Effect of Blending and Storage Conditions on the Microbial Quality and Sensory Characteristics of Soy-Tiger Nut Milk Beverage

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ABSTRACT
Effect of blending and storage conditions on soy-tigernut milk beverage was carried out. Samples of milk analogue were developed using varying proportions of soymilk and tigernut extracts. Five blends of soy-tigernut milk was formulated using varying proportion of tigernut milk extract (10, 20, 50, 80 and 90%) with soy milk. Two control samples were produced (100% milk of soy bean and tigernut respectively). Soy-tigernut milk beverage blends were subjected to sensory evaluation using 9-point hedonic scale. Two blends of the milk beverage having the highest mean score on general acceptability was subjected to three storage conditions (ambient, refrigeration and freezing conditions) for four weeks. Significant difference ($P<0.05$) was discovered on the sensory attributes of the soy-tigernut beverage blends evaluated. Samples contained 80% and 90% tigernut milk substitution had mean score of 7.47 and 8.67 respectively were the most preferred blends. The highest value of total aerobic plate count and coliform under a different storage conditions at fourth week of storage was below 10^6cfu/ml and 10cfu/ml safety limit stipulated by Food and Drug Administration regulation for aerobic plat count and caliform bacteria in food respectively.
Keywords: Blending, Storage Conditions, Microbial Quality, Sensory Characteristics, Soy-Tigernut – Milk Beverage.

INTRODUCTION
Milk has been recognized as an important food for infant and growing children [(1)] because milk is an excellent source of nutrients such as vitamins, amino acids, fats, minerals, proteins and sugar, making it an excellent medium for microbial proliferation [(2)]. In developing countries, the cost of diary milk and their products e.g. 'nono', cheese, etc are beyond the reach of common man. The high cost of milk in developing countries has led to the development of alternative source of milk from plant minerals. An inexpensive substitute in the form of a milk or beverage made from locally available plant foods, high in protein, with satisfactory quality milk could play an important role to reduce protein malnutrition [(3)]. Most of researches have centered on the use of soybean as alternative source of milk analogue or substitute with little attention on the use of other under-utilized crops such as bambaranut, tigernut, melon, etc.

Food is a chemically complex matrix, and predicting whether or how fast microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Food products should not contain microorganisms, their toxins, or metabolites in quantities that present an unacceptable risk for human health [(4)]. Regulation EC No. 178/2002 sets down general food safety requirements, according to which food must not be placed on the market if it is unsafe [(5)]. The shelf-life of food products is an integral part of food safety. Several factors encourage, prevent or limit the growth of microorganisms in foods; the most important are water availability, pH, and temperature [(6 -7)] Microbial quality of beverage drink, dairy products and other food is determined by significant total microbial count, coliform count and presence of pathogenic microorganisms. Microbial quality determination is completely used to reflect hygienic practice in food production. Milk analogue can serve as a good medium for the growth of many microorganisms especially bacterial pathogens; therefore, its quality control is considered essential to the health and welfare of a community. As reported by Foster [(8)], the threat posed by diseases spread through contaminated food is well known and the epidemiological impact of such diseases is considerable. The presence of these pathogenic microorganisms in beverage milk drink developed from under-utilized crops has emerged as a major public health concern especially for consumers. Bacterial contamination of milk analogue can originate from different sources: bad water, air, preparation equipment, unhygienic of the handler, poor post-pasteurization handling such as bottling and storage systems, among others.

