Changes in viscosity of milk samples during storage. BCM = Breadfruit-corn milk, SMA=Soymilk

The initial titratable acidity of breadfruit-corn milk of 0.423% was higher than 0.252% of soymilk as in Fig. 3. The titratable acidity value of the breadfruit-corn milk was higher than 0.23% to 0.36% for breadfruit milk reported by Onweluzo and Nwakalor (2009). The acidic nature of maize protein (zein) as reported by Hosney (1994) could be responsible for higher total titratable acidity of the breadfruit-corn milk. Increase in acidity is probably caused by oxidative changes in lipids and other organic compounds in food (Adoga, 2006), or may be as a result of anaerobic microbial activities resulting in the formation of lactic acid and other organic acids (Adeiye*et al*., 2013). The initial higher acidity of the African breadfruit-corn milk may have retarded such activities thus conferring better chemical stability. This conforms to other reports by Sanni*et al.* (1999) and Bucker *et al.* (2008) on increase in titratable acidity during storage with extent of increase influenced by the type of lactic acid bacteria present.

The initial viscosity of 0.487 pa.s of the breadfruit-corn milk was higher than the 0.475 pa.s of soymilk as shown in Fig. 4. The viscosity of the breadfruit-corn milk was stable during the first week, reducing slightly during the second week and stabilized once more during the third week. The viscosity of the soymilk was relatively stable during the first three weeks. However, the viscosity of both milks converged at 0.30 pa.s by the end of the four week study. Viscosity of food system is usually affected by sugar and other macromolecules through their interaction with the solution or solvent (Zapsalis and Beck, 1985). The initial higher viscosity value of breadfruit-corn milk could be due to the higher total solids and for the fact that it contained higher concentration of suspended solids (Onweluzo and Nwakalor, 2009), possibly due to addition of corn milk. It is noteworthy that the rapid drop in the pH of the soymilk may have reduced the level of degradation of the total solids, hence gradual loss of viscosity. The seeming direct relationship between total solids and viscosity of colloids is evinced from Fig. 1 and Fig. 4 of this study. Viscosity of any milk product is important in determining the rate of creaming of the milk and the rate of mass and heat transfer (Adeiye*et al*., 2013).

Changes in microbiological quality

The total viable count (TVC) of breadfruit-corn milk of $9.5\text{x}10^3$cfu/ml was lower than the $4.7\text{x}10^4$cfu/ml of the soymilk as reflected in Table 1. The total viable count of both milk persistently increased during the 28 days study with breadfruit-corn milk recording $1.0 \text{ x } 10^7$cfu/ml against the $1.4 \text{ x } 10^7$cfu/ml of the soymilk. The apparent convergence in microbial load towards the end of this study may be attributed to the sharp drop in the pH of soymilk during the first two weeks of storage. The initial values were higher than the $2.8 \text{ x } 10^2$cfu/ml total viable count of tiger nut milk (Ukwuru and Ogbodo, 2011) and $1.2 \text{ x } 10^2$cfu/ml of vita milk (Nwakalor, 2014). Kolapo and Oladimeji (2008) reported total bacteria count of $3.84 \text{ x } 10^4$cfu/ml for soymilk and $3.0\text{x}10^4$cfu/ml of soy-corn milk. The difference in microbial qualify may be due to preparation method, heat treatment, use of preservatives, packaging method and storage conditions (Odu*et al*., 2012; Adeiye*et al*., 2013; Nwakalor, 2014). The lower microbial growth of the breadfruit-corn milk might be due to the lower pH of 4.68 as compared to the pH 6.23 of the soymilk, since acidic medium can be antagonistic to the growth of pathogens (Onweluzo and Nwakalor, 2009). Although Kolapo and Oladimeji (2008) stated that fortifying soymilk with maize did not alter the microbiological quality of the resultant product, the total bacteria count of the soymilk varied, albeit marginally, with that of the soy-corn milk as indicated in their report.

