

Production and Proximate Composition of Yoghurts from Tiger nuts and Soybean using Lactic Acid Bacteria Starter Cultures

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Abstract: Traditionally, yoghurt is fermented whole milk. It is believed to possess nutritional and therapeutic properties. Appropriate aliquot of commercially prepared yoghurts was sourced and used to isolate LAB bacteria using standard procedures. The morphological characteristics of the isolates were studied and recorded. They were later stored in MRS agar slants at 4°C in the refrigerator for further use. Biochemical and sugar fermentation tests were carried on the isolates and the isolates were identified as *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. They were then inoculated into extracted, sterile tiger nut and soy milks for the production of yoghurts. For pH, it was observed that the highest pH at zero hour was 6 from MILK COM while the lowest after 8 hours of fermentation was 3.6 from both TGN LAB and TGN COM respectively. For temperature, 29.0°C was the lowest temperature at the beginning of the fermentation from MILK LAB while the highest temperature at the end of the fermentation was 31.5 °C from TGN LAB, TGN COM, SOY LAB, SOY COM and MILK LAB respectively. The protein content of SOY LAB yoghurt (3.69) was the highest while those of MILK LAB and MILK COM (statistically the same) were the lowest. TGN LAB yoghurt had the lowest (3.09) fat content while MILK LAB (Control 1) had the highest (3.87) value. The fiber content of the four samples produced from soy and tiger nut milks were of the same value statistically while MILK LAB and MILK COM have zero fibre. The lowest carbohydrate content (3.41) was found in yoghurt made from SOY LAB while the highest value (4.90) was in TGN LAB yoghurt. Yoghurt made from TGN LAB has the highest acceptability (7.37). Yoghurts made from soy and tiger nut milks have higher carbohydrate and protein contents than those made from animal milk. They also possess better nutritional values such as lower fat and higher fibre contents than the dairy yoghurts. Yoghurt made from tiger nut milk had best acceptance to the panelists than the dairy yoghurts.

Key words: Fermentation, LAB, starter culture, Soy milk, Tiger Nut Milk, yoghurt

INTRODUCTION

Yoghurt is a drink produced when bacteria ferment milk (International Standard Organization, 2003). It is believed to have therapeutic and nutritional values (Hughes and Hoover, 1991). Several efforts have been made to produce an alternative to milk and milk products from leguminous plants (Rao *et al.*, 1988; Terna and Musa, 1998). In the developing countries and indeed in sub-saharan Africa (except east Africa), the production of milk and its products is limited, rare and financially tasking (Fashaken and Unokiweri, 1992). The shortages have so much adverse effect on the protein intake of the young old and old persons. Yoghurt and the related fermented milk products have been very popular for a long

time in Mediterranean countries North Africa), (the Balkans, in central and southwest Asia (Turkey, Mongolia, Iraq, Syria and Iran) and in central Europe too. In many of the above countries, people still manufacture yoghurt using the traditional methods. Although the consumption has been increasing not only in European countries, but also in the United States. This has made it possible for the production at industrial-scale. At the moment, new forms of fermented milk are in place which are made by the addition of fruits or with flavouring materials, rich in vitamins. They also contain selected intestinal bacteria like *Bifidobacterium*, *Lactobacillus acidophilus* and some other species (Kurmman, 1984; Puhan, 1988).

Some milk and milk products based on legumes have been developed in efforts to increase the availability of milk-like products, mainly in areas where milk is in limited supply. Because legumes are important sources of good inexpensive protein, addition of imitation milk products from them may help to reduce the problem of protein malnutrition (Rao *et al.*, 1988).

Lactic acid fermentation of legume based milks has been used as one of the ways to extend the storage time of the product, create variety, enhance the nutritional value and also increase the acceptance of the product by consumers. Products from yoghurt have been produced by some people using soybeans (Terna and Musa, 1998). The production of *waragusi* (a soft unripe cheese-like product from water melon milk) an analogue of *warankasi* (unripe cheese product from cow milk) have been reported by Fashakin and Unokiwedi (1992). Since tiger nut (family: Liliaceae) and coconut (*cocos nucifers*, family: Palmae) grow extensively in Nigeria and are eaten as snacks usually for the pleasure of it, they may be good sources of raw material for the development of milk-like products.

