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Quality Characteristics of Probiotic (*Lactobacillus acidophilus*) Beverage from Hydrolyzed Tigernut Milk Supplemented with Beetroot Juice

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Abstract Physicochemical and sensory properties of partially hydrolysed tigernut milk and beetroot beverage fermented with probiotic *Lactobacillus acidophilus* was evaluated. Ability of the blended beverage (hydrolysed tigernut and beetroot juice) to support the growth of *L. acidophilus* was also determined. Partially hydrolysed tigernut milk produced using alpha amylase and amyloglucosidase was blended with 10, 20, and 30% beetroot juice designated as $HTNB_{10}$, $HTNB_{20}$ and $HTNB_{30}$ respectively while sample without beetroot juice (HTN_{100}) served as control. Analysis were carried out using standard analytical methods. Hydrolysis of the tigernut milk resulted in significant ($P<0.05$) decrease in starch from 29.70 - 17.14% and increase in sugar from 9.08 - 20.23 ^{0}Brix. pH significantly ($P<0.05$) decreased with time irrespective of the concentration of beetroot juice. Decrease was from 6.54 – 5.05, 6.52 – 5.38, 6.52 – 5.46 and 6.46 – 5.66 for the control (HTN_{100}), $HTNB_{10}$, $HTNB_{20}$ and $HTNB_{30}$ respectively. Titratable acidity (TTA) as % lactic acid increased respectively, from 0.19 - 0.67, 0.30 – 0.66, 0.31 – 0.69 and 0.31 – 0.42 for HTN_{100}, $HTNB_{10}$, $HTNB_{20}$ and $HTNB_{30}$. All the samples supported *L. acidophilus* growth with significant ($P<0.05$) increase from <2.00 - 6.22, 2.81 - 6.37, 2.30 - 6.94 and 2.45 - 6.29 $\log_{10}$ CFU/ml respectively, for HTN_{100}, $HTNB_{10}$, $HTNB_{20}$ and $HTNB_{30}$. Assessors' degree of likeness for the sensory attributes varied respectively, from 4.3 - 5.45, 4.8 - 5.2, 4.85 - 5.56, 5.8 - 7.0 and 5.11 - 5.74 for colour, aroma, mouthfeel, taste and general acceptability. The partially hydrolysed tigernut and beetroot beverages supported the growth of the *L. acidophilus* (6 Log_{10} CFU/ml). The physicochemical properties of beverage were of satisfactory levels and could be recommended as a potential probiotic product. The addition of 20% of beetroot juice will be recommended based on the assessors' degree of likeness.

Keywords: *Tigernut, partial hydrolysis, beetroot, Lactobacillus acidophilus, beverage, physicochemical and sensory properties*

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1. Introduction

Milk is one of nature's complete food, supplying minerals, vitamins, short chain fatty acids and several amino acids to the world [1,2]. The high cost of dairy and dairy products coupled with physiological and religious reasons for which some persons do not consume them, has necessitated researches for alternatives. Plant based milks such as soymilk, almond, coconut milk etc. are been researched on. Tigernut milk is a promising alternative and promoting its consumption will play an imperative role in the health, nutrition, and economy of many developing countries [3].

Tigernut plant (*Cyperus esculentum* L.) is a perennial grass-like plant that produces spheroid tubers cultivated in many parts of Africa, South America, Europe and Asia. It is a pale yellow kernel surrounded by a fibrous sheath and known by other names like chufa, yellow nutsedge, earth almond and ground almond [4]. In Nigeria, it is cultivated mostly by rural farmers in the northern part of the country and is known by different names as "aya" in Hausa; "aki awusa" in Igbo; "ofio" in Yoruba and "isipaccara" in Effik. This edible, sweet, nutty-flavoured tuber is an important food crop which may be eaten raw as snack, baked as a vegetable, roasted or dried and ground into flour for incorporation into many products such as ice cream, sherbet, flour for bakery products and milky drinks [4]. When blended with water and sieved, an aqueous milky

beverage called tigernut milk ('Horcha' in Spanish) [5] is produced.

Tigernut milk is very popular in Nigeria. The milk is a viscous cream coloured liquid with a very short shelf-life (48 h) when produced traditionally [6]. It has been reported to be of great health benefits. Its rich minerals content makes for bones, tissue repair, muscles, the bloodstream and body development [7]. Other health benefits are the prevention of colon cancer, coronary heart diseases, obesity, diabetes and gastro-intestinal diseases [8,9]. Tigernut has a total carbohydrate content of 47.9 – 75.88% of which starch is 17.2-39.2% [10], imparts undesirable sensory attributes such as chalky mouthfeel to the products [11].