Pasteurization is effective in eliminating all but the thermoduric microorganisms of the genera *Microbacterium,* *Micrococcus, Streptococcus, Lactobacillus, Bacillu, Clostridium*, the coliform, and occasionally some Gram-negative rods [9]. Psychrotrophs can grow at refrigeration temperatures below 7^{o}C, produce enzymes, toxins and other metabolites [9]and contribute to high standard plate counts in both raw and pasteurized milk analogue. Unlike most dairy products, milk analogue is currently handled and stored at low temperatures; these organisms hinder efforts to increase the shelf-life of pasteurized product [10].Pasteurization cannot guarantee the absence of microorganisms, when they are present in large numbers in raw material or due to post-pasteurization contamination. Examination for the presence and number of specific micro-organisms is, therefore, an integral part of any quality control or quality assurance plan. This present work aimed to investigation of microbial safety and consumer acceptability of soy-tigernut milk beverage.

MATERIALS AND METHODS

Materials

Two of the local raw materials (Soybean and Tigernut) were purchased from Owerri Main Market and Ama-Hausa Market respectively in Owerri. Sucrose (sweetener) and food grade emulsifier was purchased from De-Cross Ltd. Lagos. Sterile pre-formed plastic bottles were obtained from Industrial Layout for packaging and storage.

Preparation of Soymilk

Soymilk was prepared by modified method described by Belewu and Belewu[11]. One kilogram (1kg) of soybeans was soaked for 12hrs in a 3L of warm distilled water. It was later blanched with 6L of water (65oC) for about 5 minutes so as to inactive lipoxygenase and other anti-nutritional factors. The lanched beans were drained, dehulled and ground with 750ml of portable water in a Q-Link auto-clean blender (Model – 365XG). The resulting slurry was filtered through a muslin cloth and the extract (milk) obtained was stored in a sterile white container for further processing. The flow chart for soymilk production is shown in figure 1.

Soybean Seeds
↓
Sorting and Washing
↓
Soaking (12 Hours)
↓
Blanching (for 5 Minutes)
↓
Draining
↓
Dehulling
↓
Grinding/Milling
↓
Diluting with Water (1:3)
↓
Sieving/Filtering
↓
Cooling
↓
Soymilk (Unpasteurized)

Figure 1: Flow Chart for Unpasteurized Soymilk Production (SMD)

Tiger-Nut Milk Preparation

The method described by Udeozor[12] (Figure 2) was modified for the extraction of tiger-nut milk. One kilogram (1kg) of the fresh tiger nuts was manually sorted and cleaned to remove foreign particles and unwanted materials. The nuts were soaked in portable water at ratio nut: water (1:3) overnight. The soaked nut was milled into slurry using fabricated attrition several times with addition of 3L of water. The slurry was pressed using muslin cloth to extract the milk. The extracted liquor homogenized using Q-Link Blender (Model 365XG), rapidly cooled and stored in a white sterile 4L plastic container.

Production of Soy-Tigernut Milk Beverage (STMB), Packaging and Storage

Unpasteurized Soy Milk (SM) and Tigernut Milk (TM) were mixed together using varying proportion of 50:50 (SM:TM),90:10 (SM : TM),10:90 (SM : TM),80:20 (SM : TM), 20:80 (SM : TM) respectively. Two control samples (pasteurized) were made from a single milk extract from soybean and tigernut respectively. Each blend and single mix was sweetened with sugar syrup of 4% suspension solution of sucrose in water and final emulsified with lecithin. The resultant mixture was homogenized at maximum speed in a Q-Link Blender (Model 356 XG) for 10 minutes. The product was pasteurized at 65^{0}C for 30 minutes and bottled in PET sterile performed bottles and re-sealable nylon 66 respectively and stored for two (4) weeks under freezing, ambient and refrigeration conditions. The Figure 3 shows the flow chart for the preparation of soy-tiger nut milk beverage.

Storage System of Soy-Tigernut Milk Beverage

The two most rated sample blends (sensory evaluation) were packaged in a PET pre-form bottle and labeled accordingly storage condition. Samples were stored under three different storage conditions namely: tropical ambient temperature (27 ± 4^{o}C); refrigeration (4^{o}C) and freezing (-18^{o}C) for period of four (4) weeks. Sensory examination was done at the point of production while microbiological assessment was done at every seven (7) day interval during the storage period.