Tiger nut and soybean apart from reducing the risk factors found with dairy milk, are also important for strong bones, muscles, tissue repairs, growth and development of the body. They are also rich in vitamins E and C. They are also recommended for people who have challenges with indigestion, flatulence and diarrhoea because they contain digestive enzymes like catalase, amylase and amylase. The high content of oleic acid affect cholesterol thus preventing heart attacks, thrombosis and activates blood content of soluble glucose. The plant based milk also gives the body enough Vitamin E which is essential for fertility in both genders. The milk has a relatively high antioxidant capacity which can protect the

body against malnourishment. The aim of this work was to produce yoghurts from Tiger nuts and Soybean using Lactic Acid Bacteria (LAB) Starter Cultures and determining their proximate compositions.

MATERIALS AND METHODS

Collection of Soy bean and Tiger nut samples

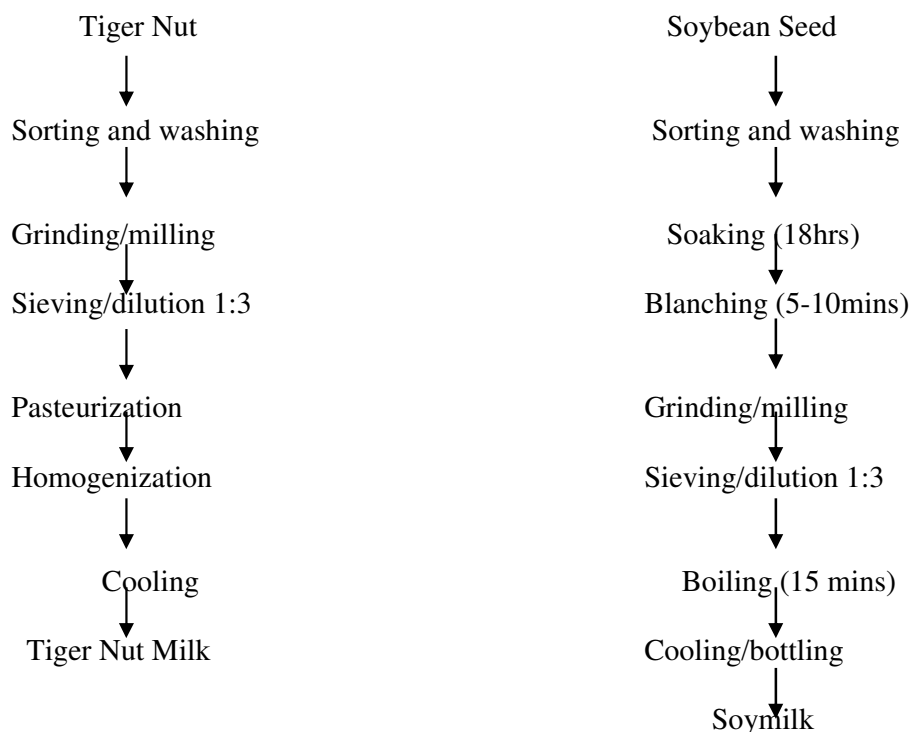
The soybean seeds and tiger nuts were purchased from markets within Umuahia metropolis. Two brands of commercial powdered milk were also purchased for the production of yoghurt. Laboratory and other facilities were provided by CesLAB Analytical Laboratories, Umudike. The starter cultures were isolated from commercial yoghurt samples and LAB commercial starter culture.