Hydrolysis of the starch in tigernut milk is necessary to overcome processing and sensory challenges such as gelatinization during pasteurization and increased viscosity related to starch content in tigernut milk. It will also provide sugars which can be metabolised by probiotic organisms such as *Lactobacillu*s to produce a functional beverage. There are different methods of starch hydrolysis but enzymatic hydrolysis has been reported to be safest and best for the consumer and the product [11].

In developing countries like Nigeraia, beetroot (*Beta vulgaris*) known as garden, table or red beets or informally called beets is consumed in different forms [12]. It can be boiled and consumed along with salad vegetables, processed into juice or fermented into wine [13]. Beetroot has been established to possess potential health benefits owing to its content of vitamins and minerals especially inorganic nitrates [14]. Its potential in lowering blood pressure and risk of cardiovascular event in humans was reported by Hobbs *et al*., [14]; Coles and Clitton, [15]. 2012. The composition of beetroot makes it a good compliment for many drinks and beverages. Beetrrot has natural colourant that has been used as food colour in ice creams, sweets, yoghurt and other confectionaries [16], [17]. According to Banigo *et al*., [18], the inclusion of beetroot into milk-analogues like tigernut milk will harness the nutritional and flavour properties of the milk especially with the preponderance of milk drinks with strong fruit content that are often unaffordable by the average Nigerian. Blending tigernut milk with nutrient dense beetroot juice to dilute the starch instead of plain water will create a novel product with potential health benefits.

Lactic fermentation of the blend can proffer more health benefits depending on the bacterial strains and the probiotic effects. Some researchers have isolated LABs from tigernut [19,20,21]. Some studies on the isolated have revealed probiotic potentials [22]. Although products such as yoghurt and other fermented milk products contain probiotic bacteria, majority of the products in the market do not maintain recommended number (10^6) of the probiotic bacteria. Therefore, the population of probiotic bacteria in a product is an important requirement for a product to be labelled a probiotic [23]. There are no reports on the effect of lactic fermentation on the physicochemical and sensory properties of beverages formulated with blends of partially hydrolysed tigernut and beetroot. Therefore, this study was aimed at the quality characteristics of probiotic *L. acidophilus* fermented partially hydrolysed tigernut and beetroot beverage.

2. Materials and Methods

2.1. Tigernut and Beetroot

Tigernuts (*Cyperus esculentus* L.) was purchased from Mile 3 Market, Port Harcourt, while Beetroot was purchased from Fruit Garden, D-line, Port Harcourt, Rivers State, Nigeria. These samples were collected in sterilized polythene bags and transported in cool boxes containing ice blocks to the Food Microbiology Laboratory in the Department of Food Science and technology, Rivers State University.

2.2. Enzymes

Bacterial alpha amylase and amyloglucosidase were obtained from Novozymes (Switzerland AG).

2.3. Microbial Cultures and Media

Lactobacillus acidophilus (Nature source, UK) was the probiotic lactic acid bacteria used. The microbial media used were: peptone water as diluent for serial dilution, De Mann Rogosa Sharpe (MRS) agar and broth (Oxiod, UK) for growth and enumeration.

2.4. Reagents

Analytical grade reagents used included Hydrochloric acid (HCL), Calcium hydroxide $Ca(OH)_3$, Sodium hydroxide (NaOH).

2.5. Preparation of Tigernut Flour

The method described by Ade-omawaye *et al*., [24], was used in the production of tigernut flour. Fresh tigernuts were sorted, washed with distilled water oven dried on a sterile foiled tray at 60°C for 24 h and milled into powder using attrition mill. The powder was sieved using a 0.45 mm mesh size and packaged in a container. The oven dried tigernut and tigernut flour are shown in plate 1 and plate 2 respectively.

