

## Original Research Article

# Microbiological Evaluation of Milk Beverages Produced From African Breadfruit, Tigernut, Coconut and Date Fruit

### ABSTRACT

This study was carried out to extract milk from African breadfruit, tigernut, coconut and date fruit and to observe the changes in the microbial load of milk during storage. The formulated plant milk beverages were compared to conventional plant milk beverage "Vita milk" which served as the control sample. Microbial counts, characterization and identification were carried out on the beverage samples every 7 days for up to 35 days. The total viable count of the toasted breadfruit-based samples ranged from  $3.7 \times 10^2$  cfu/ml to  $5.1 \times 10^2$  cfu/ml and the values were lower than those recorded from the cooked breadfruit-based samples which ranged from  $3.95 \times 10^2$  cfu/ml to  $6.10 \times 10^2$  cfu/ml. There were no presence of coliform bacteria in the formulated beverage samples and the reference sample from 1 to 21 days of storage. After these periods, coliform bacteria were recorded in 3 samples of the formulated plant milk and in all the formulated beverage samples after 35 days of storage. The percentage occurrence of *Staphylococcus* species according to the result was low on the 1<sup>st</sup> day of storage (18.2%), but increases with storage period up to 28.7% after 35 days of storage. The percentage occurrence of *Aspergillus* species which is another organism of public health concern as recorded from the beverage samples throughout the period of storage were low and below the specified tolerable set by Codex Alimentarius which is  $< 50$ ug/ml. The overall microbial qualities of the formulated beverage were safe and stable for the 1<sup>st</sup> 21 days of storage and up until 28 days for the control sample. The study revealed that milk beverages formulated from these plants gave products that are comparable to the conventional soy bean based vitamilk. The microbiological stability of the formulated plant milk samples were relatively less compared to the vitamilk.

Keywords: Coliform, Nutrient, Microbial, Milk beverages, African breadfruit, Date fruit

### 1. INTRODUCTION

Hygienic practice in food production is been reflected by microbial quality determination. Growth of many microorganisms especially bacterial pathogens can be increased in milk as it serves as a good medium for their growth; thus, its quality control is considered important to the health and welfare of a community. The risk with diseases spread through contaminated food is known and the impact of such diseases is considerable. Beverage milk drink developed from under-utilized crops which are high in pathogenic microorganisms has emerged as a serious public health concern mostly for consumers. Different sources such as: bad water, air, preparation equipment, unhygienic of the handler, poor post-pasteurization handling such as bottling and storage systems, among others can cause bacterial contamination of milk analogue [1].

Tiger-nut (*Cyperus esculentus*) belong to the family, Cyperaceae. The plant was first known by the Arabs, first in the Valencia region. It is a local crop for most of the Western Hemisphere as well as Southern Europe, Africa, Madagascar, the Middle East and the Indian Subcontinent [2]. It has different names in Nigeria, as "Aya" in Hausa, "Imumu" in Yoruba and "Aki Hausa" in Igbo. Tiger nuts can be eaten in different forms like: raw, roasted, dried, or baked. It serves as a good plant for the preparation of "kunu

aya” (a local beverage in Nigeria) [3]. Tiger nut milk is a highly nutritional and energetic drink which can be consumed by both the old and young people [2], is a good source of energy contents (starch, fat, sugar and protein), minerals (phosphorus, potassium) and vitamins E and C (4,5). Due to its short shelf life and lack of information on its nutritional potentials, the milk is underutilized [6]. Other than its use as a beverage, tiger nut milk is beneficial to diabetic patients [7]; reduces cholesterol level or aids lose weight [5,8].

African breadfruits seed (*Treculia africana*) is a leguminous crop which is known in Nigeria as afon, ediang or ekwa [9]. Breadfruit is rich in carbohydrate and contains 2.2 – 5.9% protein on a dry weight basis. It is a rich source of amino acids, especially histidine and lysine, which are very important for infant growth. Breadfruit has been reported to be a rich source of vitamins and minerals such as phosphorous, copper, magnesium, potassium, calcium, iron, and manganese depending on the cultivar [10]. Phytate, oxalate, and tannin, ascorbic acid, and high contents of carotenoids which are bioactive compounds can be obtained from breadfruit [11]. Breadfruit undergoes physiological deterioration after harvesting. The fruit can be processed into flour as a way of reducing post-harvest losses and increasing the utilization of breadfruit, which is more shelf stable.