Isolation and Identification of Lactic Acid Bacteria

The isolation of LAB from procured commercial yoghurt was carried out using the method discussed by Nyanga *et al.* (2007) by inoculating 0.1 ml of the commercial yoghurt by direct plating on De Man Rogosa Sharp (MRS) in duplicates. It was then spread with a sterile bent glass rod. The culture plates were labeled and incubated in an electric incubator (Goodcare England) at 37°C and for 48 hours. The morphological characteristics of the isolates were observed and recorded. The isolates were sub-cultured on MRS Agar and later stored in MRS agar slants at 4°C in the refrigerator until required for further use.

Extraction of Tigernut and Soybean Milk

The direct water extraction technique according to Amanze and Amanze (2011) was used. The milk extracts of the test (soybean seed and Tiger nut) were produced separately using the flow charts below and later mixed in the appropriate ratios to use in yoghurt production.



Selection of Starter Cultures

The identified LAB were screened and selected based on the following properties:

Determination of Lactic Acid Production

One loopful of 24 hrs old standardized culture of the LAB isolates containing 10^6 CFU/mL were inoculated into 20 mL of MRS broth, and incubated for 24 hrs. The production of lactic acid was determined by titrating 20 mL of MRS broth containing Lactic acid contents = $\frac{M1 \text{ NaOH} \times N \text{ NaOH} \times M.E. \times 100}{\text{Volume of sample}}$

Where M1 NaOH = Volume of NaOH used,

N NaOH = Normality of NaOH solution,

M.E. = Equivalence Factor

Determination of Diacetyl Production

One loopful of 24 hrs old standardized culture of the LAB isolates containing 10^6 CFU/mL was inoculated into 25 mL of MRS broth, and incubated for 24 hrs. Diacetyl production was determined by transferring 25 mL of MRS broth containing LAB

LAB isolates after 24 hrs with 0.1M of NaOH and 1 mL of phenolphthalein indicator (0.5% in 50% alcohol). The titratable acidity was calculated as lactic acid (% v/v). The milliliter of 1N NaOH can be estimated as 90.08 mg of lactic acid. The lactic acid was calculated according to AOAC (2000).

isolates after 24 hrs into 100 mL of conical flasks. Both flasks were titrated with 0.1N HCl to a greenish yellow end point using bromophenol blue as indicator (AOAC, 1990). Hydroxylamine was used for residual titration.

$$AK = \frac{(b-s) (100E)}{W}$$

Where

K = Percentage of diacetyl

B = No of mL of 0.1N HCl consumed in titration of sample;

E = Equivalence factor

W = Volume of sample

S = No of mL of 0.1N HCl consumed in titration of samples.

Production of Yoghurt with Selected Starter Cultures

The yoghurt samples were prepared according to the method of Aforijiku *et al.* (2020) with slight modification. The inoculum size (10^6 CFU/mL) of the selected LAB starter cultures were obtained using Mcfarland standard 0.5. However, sterile glass bottles containing 100 mL of raw tiger nut and soy milk samples were pasteurized at 85°C for 30 minutes with the use of a water bath, and cooled to 37°C. They were inoculated with 1.0 mL of the starter cultures containing inoculums size of 10^6 CFU/mL. Each 100 mL of pasteurized tiger nut milk and soy milk was inoculated with selected starter cultures of inocula size of 10^6

$$\% \text{ total solid} = \frac{W_2 - W_1}{V} \times 100$$

W = weight of sample

W_1 = weight of empty moisture can

W_2 = weight of moisture can with sample dried to constant weight

% Moisture content = 100 - % T.S (Total solid)

Determination of ash content

This was determined using the furnace incineration gravimetric method of Harold *et*

$$\% \text{ ash} = \frac{W_2 - W_1}{V} \times \frac{100}{1}$$

W = weight of sample analysed

W_1 = weight of empty crucible

W_2 = weight of crucible and ash from the sample

Determination of fat content

The Rose-Gotish gravimetric method of Kurt *et al.* (1996) was used. Five grams of the sample was treated with different petroleum based fat solvents in a Rose-

CFU/mL at equal proportion of 1:1. After inoculation, the contents were thoroughly mixed, and incubated at 35°C for 8 hrs using a thermostatically controlled water bath, and cooled to 4°C. However, the yoghurt samples were stored at 4°C (cold storage).