2.6. Partial Hydrolysis of Tigernut Starch

Tigernut starch was hydrolyzed following the method of Barber *et al*., [25]. Briefly, tigernut slurry from 500 g of the flour homogenised in 0.1M calcium hydroxide ($Ca(OH)_3$, after pH adjusted to 7.5 with 0.5N HCL was heat in a water bath at 45-48°C for 10 min with continuous stirring. While heating, the first, second and third stages of enzyme hydrolysis were carried out respectively, at 45-48°C for 10 min 62°C for 30 min and 92°C for 15 min with bacterial alpha amylase and amyloglucosidase were respectively added and mixed thoroughly. Thereafter, the mixture was brought to boil to inactivate the enzymes and cooled to a temperature of 57°C. The partially hydrolysed tigernut beverage was

filtered using a muslin cloth and packaged and stored in well labelled plastic containers in a refrigerator. Sugar and starch concentrations were carried out before, during and after the hydrolysis.

2.7. Preparation of Beetroot Juice

The method described by Emelike *et al*., [13] was used in the preparation of beetroot juice. Fresh Beetroot was sorted, washed with clean water to remove extraneous materials, milled, extracted under pressure, sieved, packaged and stored in the refrigerator till needed. Plate 4 showed the beetroot and its extract.

2.8. Preparation of Tigernut Milk for Probiotic Activation

The method described by Maduka *et al*., [22] was used in the production of tigernut milk. Fresh tigernuts were sorted, washed with clean water, milled, wet sieved and the extract was packaged in a clean labelled container for use in the activation of the probiotic *L. aciduphilus*. The fresh tigernut and its milk extract are shown in Figure 1b.

2.9. Formulation of Hydrolysed Tigernut-Beetroot (HTNB) Beverage Blends

Formulation of hydrolysed tigernut-beetroot beverage (HTNB) was obtained by blending different percentages of Hydrolysed tigernut beverage and Beetroot juice as shown in Table 1. The controls samples A and E were properly mixed, homogenized and pasteurized at 70^0C for 5 min.

Table 1. Blends of Hydrolysed tigernut-beetroot beverage

Sample code	Tigernut (%)	Beetroot (%)
HTN_{100}	100	0
$HTNB_{10}$	90	10
$HTNB_{20}$	80	20
$HTNB_{30}$	70	30

HTN_{100}= 100% hydrolysed tigernut beverage,
$HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice
$HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice
$HTNB_{30}$ = 70% hydrolysed tigernut beverage and 30% Beetroot juice

2.10. Activation of *Lactobacillus acidophilus* and Fermentation of Hydrolysed Tigernut - Beetroot (HTNB) Beverage

The pure culture of *Lactobacillus acidophilus* was activated in a freshly prepared MRS agar and a colony transferred with a sterile loop into pasteurized tigernut milk as described by Barber *et al*., [25]. Before pasteurization, 5% of sugar was added to bring brix of tigernut milk to 22 ^{0}Brix for faster growth and replication of the probiotic *L. acidophilus*. The inoculated milk was incubated at 42^0C for 18 - 24 h under anaerobic conditions. Thereafter, 1 ml of the tigernut milk with an inoculum size of 8 $\log_{10}$CFU/mL was used as starter for the fermentation of the hydrolysed tigernut-beetroot (HTNB) beverage beverage.

2.11. Determination of Physical Properties of HTNB beverage

2.11.1. Determination of pH and Titratable Acidity (TTA)

The pH and TTA (% lactic acid) of the beverage was determined according to the standard AOAC method [26]. Twenty (20 ml) of each sample was transferred into a beaker and the pH was determined using a pH meter (TS 625, USA) after calibration and stabilization with standard buffer of pH 4.0 and 7.0. Thereafter, 3 drops of phenolphthalein were added as the indicator and the mixture was titrated against 0.1 M NaOH. Acidity was expressed as % lactic acid with each ml of the 0.1 M NaOH equivalent to 0.09 of lactic acid.

2.11.2. Determination of Viscosity

The viscosity of the beverage was determined with the aid of a rotary digital viscometer (NDJ.8S, China) using spindle number 2 at 12 rpm. Two hundred and fifty (250 ml) of the beverage was transferred into a 25 ml beaker. The content of the beaker was introduced directly unto the rotating spindle and the value of the viscosity displayed on the LCD screen in Pa/s was recorded [27].

2.11.3. Determination of Total Soluble Solids

The total soluble solids of the beverage stored at 28±2°C and 4±2°C were determined in duplicate using Abbe 60 Refractometer and the results expressed as degree of brix (°Brix) using the procedure described by Danbaba *et al*., [28].