Coconut (*Cocos nucifera* L.) is the mostly grown and used nut in the world, which belongs to the palm family (Arecaceae). It is an important commercial crop in many tropical countries, contributing majorly to their economies [12]. Coconut milk contains a liquid extract obtained from the solid endosperm of mature coconut and can be mixed with water for consumption as a beverage. This product contains lauric acid as a functional component, which can support the development of brain functions, maintain elasticity of blood vessels and stimulate immune system defenses [13].

Dates (*Phoenix dactylifera*) are an essential staple food in the diet of population living in the arid and semi-arid regions of North Africa, Middle East and South-Asian countries [14]. Dates contribute significantly to improve the diet of consumers due to its high nutritional value. The fruit contain carbohydrates (44-88%), fats (0.2-0.4%), fiber (6.4-11.5%), minerals, vitamins and higher concentration of protein (2.3-5.6%). According to various researches, many bioactive compounds such as sterols, anthocyanins, phenolics, carotenoids, procyanidins and flavonoids, which are thought to have beneficial effects on human health are present in date fruit [15].

Several works have been done on the microbial and shelf life of plant extracts. However, beverages from blend of African breadfruit, tigernut, coconut and date fruit have not be developed, likewise no existing reports on the microbial and shelf life of beverages formulated from a blend of these plant extracts. Therefore, the present study has been designed to extract milk from African breadfruit, tigernut, coconut and date fruit and to observe the changes in the microbial load of milk during storage.

## **2. MATERIALS AND METHODS**

### **2.1 Source of raw materials**

African breadfruit seeds, tiger nuts, coconut and date fruits were purchased at Oyibo market, old Aba Road Port Harcourt, River state, Nigeria. The chemical additives that were used in this research were purchased from Pokobros Food and Chemical Industries Limited, No.1 Harbour Industrial Layout, Port Harcourt, Rivers state Nigeria and they were of analytical standards.

### **2.2 Preparation of raw materials**

The raw materials were prepared and processed in the Food Processing Laboratory of Food Science and Technology Department, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria according to the method of Ogbuele *et al.* [16]. The breadfruit seeds were sorted to remove bad seeds and other solid contaminants. Two (2) kg of the sorted seeds was divided into two parts of 1kg each. One part was thoroughly washed, par-boiled/blanched in hot water for 10-15 min and drained after. The par-boiled seeds were de-hulled manually to get the per-boiled dehulled seeds. The second part of the sorted breadfruit seeds were washed drained and roasted at 72<sup>0</sup>C for 30 min, then de-hulled and winnowed to separate the seeds from husks to get the roasted breadfruit seeds. The tiger nuts were sorted to remove bad nuts and contaminants and 2 kg sorted nuts were soaked in worm water for 2 h. The coconut seeds were cracked manually and their endocarp or meat detached from the pericarp using a knife. Two (2) kg of the endocarp were measured, cut into smaller pieces and washed, while the dried date fruits were sorted to remove bad fruits and contaminants, then two (2) kg of the sorted date fruits were chopped into smaller sizes and the enclosed seeds removed and washed.

### **2.3 Processing of raw materials**

African bread fruit milk was processed according to the method of Okafor and Ugwu [17].

Tiger nuts milk was processed according to the method of Udeozor [18].

Coconut milk was processed according to the method of Okafor and Ugwu [17].

Date fruit juice was processed as described by Jo-Ann [19].

### **2.4 Formulation of plant milk beverages**

Two groups of the beverages were formulated using Roasted Breadfruit milk (rBFM), Tiger nut milk (TM), Coconut milk (CM) and Date fruit Juice (DFJ) in three different ratios of (3:5:1:1), (2:6:1:1) and (1:7:1:1) in one group and Cooked Breadfruit milk (cBFM), TM, CM and DFJ using the same ratio in another group. After which 0.3% CMC solution were added to the blends to serve as stabilizer and the formulation were thoroughly mixed, homogenized and pasteurized at 72<sup>0</sup>C for 5 min in a water bath and allowed to cool. Exactly 0.2% Potassium sorbate solution and 0.05% Citric acid solution were added to the blends as

preservatives. The beverages samples were homogenized, filled into screw capped plastic bottles and stored at room temperature.