Determination of Proximate Composition of Yoghurts

The produced yoghurt was analyzed for proximate composition using conventional methods as follows:

Determination of Total solids/moisture content

The oven drying gravimetric method (Bradley, 2010) was used. The total solid and moisture content was calculated as shown below:

al. (1991). The ash content was calculated as shown below:

Gotish apparatus until all the oil fat was extracted. The weight of extracted fat was calculated by difference in weight using the formula below.

$$\% \text{ fat} = \frac{W_2 - W_1}{V} \times \frac{100}{1}$$

W = weight of sample analysed

W₁ = weight of empty extraction flask

W₂ = weight of flask with oil (fat) extract

Determination of protein content

Protein content was determined using the Kjeldahl method (Halold *et al.*, 1991). The formula below was used to calculate the nitrogen and protein contents accordingly.

$$\% N_2 = \frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{NF}{V_a} \times T - B$$

W = weight of sample analyzed,

N = normality (conc.) of titrant acid solution,

N_f = total volume of digest per sample,

V_a = volume of digest distilled,

T = titre value of sample,

B = titre value of reagent blank

Determination of crude fibre content

The Weide method of Halold *et al.* (1991) was used. The crude fibre content was calculated using the formular:

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{V} \times \frac{100}{1}$$

Where: W₂ = weight of crucible and sample after boiling and drying,

W₃ = weight of crucible and sample after burning to ashes,

W = weight of sample analyzed

Determination of carbohydrate content

Carbohydrate content was calculated directly by differences between 100 and the sum of other proximate parameters (Halold *et al.*, 1991). The formula below was used:

$$\% \text{ CHO} = 100 - \% [\text{protein} + \text{fat} + \text{fibre} + \text{ash} + \text{moisture}]$$

Sensory evaluation

A 9-point hedonic scale was used to measure the sensory qualities (aroma, appearance, taste, texture, general acceptability) of the product (Larmond, 1977) using 11-member untrained panelist comprising of students of Microbiology Department that were familiar with yoghurt.

The scale used was:

1-Disliked extremely,

2-Disliked very much,

3-Disliked moderately,

4-Disliked slightly,

5-Neither liked nor disliked,

6-Liked slightly,

7-Liked moderately,

8-Liked very much,

9-Liked extremely.

Statistical Analysis

Experiments were performed in triplicate.

The data were analyzed using one-way analysis of variance (ANOVA) and by SPSS

18.0. (P < 0.05) was used for determining and comparison of significant difference of the proximate analyses results.

RESULTS

The results obtained from the various experiments and analysis of the different yoghurt samples are shown in the tables below. The characteristics of the three bacterial isolates (Table 1) from microbiological examination of the commercial yoghurt sample. They are *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophiles*.

In Table 2, the changes in fermentation parameters of yoghurt samples which includes pH, temperature, optical density and titratable acidity are presented. For pH, it was observed that the highest pH at zero hour was 6 from MILK COM while the lowest after 8 hours of fermentation was 3.6 from both TGN LAB and TGN COM respectively. For temperature, 29.0°C was the lowest temperature at the beginning of the fermentation from MILK LAB while the highest temperature at the end of the fermentation was 31.5 °C from TGN LAB, TGN COM, SOY LAB, SOY COM and MILK LAB respectively. For Optical Density, the lowest value at the beginning of fermentation was 1.133 from MILK LAB and MILK COM respectively while the highest value after fermentation was 1.384 from TGN COM. For Titratable Acidity, the lowest value at the beginning of the fermentation was 0.52 from MILK LAB

while the highest value after fermentation (1.38) was from TGN LAB and TGN COM respectively.