2.11.4. Determination of Starch Content

Starch content of the sample was determined following the method described by Onwuka, [29]. Briefly, 3 g of flour sample was mixed with cold water and allowed to stand for 1 h. Hydrochloric acid (20 ml) and of distilled water (150 ml) was added and refluxed for 2 h in a 250 ml round bottom flask. Mixture was made up to mark with distilled water after cooling and neutralizing with NaOH. Glucose content was determined using anthrone reagent. Series of glucose solution was prepared such that 1 ml contained 0.004 - 0.2 mg for the standard glucose calibration curve. For colour development, 5 ml of anthrone reagent was added to each of the standard solution and a test sample in test tubes that was allowed to boil in water bath for 20 min after thoroughly mixing. Absorbance of the cooled mixture was read at 620 nm against a blank containing only 1 ml of water and 5 ml of anthrone reagent. The concentration of the test samples was obtained from the absorbance by interpolation. The mass of the glucose was obtained by calculation involving the concentrations and dilutions made. The mass of starch was consequently expressed as the mass of glucose multiplied by 0.9.

2.11.5. Enumeration of Probiotic Starter Culture in Tigernut Beverage

The probiotic starter culture: *L. acidophilus* in tigernut beverage was enumerated following the conventional

method described by Obinna-Echem *et al.*, [30] with slight modification. A 10-fold serial dilutions of up to 10^6 was made from stock of 10 ml of sample in 90 ml of sterile diluent. Aliquot of 0.1 ml from the dilutions were spread plated in duplicate onto MRS agar and incubated anaerobically (using an anaerobic pack) at 42°C for 24 h. Thereafter, plates containing countable colonies were counted in an electronic counter and the number of *L. acidophilus* was expressed as colony forming units per ml (CFU/mL).

2.12. Sensory Evaluation of the HTNB Beverage

Assessors degree of likeness of the sensory attributes (Appearance, Aroma, Texture, Taste, Consistency and General Acceptability) of the HTNB was carried out using a 9-point hedonic scale as described by Obinna-Echem *et al.*, [31]. A 20 member panelists were selected from among the lecturers, laboratory technologists, and students of the department of Food Science and Technology, Rivers State University who are familiar with non-dairy beverages. The samples were served to all members of the panel with a glass of water to rinse their mouth during the tasting exercise. The degree of likeness was expressed as: 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much and 9 = Like extremely.

2.13. Statistical Analysis

The data obtained were analysed statistically using IBM SPSS version 23 statistical package Two-way analysis of variance (ANOVA) was carried out and the means were separated using Tukey's multiple comparison test, at a significance level of $P<0.05$.

3. Result and Discussion

3.1. Effect of Partial Hydrolysis on the Starch Content of Tigernut Beverage

Figure 1 showed the starch content of tigernut beverage at various stages of hydrolysis with fungal bacteria alpha amylase and Beta glucosidase. The starch and sugar content before hydrolysis was 29.70% and 9.08^0Brix respectively. During hydrolysis at the different temperatures, the starch content significantly ($P<0.05$) decreased from 28.40 - 17.85% at 48 and 92°C respectively, while the soluble sugar content varied from 9.08 – 20.23^0Brix. Hydrolysis at 45 - 48°C had significantly ($P<0.05$) the highest starch and the least sugar content compared to other temperature, while the reverse was the case at 92°C. while the final starch and sugar content after cooling were 17.14% and 20.23^0Brix respectively. These values did not differ significantly ($P>0.05$) from those at 92°C.

Starch is a complex carbohydrate, its hydrolysis with enzyme alpha amylase and amyloglucosidase resulted in production of glucose which remains as a reducing sugar in the medium. The significantly ($P<0.05$) least starch and highest sugar content in the sample hydrolysed at 92°C indicated that the temperatures at which these enzymes are exposed to the slurry, influenced the conversion rate of the carbohydrates (starch to glucose). This is in agreement with the report by Turini *et al.*, [32], where alpha amylase and amyloglucosidase enzyme converted cellulose to a reducing sugar. Alpha–amylase hydrolysed starch faster as these enzymes only break the α-1, 4 bonds along the amylose and amylopectin chains [33]. Iodine test carried out showed change in colour from blue black to purple-brown colouration, which is also an indication of the decrease in starch and increase in sugar content. The increased sugar content is required for microbial growth in fermented products.