**Table 1. Proportions of extracts for plant milk formulation**

Beverage brand	Proportion of the milk components in the beverage samples (%)				
	Samples	Breadfruit	Tiger nut	Coconut	Date fruit
Roasted	621	30	50	10	10
	742	20	60	10	10
	852	10	70	10	10
Cooked	536	30	50	10	10
	482	20	60	10	10
	941	10	70	10	10

## 2.5 Microbial analysis

### 2.5.1 Microbial analysis of stored samples

Microbial counts, characterization and identification were carried out on the beverage samples every 7 days for up to 35 days.

### 2.5.2 Preparation of diluents, media and samples for analysis

Diluents (peptone water), media (Nutrient Agar, MacConkey Agar and Potato Dextrose Agar) and the samples were prepared according to Cheesbrough [20].

### 2.5.3 Enumeration of microbial population

Total plate counts for the nutrient and MacConkey Agar were done by counting colonies at the reverse side of the culture plates as described by Harrigan [21].

### 2.5.4 Characterization and identification of microbial isolates

Microbial isolates (bacteria and fungi) were characterized based on colonial, microscopic and biochemical characteristics to know the genera using the method as described by Cheesbrough [20]. The identities of the isolates were determined using a reference manual by Berishir [22].

### **2.5.5 Determination of coliform count**

Analysis of coliform in the beverage samples was done according to the method described by Harrigan [21].

## **3. RESULTS AND DISCUSSIONS**

### **3.1 Microbial composition of the plant milk samples**

Table 2 shows the microbial counts recorded for the plant milk samples from the 1<sup>st</sup> day of study to 35<sup>th</sup> day of storage. The total viable count of the toasted breadfruit-based samples ranged from  $3.7 \times 10^2$  cfu/ml to  $5.1 \times 10^2$  cfu/ml and the values were lower than those recorded from the cooked breadfruit-based samples which ranged from  $3.95 \times 10^2$  cfu/ml to  $6.10 \times 10^2$  cfu/ml. The observed variation of total viable count in these two groups of samples may be attributed to the method of heat pretreatment given to the breadfruit seeds before extracting milk from them, which have been reported to have varying effects on the microbial load of the treated seeds and handling of the extracted milk during formulation and storage [23]. However, the total viable count recorded for both group of formulated plant milk samples were higher than the value recorded for the reference sample ( $2.2 \times 10^2$  cfu/ml). This observation may be due to some factors which include; method of production, level of hygiene during preparation, microbial load of raw materials and pH of the sample [24]. The initial values of total viable count recorded from the plant milk samples were higher than the  $2.8 \times 10^2$  cfu/ml total viable count for tiger nut milk reported by [25], and  $1.2 \times 10^2$  cfu/ml total viable count reported for vita milk (the reference sample) as reported by [24]; but were lower than  $3.84 \times 10^4$  cfu/ml total viable count for soymilk and  $3.0 \times 10^4$  cfu/ml total viable count for soy-corn milk as reported by [26]. The difference in microbial quality may be due to the preparation method, type of heat treatment used, nature of preservatives used, packaging material and storage conditions [24]. The total viable count of all the samples decreased slightly during the first 14 days of storage and increased subsequently until the 35 days storage period. The initial decrease in total viable count may be attributed to the effectiveness of the preservatives used and also the dormancy period for some of the inherent microorganisms in the samples to acclimatize to their new environment, while the subsequent increase of total viable count in the later stages of storage may be due to the multiplication of microorganisms within the plant milk samples and also the decrease in potency of the preservatives used in the plant milk samples [26]. The microbial load of the plant milk samples were also observed to be inversely related to total titrable acidity and directly related to pH of the beverage samples and this agreed with the findings of [23]. The application of potassium sorbate at 0.2% level and citric acid at 0.05% level to the formulated samples might have discouraged intense growth of microorganism, given that the samples were stored at ambient conditions. There were no fungal growths in the beverage samples up until the 14<sup>th</sup> day of storage. However, there were fungal count of  $1.02 \times 10^2$  cfu/ml for all the toasted breadfruit-based samples and  $1.05 \times 10^2$  cfu/ml for the cooked breadfruit-based samples after 21 days of storage which rose to the range of  $1.52 \times 10^2$  cfu/ml to  $2.03 \times 10^2$  cfu/ml for the toasted breadfruit