The result of the proximate composition of the yoghurt samples is shown in Table 3. The protein content of SOY LAB yoghurt (3.69) was the highest while those of MILK LAB and MILK COM (statistically the same) were the lowest. For fat content, TGN LAB was the lowest (3.09) while MILK LAB (Control 1) was the highest (3.87). The fiber content of the four samples produced from soy and Tiger Nut milks were of the same value statistically while MILK LAB and MILK COM was found to be fibre-zero. SOY COM yoghurt has the lowest ash content (1.83) while the highest content was found in MILK LAB yoghurt (2.96). The lowest moisture content recorded was in SOY LAB yoghurt (86.74) while the highest value was found in SOY LAB yoghurt. The lowest carbohydrate content (3.41) was found in yoghurt made from SOY LAB while the highest value (4.90) was in TGN LAB yoghurt.

In Table 4 is presented the result of the sensory evaluation of the yoghurt samples. Yoghurt made from TGN LAB has the highest acceptability (7.37) while that made from TGN COM had the lowest acceptability (6.16).

Table 1: Morphological and biochemical characteristics of Lactic Acid Bacteria isolates

S/N	Colony features	Microscopy	Arrangement	Gram stain	Motility	Spore	Cell shape	Urease	Catalase	Oxidase	Coagulase	Indole	Methyl-red	Voges-proskauer	Glucose	Sucrose	Lactose	Maltose	Mannitol	Fructose	Diacetyl (g/l)	Lactic acid contents	Probable Isolate
1	Round white rough raised translucent colonies	Rod shaped cocco-bacilli	Occurring singly or in chains	+	-	-	Rod	-	-	-	-	-	+	-	+	+	+	+	+	+	0.84±0.0	0.84±0.00	<i>L. acidophilus</i>
2	Rounded colonies, flat with non defined border	Rod shaped with rounded ends	Single or short chains of 3-4 cells	+	-	-	Rod	-	-	-	-	-	+	-	+	-	+	-	-	+	0.60±0.01	0.80±0.07	<i>L. bulgaricus</i>
3	Circular opalescent white colonies with well defined borders	Spherical/ ovoid cells	In pairs or chains	+	-	-	Rod	+	-	-	-	-	+	-	+	+	+	+	-	+	0.75±0.00	0.70±0.01	<i>S. thermophilus</i>

Table 2: Changes in fermentation parameters of yoghurt samples

	pH					TEMPERATURE					OPTICAL DENSITY (OD)					TITRATABLE ACIDITY (TTA)				
	0H	2H	4H	6H	8H	0H	2H	4H	6H	8H	0H	2H	4H	6H	8H	0H	2H	4H	6H	8H
TGN LAB	5.5	4.6	3.8	3.6	3.6	30.5	31.0	31.0	31.5	31.5	1.142	1.189	1.233	1.222	1.220	0.67	1.00	1.30	1.38	1.38
TGN COM	5.4	4.4	3.9	3.8	3.6	29.5	30.5	31.0	31.5	31.5	1.286	1.336	1.391	1.386	1.384	0.70	1.13	1.25	1.32	1.38
SOY LAB	5.4	4.8	4.5	4.4	4.3	30.0	30.5	31.0	31.0	31.5	1.149	1.193	1.243	1.243	1.242	0.75	0.92	1.12	1.17	1.25
SOY COM	5.5	4.8	4.5	4.4	4.4	30.0	31.0	31.0	31.5	31.5	1.147	1.194	1.240	1.240	1.240	0.68	0.92	1.10	1.17	1.22
MILK LAB (control 1)	5.9	5.5	5.2	5.1	5.1	29.0	30.0	30.5	31.0	31.5	1.133	1.176	1.221	1.220	1.219	0.52	0.60	0.82	0.87	0.88
MILK COM (control 2)	6.0	5.5	5.3	5.2	5.2	29.5	30.0	30.5	31.0	31.0	1.133	1.179	1.242	1.225	1.224	0.53	0.60	0.77	0.87	0.88

Key:

TGN LAB = Tigernut milk fermented with laboratory isolates, TGN COM = Tigernut milk fermented with commercial starter culture