Table 1: Microbiological quality of milk samples during 28 days storage

Sample	Storage period (days) Microbial Count (cfu/ml)									
	1		7		14		21		28	
	TVC	Mould	TVC	Mould	TVC	Mould	TCV	Mould	TVC	Mould
BCM	$9.5\text{x}10^3$	Nil	$1.5\text{x}10^4$	$1.05\text{x}10^2$	$2.0\text{x}10^4$	$2.0\text{x}10^3$	$1.5\text{x}10^6$	$2.5\text{x}10^4$	$1.0\text{x}10^7$	$1.5\text{x}10^5$
SMA	$4.7\text{x}10^4$	Nil	$4.9\text{x}10^4$	$3.0\text{x}10^2$	$9.05\text{x}10^4$	$9.5\text{x}10^3$	$2.1\text{x}10^6$	$8.5\text{x}10^4$	$1.4\text{x}10^7$	$2.0\text{x}10^5$

BCM = Breadfruit-corn milk, SMA = Soymilk

The titratabale acidity of the milk samples directly related to microbial growth and inversely related to pH of samples during storage and this agreed with the findings of Walia*et al.* (2013). The shelf life of pasteurized milk products subjected to ultra high temperature (UHT) is usually extended especially if adequately stored (Saidu, 2005). The effects of certain preservatives at various concentrations, within their permissible levels, along with pasteurization and refrigeration storage on the microbial quality of soymilk were provided by the Codex Alimentarius Commission (FAO/WHO, 2002a, b). The application of benzoates and sorbates at 0.02% to the samples under review might have discouraged intense growth, given that they were stored at ambient condition.

There was no mould growth in the samples on day 1. However there was mould count of $1.0 \text{ x } 10^2$cfu/ml for breadfruit-corn milk and $3.0\text{x}10^2$cfu/ml for soymilk on day 7, which rose to $1.5 \text{ x } 10^5$ and $2.0 \text{ x } 10^5$ respectively on day 28. The values were higher than the $1.6 \text{ x } 10^2$cfu/ml to $3.2 \text{ x}10^2$cfu/ml mould count of Ukwuru and Ogbodo (2011) for tiger nut milk stored for 60 days at 4^0C, but correlated with the $2.5 \text{ x } 10^4$cfu/ml total fungal count of soymilk and $2.2 \text{ x } 10^4$cfu/ml of soy-corn milk reported by Kolapo and Oladimeji (2008). The difference may be due to dissimilar moisture content, process variables and storage conditions. The lower level of microbial growth in the African breadfruit-corn milk was in agreement with previous reports of higher levels of deterioration in soymilk based products during storage than in maize based beverages (Iwuoha and Umunnakwe, 1997; Omueti and Ajomale, 2005). This may be due to the higher acidity of the African breadfruit-corn milk which conferred on it pH value within the working range of potassium sorbate against moulds. High moisture content of the milk samples are of important consideration. The higher moisture content of the soymilk may be another drawback since high moisture content could affect stability and safety of food with respect to microbial growth and proliferation. Moisture content of between 57.34% and 80.34% could affect the stability and safety of food with respect to microbial growth and proliferation Udeozor (2012).The moisture content of the milk samples used in this study fall within this range which suggests cold storage to retard the rate of microbial growth. The use of preservatives (Adeiye*et al*., 2013) and cold storage (Udeozor, 2012) has been suggested for the prolongation of stability of groundnut milk and other high moisture beverages. However, the mould count of the products during the study period falls within the limits of acceptance of 2.0x 10^5cfu/ml for dairy milk by Codex Alimentarins Commission (FAO/WHO, 2002 a, b). This could be attributed to good process control as well as the use of chemical preservatives. Cold storage, no doubt, could have resulted in better microbial quality.

5. CONCLUSION

The results of this research work show that blending corn milk and breadfruit milk resulted in product with comparatively better stability than soymilk which is a reference plant milk. The effect of storage on the chemical and microbiological changes of the milk samples revealed that the African breadfruit-corn milk was relatively more stable than the soymilk, probably due to higher acidity of the former. There is no doubt that improving the processing and packaging method of this milk, as in the industrial processing of soymilk in addition to cold storage, will extend its keeping quality.

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