samples and  $2.10 \times 10^2$  cfu/ml to  $3.01 \times 10^2$  cfu/ml for the cooked breadfruit samples after the 35 days storage period. Also, fungal counts were recorded for the reference sample from the 21<sup>st</sup> day of storage at the level of  $1.03 \times 10^2$  cfu/ml which also rose to  $1.26 \times 10^2$  cfu/ml on the 35<sup>th</sup> day storage period. These values were within the range of  $1.6 \times 10^2$  cfu/ml to  $3.2 \times 10^2$  cfu/ml fungal counts reported by [25] for tiger nut milk stored for 60 days but were lower than the  $2.5 \times 10^4$  cfu/ml fungal count of soymilk and  $2.2 \times 10^4$  cfu/ml fungal counts for soy-corn milk stored for 35 days as reported by [26]. There were no presence of coliform bacteria in the formulated beverage samples and the reference sample from 1 to 21 days of storage. After these periods, coliform bacteria were recorded in 3 samples of the formulated plant milk viz; 852, 482 and 941 and in all the formulated beverage samples after 35 days of storage. The absence of coliform bacteria in the first 21 days of storage could probably be attributed to some factors which include; the heat treatment (boiling and pasteurization) that were given to the plant milk samples during processing as coliforms cannot withstand high temperatures. Also, high level of hygiene during preparation and good packaging of the products may have contributed to their absence during this period. Similar results were reported by [27] in peanut beverages that were sterilized under two heat treatment conditions. Heat is known to improve the availability of some nutrients, inactivate enzymes that speed up nutrient damage, destroy undesirable microorganisms and food contaminants (coliform inclusive), as well as favorably change the physical attributes of the food such as colour, texture and flavour. The presence of coliform in some of the formulated plant milk samples after 21 days of storage and in all the samples after 35 days of storage could be attributed to chance contamination during processing, analysis and/or possible leakage of the packaging material. The presence of coliform is evidence of fecal contamination and as such is of public health importance in determining the safety of food products [28]. Coliforms are also indicator microorganisms for milk and milk products, which their presence indicates fecal contamination thus making them microorganisms of public health concern [29].

		Beverage Samples Studied						
Storage Period(days)	Microbial Count(cfu/ml)	621	742	852	536	489	941	804

1	TVC	$5.10 \times 10^2$	$2.58 \times 10^2$	$3.67 \times 10^2$	$4.63 \times 10^2$	$3.95 \times 10^2$	$6.10 \times 10^2$	$2.24 \times 10^2$
	FC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	CFC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
7	TVC	$4.23 \times 10^2$	$2.01 \times 10^2$	$3.15 \times 10^2$	$3.81 \times 10^2$	$3.21 \times 10^2$	$4.10 \times 10^2$	$2.02 \times 10^2$
	FC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	CFC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
14	TVC	$4.02 \times 10^3$	$1.91 \times 10^3$	$2.95 \times 10^3$	$3.34 \times 10^3$	$2.86 \times 10^3$	$3.86 \times 10^3$	$1.98 \times 10^3$
	FC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	CFC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
21	TVC	$4.31 \times 10^4$	$2.40 \times 10^4$	$3.26 \times 10^4$	$3.60 \times 10^4$	$3.34 \times 10^4$	$4.01 \times 10^4$	$2.48 \times 10^4$
	FC	$1.02 \times 10^2$	$1.02 \times 10^2$	$1.02 \times 10^2$	$1.05 \times 10^2$	$1.05 \times 10^2$	$1.05 \times 10^2$	$1.03 \times 10^2$
	CFC	Nil	Nil	Nil	Nil	Nil	Nil	Nil
28	TVC	$4.70 \times 10^4$	$2.94 \times 10^4$	$3.81 \times 10^4$	$4.72 \times 10^4$	$4.02 \times 10^4$	$5.12 \times 10^4$	$2.50 \times 10^4$
	FC	$1.20 \times 10^2$	$1.31 \times 10^2$	$2.50 \times 10^2$	$1.52 \times 10^2$	$1.60 \times 10^2$	$1.65 \times 10^2$	$1.03 \times 10^2$
	CFC	Nil	Nil	$1.35 \times 10^2$	Nil	$1.50 \times 10^2$	$1.18 \times 10^2$	Nil
35	TVC	$4.90 \times 10^5$	$3.01 \times 10^5$	$3.90 \times 10^5$	$3.96 \times 10^5$	4.78	5.26	2.54
	FC	$2.03 \times 10^2$	$2.50 \times 10^2$	$1.52 \times 10^2$	$2.10 \times 10^2$	$3.01 \times 10^2$	$2.80 \times 10^2$	$2.16 \times 10^2$
	CFC	$1.76 \times 10^2$	$1.82 \times 10^2$	$2.01 \times 10^2$	$1.63 \times 10^2$	$1.43 \times 10^2$	$1.45 \times 10^2$	$1.26 \times 10^2$