SOY LAB = Soymilk fermented with laboratory isolates, SOY COM = Soymilk fermented with commercial starter culture

MILK LAB = Commercial milk fermented with laboratory isolates, MILK COM = Commercial milk fermented with commercial starter culture

Table 3: Proximate composition of yoghurt samples

Sample	Protein	Fat	Fibre	Ash	Moisture	Carbohydrate
TGN LAB	2.66 ^c ±0.27	3.09 ^a ±0.11	0.36 ^a ±0.04	1.97 ^c ±0.03	87.01 ^b ±0.09	4.90 ^d ±0.31
TGN COM	2.44 ^b ±0.10	3.10 ^a ±0.05	0.37 ^a ±0.02	1.98 ^c ±0.07	87.31 ^b ±0.13	4.81 ^d ±0.03
SOY LAB	3.69 ^d ±0.10	3.85 ^b ±0.01	0.39 ^a ±0.03	1.92 ^b ±0.06	86.74 ^b ±0.14	3.41 ^a ±0.18
SOY COM	3.63 ^d ±0.10	3.81 ^b ±0.04	0.38 ^a ±0.03	1.83 ^a ±0.13	86.86 ^a ±0.05	3.44 ^a ±0.12
MILK LAB (Control 1)	1.85 ^a ±0.10	3.87 ^c ±0.03	0.00±0.00	2.96 ^c ±0.16	87.45 ^b ±0.09	3.86 ^b ±0.26
MILK COM (Control 2)	1.75 ^a ±0.06	3.85 ^b ±0.01	0.00±0.00	2.56 ^d ±0.06	87.54 ^b ±0.07	4.29 ^c ±0.11

Values with different superscripts down the columns are significantly different at P≤0.05)

Key:

TGN LAB = Tigernut milk fermented with laboratory isolates, TGN COM = Tigernut milk fermented with commercial starter culture, SOY LAB = Soymilk fermented with laboratory isolates, SOY COM = Soymilk fermented with commercial starter culture, MILK LAB = Commercial milk fermented with laboratory isolates, MILK COM = Commercial milk fermented with commercial starter culture

Table 4: sensory evaluation of yoghurt samples

Sample	Appearance	Aroma	Taste	Texture	Acceptability
TGN LAB	7.27±0.03	7.10±0.01	7.35±0.05	7.29±0.02	7.37±0.06
TGN COM	7.27±0.03	7.58±0.03	7.07±0.06	7.16±0.05	6.16±0.05
SOY LAB	6.65±0.05	6.55±0.05	6.35±0.05	6.55±0.05	6.74±0.74
SOY COM	6.29±0.02	6.84±0.05	7.03±0.06	7.03±0.06	6.96±0.06
MILK LAB (Control 1)	6.45±0.05	6.71±0.10	6.65±0.05	6.74±0.05	6.65±0.05
MILK COM (Control 2)	6.84±0.05	6.39±0.02	6.84±0.05	6.84±0.05	6.74±0.05

Key:

TGN LAB = Tigernut milk fermented with laboratory isolates, TGN COM = Tigernut milk fermented with commercial starter culture, SOY LAB = Soymilk fermented with laboratory isolates, SOY COM = Soymilk fermented with commercial starter culture, MILK LAB = Commercial milk fermented with laboratory isolates, MILK COM = Commercial milk fermented with commercial starter culture

DISCUSSION

The occurrence of *Lactobacillus bulgaricus*, *L. acidophilus* and *Streptococcus thermophilus* in the commercially prepared yoghurt samples is in agreement with the result of Olawuyi, (1987). Commercial yoghurts are usually prepared using TGN LAB yoghurt recorded the least pH value (3.6) at the end of the fermentation. Low pH value and high titratable acidity were also reported by Almelda *et al.* (2007) and Gesinde *et al.* (2008). This could be due to the accumulation of some organic acids from the activities of Lactic Acid bacteria starter cultures. The low pH recorded in TGN LAB is an indication that the yoghurt