Fresh Tiger Nuts
↓
Sorting and Washing
↓
Soaking (1:3 Nut-Water overnight)
↓
Grinding/Milling (with addition of 3L water)
↓
Extracting/Sieving (using cheese cloth)
↓
Homogenizing
↓
Cooling
↓
Bottling (for further use)
↓
Tigernut Milk

Figure 2: Flow Chart for Tigernut Milk Drink Production

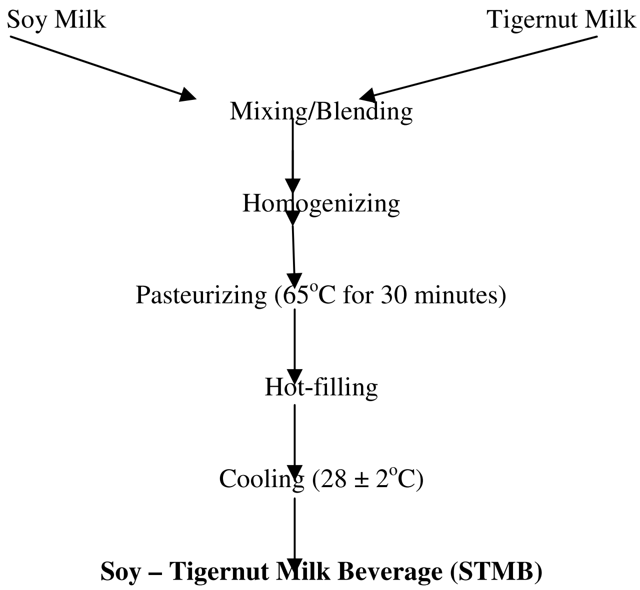

Figure 3: Flow Chart for the Preparation of Soy – Tigernut Milk Beverage (STMB)

MICROBIAL EVALUATION

Determination of Microbial Population

Microbial counts were done according to ICMSF [7] on each under investigation. Ten grams (10ml) of sample content was dispersed in 90ml of sterile distilled water, homogenized by shaking vigorously and further diluted up to 10^6. An aliquot portion (0.1ml) of the 6th dilution was inoculated in duplicate onto surface dried-nutrient and MacConkey agar. A 0.1ml of the 4th dilution was transferred in duplicate on Sabouraud dextrose agar. The plate was spread evenly using a sterile spreader and incubated for 48 hours at 28°C for heterotrophic fungi and at 37°C for 24 hours for bacterial and coliforms [13 - 14].

Identification of Bacteria and Fungi Isolates

Bacterial colony count was done using the Gallenkamp electric colony counter. A magnifying lense was used in counting fungal colonies. The identity of the bacteria isolates was determined based on the colonial, microscopic and biochemical characteristics [14 – 15]. The characteristics of the bacterial isolates was matched against those in Bucharian and Gibbon [16] and those of the yeast were matched with features presented in Harrigan and McLance[15]. Cultures of mould isolated were identified based on macro and micro morphology, reserve and surface coloration of colonies grown on SDA [15].

Microscopic Examination of Microbes

The traditional method in the microscopic examination of bacteria in the laboratory is the Gram's staining method. The description of the staining method was extracted from Cheesbrough [14], and the method for the microscopic study of fungi was extracted from Harrigan and McLance[15].

Sensory Evaluation

Consumer-oriented test was conducted to determine produce acceptability using scoring test with the aid of 9-points hedonic scale. The sensory characteristics of the product such as colour, taste, aroma, mouth-feel and general acceptability were examined by a team of twenty (20) semi-trained panelists drawn from staff of the college who are familiar with the product. Sample presentation was randomized and presented to each panelist at the same time using identical containers. Sensory Booth was fitted with lightening that support visual judgment of the panelist on colour attribute of the new product. Each panelist was asked to score each coded sample based on a nine-point hedonic scale.