**Table 2. Microbial count of the plant milk samples stored at different period**

Values are means of triplicate samples. Values with different superscript in the same column are significantly different at ( $P > 0.05$ ). Samples 621,742 and 852 are toasted breadfruit milk-based samples blended with breadfruit milk, tiger nut milk, coconut milk and date fruit juice at the ratios of 3:5:1:1, 2:6:1:1 and 1:7:1:1 respectively and samples 536, 489 and 941 are cooked breadfruit milk-based samples blended with breadfruit milk, tiger nut milk, coconut milk and date Coliform Count

Table 3 shows the occurrence of bacterial isolates from the plant milk samples studied. Three different bacterial species namely; *Lactobacillus*, *Staphylococcus* and *Micrococcus* were isolated from the plant milk samples. *Lactobacillus* species were persistent in all the plant milk samples studied, while the presences of *Staphylococcus* and *Micrococcus* species were not persistent as they were not isolated from some of the plant milk samples. This observation may be due to the slightly acidic pH of the formulated plant milk samples resulting from the citric acid used as preservative which might not be favorable for the *Staphylococcus* and *Micrococcus* species, but favors the *Lactobacillus* species [30]. Also, *Staphylococcus* species may not be persistent because its presences were basically from environmental contamination during processing which will definitely vary [31]. However, the presences of the *Staphylococcus* species were not observed in the reference sample up till day 21 of storage, while *Micrococcus* species were not recorded at all in the reference sample throughout the storage period. This observation may be due to the high level of hygiene observed during the preparation of the reference sample, method of heat pretreatment used, preservatives used and packaging methods used which all have effect on the microbial quality of liquid food products [32].

**Table 3. Occurrence of bacterial isolates from the plant milk samples**

Storage Period(days)	Bacterial Isolate	621	Beverage 742	Samples 852	Studied 536	489	941	804
1	Lac	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Staph	+ve	+ve	+ve	+ve	+ve	+ve	-ve
	Micro	+ve	-ve	+ve	+ve	-ve	+ve	-ve
7	Lac	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Staph	+ve	+ve	+ve	+ve	+ve	+ve	-ve
	Micro	+ve	-ve	+ve	+ve	-ve	+ve	-ve
14	Lac	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Staph	+ve	+ve	+ve	+ve	+ve	+ve	-ve
	Micro	+ve	-ve	+ve	+ve	-ve	+ve	-ve
21	Lac	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Staph	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Micro	+ve	+ve	+ve	+ve	+ve	+ve	-ve
28	Lac	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Staph	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Micro	+ve	+ve	+ve	+ve	+ve	+ve	-ve
35	Lac	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Staph	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Micro	+ve	+ve	+ve	+ve	+ve	+ve	-ve

Values are means of triplicate samples. Values with different superscript in the same column are significantly different at ( $P < 0.05$ ). Samples 621, 742 and 852 are toasted breadfruit milk-based samples blended with breadfruit milk, tiger nut milk, coconut milk and date fruit juice at the ratios of 3:5:1:1, 2:6:1:1 and 1:7:1:1 respectively and samples 536, 489 and 941 are cooked breadfruit milk-based samples blended with breadfruit milk, tiger nut milk, coconut milk and date fruit juice at the ratios of 3:5:1:1, 2:6:1:1 and 1:7:1:1 respectively. Sample 804 is vitamilk which is the reference sample. Lac = *Lactobacillus* species, Staph = *Staphylococcus* species and Micro = *Micrococcus* species

Table 4 shows the occurrence of fungal isolates from the plant milk samples studied. Two different fungal species were isolated from the plant milk samples namely; *Saccharomyces* species and *Aspergillus* species. Both species were found present in the plant milk samples only after 14 days of storage but not in all the samples.

The presence of the fungal isolates on 21 days and 28 days of storage were very low and limited only to the formulated plant milk samples as only *Saccharomyces* species were isolated from the reference sample during this period.

The reference sample had the two fungal isolates only after the 35 days of storage. The use of preservatives, heat treatment and packaging may have attributed to the low level of fungal isolates recorded from the plant milk samples [32]. The presence of *Aspergillus* species in the plant milk sample after some days of storage is of concern as it is one of the microorganisms which are of public health importance when ascertaining food safety. This is because of their ability to produce mycotoxins which when ingested by humans to a particular dosage are harmful to health [33].