Lactobacillus bulgaricus and *Streptococcus thermophilus* (Masci, 2013; Vasiee *et al.*, 2014). However, LAB such as *L. amylovorus*, *L. helveticus*, *L. amylophilus*, *L. casei*, *L. brevis* and *L. plantarum* could also be used for yoghurt production (Mohammed *et al.*, 2016).

will be protected from spoilage and pathogenic microorganisms thereby ensuring safety of the yoghurt. These organisms have the tendency to produce antimicrobials such as lactic acid and diacetyl which inhibit pathogens, produce desirable characteristics flavor and also increase the organoleptic quality of yoghurt (Mohammed *et al.*, 2016).

The proximate composition of the various yoghurt samples revealed a significant increase in ($P \leq 0.05$) in the protein content of the various yoghurt samples with the protein content of SOY LAB yoghurt the highest. This statement had been reported by Aforijiku *et al.* (2020) who revealed that pasteurized milk inoculated with lactic acid bacteria starters could have better crude protein and fat contents. This indicates that this yoghurt can be used as a good source of protein and a possible replacement for animal protein especially in the rural areas where the cost of animal protein is high. This finding is in agreement with the result of Akoma *et al.* (2000); Bamishaiye and Bamishaiye (2011); Gambo and Dau (2014). The high moisture content (86.74 – 87.54) of the yoghurt samples recorded in this work implies high water activity which supports microbial growth consequently reducing the shelf life of the yoghurt samples (Ajai *et al.*, 2012). This means that the yoghurt should be consumed immediately after production to avoid spoilage from possible contaminant unless they are refrigerated. The carbohydrate content showed a fairly high value as found in yoghurt made from TGN LAB. The carbohydrate content of the two different milk from plant origin show that the yoghurt can serve as a source of energy for the body and also its profile does not include lactose which makes it suitable and ideal for lactose intolerant individuals (Nelson *et al.*, 1971).

Yoghurts from Tiger nut and soy milk were found lowest in fat content than the dairy milk yoghurts. This means that dairy yoghurts high fat content contributes to hypercholesterolemia and possible cardiovascular diseases (Warenjo *et al.*, 2004). This condition could be reduced by the consumption of yoghurts made for tiger nut and soy milks.

Yoghurts made from Tiger nut and soy milks had same level of fibre contents while the dairy yoghurts have no fibre. Fiber is essential for effective gastro- intestinal

functions during digestion. It could also be effective in the treatment and prevention of many diseases including obesity, diabetes and gastrointestinal disorders (Anderson *et al.*, 1994). High fibre content is one of the comparative advantages of plant milk over animal milk and this is another advantage that yoghurts made from tiger nut and soy milks have over dairy yoghurt. The highest ash content was found in the MILKLAB yoghurt. This is an indication of presence of high minerals for body maintenance. The high ash content in the yoghurt could be attributed to the fact that fermented food constitutes a product of microbial decomposition resulting in mineralization of higher compounds (Tamine, 1977).

Sensory evaluation indicated that yoghurt made from TGN LAB had the highest level of acceptability by the panelist. This means that the yoghurt will compete favourably for acceptability with the commercially made yoghurts among the consumers.

CONCLUSION

The findings from this study demonstrates that yoghurts made from Soy milk and Tiger nut milk have higher carbohydrate (4.90) and protein contents (2.66) than those made from animal milk (3.86 and 1.85 respectively). They also possess better nutritional values such as lower fat (3.09 as against 3.87) than the dairy yoghurts. Yoghurt made from tiger nut milk had best acceptance to the panelists than the dairy yoghurts.

RECOMMENDATION

We recommend the production and consumption of yoghurts from tiger nut and soy milks by the public due to the numerous nutritional benefits they possess. This research has made known the benefits of the yoghurts made from tiger nut and soy milks. Therefore the general public should explore these new products from soybean and tiger nut.

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