Statistical Analysis

The data obtained from different analyses were subjected to various statistical analyses which include simple descriptive mean, standard deviation and Analyses of Variance (ANOVA), while Duncan's test was used to separate the means and correlation among the sample's attributes examined using SPSS 17.0 Software Inc.

RESULTS AND DISCUSSION

Sensory Evaluation of Soy-Tigernut Milk Beverage

The result of sensory characteristics (Table 1) showed significant variations (P<0.05) on all the organoleptic properties studied on milk beverage. The colour varied among the milk blends. The sample blends of 10:90 and 20:80 (Soy-tigernut) had scores 7.47 and 7.33 were the samples with most preferred colour. The perception of consumers (panelists) on the aroma, taste and mouth-feel (texture) equally varied among the milk blend and as well as from the control sample tiger milk and soy milk respectively. Samples $STMB_1$, $STMB_3$, $STMB_5$ and $STMB_6$ compared well (P>0.05) but differed significantly (P<0.05) from $STMB_2$, $STMB_4$ and $STMB_7$. Generally, the overall acceptability of the samples differed significantly from the two control samples (tiger milk and soy milk). The samples containing 80 and 20% tigernut milk were the most rated milk blends by the panelists.

Table 1:Mean Score of Sensory Characteristics of Soy – Tigernut Milk Beverage

Blend Milk Beverage	Colour of Sample	Aroma of Sample	Taste of Sample	Mouth-Feel	Overall Acceptability
$STMB_1$	7.73^{b}	7.27^{b}	6.73^{bcd}	7.40^{bc}	7.26^{bc}
$STMB_2$	6.13^{a}	5.87^{a}	5.47^{a}	6.13^{a}	6.13^{a}
$STMB_3$	4.47^{b}	7.33^{b}	7.07^{d}	7.60^{bc}	7.47^{b}
$STMB_4$	6.00^{a}	6.53^{ab}	6.00^{ab}	6.93^{bc}	6.73^{a}
$STMB_5$	7.33^{b}	6.40^{ab}	5.93^{ab}	6.67^{ab}	8.67^{ab}
TMB	8.13^{b}	7.33^{b}	6.93^{cd}	7.87^{c}	7.73^{a}
SMB	6.0^{a}	6.200^{a}	6.13^{abc}	6.67^{ab}	6.00^{a}
LSD	0.58787	0.64521	0.56470	0.6333	0.6492

Mean score not followed by the same letters in a column are significantly different (P<0.05)

<u>Key:</u>

$STMB_1$ = 50:50 Proportion of Soy – Tigernut Milk Beverage Blend
$STMB_2$ = 10:90 Proportion of Soy – Tigernut Milk Beverage Blend
$STMB_3$ = 80:20 Proportion of Soy – Tigernut Milk Beverage Blend
$STMB_4$ = 20:80 Proportion of Soy – Tigernut Milk Beverage Blend
$STMB_5$ = 90:10 Proportion of Soy – Tigernut Milk Beverage Blend
TMB = 100% Tigernut Milk Beverage
SMB = 100% Soy Milk Beverage

Microbial Quality of Soy – Tigernut Milk Beverage (STMB)

Total plate and coliform counts of bacteria in soy-tiger milk beverage was presented in Table 2. The total plate and caliform counts (cfu/ml) of bacteria on the soy-tigernut milk beverage samples are presented in Table 4. The mean total microbial count (cfu/ml) among the blends varied and ranged from $0.2 \times 10^6 – 1.2 \times 10^5$ and $0.3 \times 10^6 – 0.7 \times 10^4$ on samples contained 90 and 80% proportions of tigernut milk extract respectively. The indication from the result showed that the values of total aerobic plate counts and coliform were below the 10^6cfu/ml and 10cfu/ml safety limit respectively. The safety limit for beverage drinks [7, 17] reported that the presence of *E. coli* in food material is an indicator of faecal contamination through the use of water during processing and preparation of food. European Commission [4] stated that microbiological quality of raw materials involved in food processing may partly determine the safety of food products and the shelf-life. It should be assumed that any raw material entering a food processing firm is a potential source of microbial contamination. However, the values of total plate and zero coliform counts which are within the safety limit discovered in the work may be due to hygienic practices observed during preparation of the beverage milk extract (soybean and tigernut) and as well as during milk beverage formulation.