**Table 4. Occurrence of fungal isolates from the plant milk samples**

Storage Period(days)	Fungal Isolates	621	Beverage 742	Samples 852	Studied 536	489	941	804
1	Sacc	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Asper	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7	Sacc	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Asper	-ve	-ve	-ve	-ve	-ve	-ve	-ve
14	Sacc	-ve	-ve	-ve	+ve	+ve	+ve	-ve
	Asper	-ve	-ve	+ve	+ve	+ve	+ve	-ve
21	Sacc	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Asper	+ve	+ve	+ve	+ve	+ve	+ve	-ve
28	Sacc	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Asper	+ve	+ve	+ve	+ve	+ve	+ve	-ve
35	Sacc	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	Asper	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Values are means of triplicate samples. Values with different superscript in the same column are significantly different at ( $P < 0.05$ ). Samples 621, 742 and 852 are toasted breadfruit milk-based samples blended with breadfruit milk, tiger nut milk, coconut milk and date fruit juice at the ratios of 3:5:1:1, 2:6:1:1 and 1:7:1:1 respectively and samples 536, 489 and 941 are cooked breadfruit milk-based samples blended with breadfruit milk, tiger nut milk, coconut milk and date fruit juice at the ratios of 3:5:1:1, 2:6:1:1 and 1:7:1:1 respectively. Sample 804 is vitamilk which is the reference sample. Sacc = *Saccharomyces* species and Asper = *Aspergillus* species

Table 5 shows the percentage occurrence of microbial isolates from the plant milk samples studied. The percentage occurrence of *Staphylococcus* species according to the result was low on the 1<sup>st</sup> day of storage (18.2%), but increases with storage period up to 28.7% after 35 days of storage. The observation showed that there was contamination during handling or storage of the formulated plant milk samples as *staphylococcus* species are ambigious and can be contacted from the environment. However, the level of *Staphylococcus* species recorded during the storage period of the plant milk samples were still within the tolerable level of *Staphylococcus* species allowed in food samples according to the specification of codex Alimentarius that is  $< 10^5$ cfu/ml [34]. The level of *Staphylococcus* species in the beverage samples are of important in determining the microbial safety of the samples as they are one of microorganism of public health concern.

The percentage occurrence of *Aspergillus* species which is another organism of public health concern as recorded from the beverage samples throughout the period of storage were low and below the specified tolerable set by Codex Alimentarius which is  $< 50$ ug/ml [34]. This observation shows that potassium sorbate used as preservative in the formulated beverage samples were very effective in making the samples safe in regards to fungal contamination.

**Table 5. Percentage occurrence of microbial isolates in the plant milk samples**

Isolate	Percentage 1	Microbial 7	Occurrence (%) 14	Within the 21	Storage 28	Period(days) 35
Bacteria						
<i>Lactobacillus</i> species	52.3(7)	40.5(7)	39.0 (7)	38.3(7)	38.1 (7)	39.2 (7)
<i>Staphylococcus</i> species	18.2(6)	26.1(6)	27.3 (6)	27.8(7)	27.8 (7)	28.7 (7)
<i>Micrococcus</i> species	29.5(4)	33.4(4)	33.7 (4)	33.9(6)	34.1(6)	32.1 (6)
Total Fungi	100(17)	100(17)	100 (17)	100(20)	100 (20)	100 (20)
<i>Saccharomyces</i> species	Nill	Nill	73.6 (3)	69.2(7)	66.4 (7)	61.7 (7)
<i>Aspergillus</i> species	Nill	Nill	26.4 (4)	30.8(6)	33.6 (6)	38.3 (7)
Total	Nill	Nill	100(7)	100 (13)	100(13)	100 (14)

n = number of samples studied = (7)

#### 4. CONCLUSIONS

The study revealed that plant milk formulated from breadfruit milk, tiger nut milk, coconut milk and date fruit juice gave products that are comparable to the conventional soy bean based vitamilk. The microbiological stability of the formulated plant milk samples were relatively less compared to the vitamilk (reference sample). However, the samples containing the roasted breadfruit milk were slightly more stable than those produced with cooked breadfruit milk, probably due to the higher acidity of the roasted breadfruit milk-based samples.

#### DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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