Table 2:Total Plate and Coliform Counts of Bacteria in Soy Tiger Milk Beverage

Sample	Type of Analysis	10^{-3}	10^{-5}	Cfu/ml10^{-3}	Cfu/m10^{-5}
Sample A	Total Bacteria Count	12	2	1.2×10^5	0.2×10^6
Sample A	Coliform Count	N.G	N.G	N.G	N.G
Sample D	Bacteria Count	7	3	0.7×10^4	0.3×10^6
Sample D	Coliform Count	N.G	N.G	N.G	N.G

Note N.G = No Growth

Total Heterotrophic Count and Characteristics of Fungi Isolate

The result of total heterotrophic count and characteristics of fungi isolate are presented in Table 3. The cfumL^{-1}

of the heterotrophic varied among the sample blends and the values ranged from 0.4 X 10^3cfu/ml to 1.1 X 10^2cfu/ml and 0.2 X 10^5cfu/ml to 0.5 X 10^5cfu/ml for different several dilution factors (10^3 and 10^5) respectively. The fungi suggested in the isolates were *Fusarium spp, Saccharomyces carevisae* and *Mucor spp.*

Table 3: Total Heterotrophic Count and Characteristics of Fungi Isolate from Soy-Tigernut Milk Beverage

Sample	Total Plate Count cfu/ml (10^3)	Total Plate Count cfu/ml (10^5)	Colonial Property	Microscopic Property	Identity
$STMB_1$	1.0 X 10^2	0.3 X 10^5	Short slender cotton like mycelia with pigment	Curved sickle shaped irregular branching with thick conidial cell wall	*Fusarium spp*
$STMB_3$	1.1 X 10^2	0.5 X 10^5	Small circular moist cream colonies	Large grain positive spherical and oval budding cells	*Saccharomyces cerevisae*
$STMB_4$	0.4 X 10^3	0.2 X 10^5	Short white flomantoes mycelium radiating	Non septate hyphae. Sporangio there is septate and spore enclosed in sporangium	*Mucor spp*
$STMB_5$	0.8 X 10^3	0.3 X 10^5	Small circular moist cream colonies	Large strain positive spherical and oval budding cells	*Saccharomyces cerevisae*

$STMB_1$ = 50:50
$STMB_3$ = 10:90
$STMB_4$ = 80:20
$STMB_5$ = 20:80

Effect of Storage Conditions on Microbial Load of Soy – Tiger Milk Beverage

The total viable plate count (cfu/ml) of soy-tigernut milk beverage as affected by length and conditions of storage are presented in table 4. The result showed no visual difference in the total plate count (0.2 X 10^6 – 0.3 X 10^6cfu/ml) at first day. This may be due to the effect of good manufacturing practice and hygienic conditions observed during the preparation period. The microbial load increased with length of storage. Under ambient, the total plate count increased to 1.7 X 10^4 (cfu/ml) and the growth was too numerous to count (TNC) at the third week (21 days) of storage. At the third week, samples under refrigeration and freezing recorded maximum microbial load of 0.5 X 10^9 – 0.6 X 10^9cfu/ml and 0.3 X 10^9cfu/ml respectively. The observation from this result may be attributed to the effect of storage condition especially temperature and relative humidity of the environment of the storage system. Loss and Hotchkiss [(18)] stated that there are several intrinsic and extrinsic factors that influence the growth of microorganisms in food (especially fruit, vegetables and dairy products). The factors include product temperature, product-to-headspace, gas volume ratio, initial microbial loads and type of flora, packaging, barrier properties, stogie condition and biochemical composition of the food. All interact to determine the degree to which the microbial quality and safety are enhanced. However, practical food storage temperatures, packaging configurations, especially the product-to-headspace volume ratio, play a major role in determining the magnitude of microbial inhibition. Therefore, the results from this work indicates the potential and the possibility of soy-tiger product shelf-life extension up to three (3) weeks under ambient condition especially in the rural areas where lack of adequate storage facility and epileptic electricity supply are the major challenges. Also, this work provided information on the use of refrigeration and freezing as alternative means of storage especially where large volume of product are involved (commercially).

Table 4: Total Plate Counts of Soy-Tiger Milk Beverage under Three Storage Conditions

Storage Period/Time (Day)	Ambient ($STMB^3$)	Condition ($STMB^5$)	Refrigerator ($STMB^3$)	Condition ($STMB^5$)	Freezer ($STMB^3$)	Condition ($STMB^5$)
0	0.2 X 10^6	0.3 X 10^6	0.2 X 10^6	0.3 X 10^6	0.2 X 10^6	0.3 X 10^6
7	0.8 X 10^5	0.6 X 10^5	0.3 X 10^5	0.4 X 10^5	0.3 X 10^5	0.3 X 10^5
14	1.7 X 10^4	1.4 X 10^4	0.5 X 10^5	0.5 X 10^5	0.3 X 10^3	0.3 X 10^5
21	TNC	TNC	0.5 X 10^9	0.6 X 10^9	0.3 X 10^9	0.3 X 10^9

$STMB_3$ = 10:90% Soy – Tigernut Milk Blend
$STMB_5$ = 20:80% Soy – Tigernut Milk Blend
Note: TNC = Too Numerous to Count

Biochemical Characteristics of Bacteria Isolates from Soy-Tigernut Milk Beverage

The characteristics of bacterial isolate from soy-tigernut milk beverage are presented in Table 5. The result showed the presence of *Streptococcus spp., Straphylaoccus spp.* and *Bacillus spp*among the pathogenic organism of public health and non-public health concern. FDA [17] reported that the presence of *Bacillus spp* above 10^6cfu/ml in a food is an indicative of active growth and proliferation of the organization and consistent with the potential hazard. The result was further supported by total plate count obtained during the period of storage in different conditions that gave a maximum (0.6 x 10^9 cfumL^{-1}) at fourth week under refrigeration condition. However, these results indicate the microbial safety of stored milk beverage blends during shelf-life.

Table 5: Biochemical and Carbohydrate Fermentation Test of Bacterial Isolate

Calonia	Catalase	Oxidase	Coagula	Indole	Methyl	Voges	Citrate	H2S	Nitrate	Urea	Glucose	Sucruse	Lactose	Fructose	Maltose	Mannos	Identity of Isolate
A_1	-	-	-	-	+	-	+	-	+	-	+	+	+	-	+	-	Streptococcus spp
A_2B	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	Staphylococcus spp
B_1C	-	-	-	-	+	-	+	-	+	-	+	+	+	-	+	-	Streptococcus spp
B_2C	+	-	-	-	-	+	+	+	+	-	+	+	-	-	-	+	Bacillussspp

Key: += Positive, - = Negative

Conclusion

This work sought to determine the effect of blending and storage condition on microbial quality and sensory characteristics of soy-tigernut milk beverage blend. The 10:90% and 20:80% ratio soy-tigernut milk blends were the most preferred, when compared the mean scores of the general acceptability of the sample blends. The results obtained from the effect of storage condition on the milk beverage blends showed that samples stored at freezing and refrigeration temperature condition shows/has minimal total aerobic plate counts than those at ambient condition. Again, the absence of coliform bacteria from the samples signifies microbial safety of the formulated soy-tigernut milk beverage blends.

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