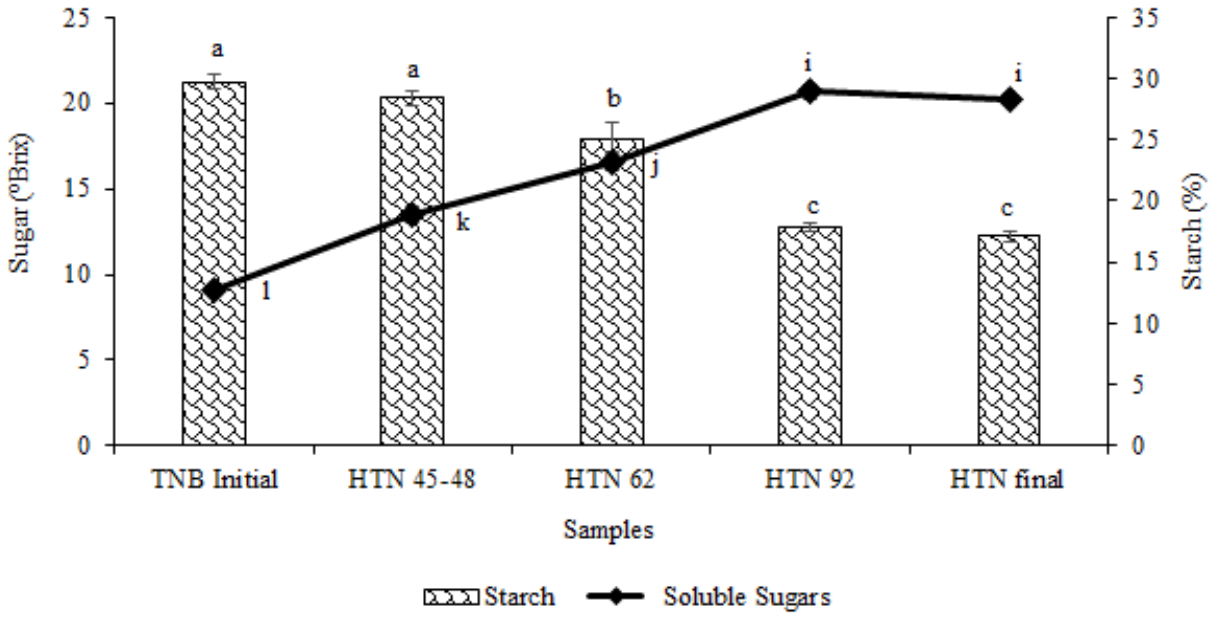

Figure 1. Effect of Partial hydrolysis on the starch and sugar content of tigernut beverage (Bars and error bar represent the mean and standard deviation of duplicate samples for starch. Markers and error bars represent the mean and standard deviation of duplicate sample for sugar. Means with different letters differ significantly ($P<0.05$). TNB: Tigernut beverage; HTN: Hydrolysed tigernut beverage at different temperatures (°C))

3.2. Effect of Beetroot Juice Concentration and Time on the Physicochemical Properties of Partially Hydrolysed Tigernut-Beetroot Beverage

The effects of beetroot juice concentration and time on the physicochemical properties of partially hydrolysed tigernut beetroot beverage were shown in Table 2, Table 3 and Table 4 respectively, for pH, total titratable acidity (TTA) and total soluble solids (TSS). There was significant ($P<0.05$) decrease in pH of the samples with time irrespective of the concentration of beetroot. The samples had significantly ($P<0.05$) the least pH after 24 h. The decrease was from 6.54 – 5.05, 6.52 – 5.38, 6.52 – 5.46 and 6.46 – 5.66 for the control (HTN_{100}), the samples with 10, 20, and 30% of beetroot juice respectively. The decrease in pH was significantly ($P<0.05$) higher in samples with greater concentration of beetroot.

Titratble acidity as %lactic acid present in the samples varied significantly ($P<0.05$). There was significant ($P<0.05$) increase in TTA of the samples with time. The samples had significantly ($P<0.05$) the highest TTA after 24 h. The values ranged from 0.19 - 0.67, 0.30 – 0.66, 0.31 – 0.69 and 0.31 – 0.42 for the control (HTN_{100}), the samples with 10, 20, and 30% of beetroot juice respectively. The increase in TTA was significantly ($P<0.05$) lower in the sample with 30% beetroot juice concentration. The initial TTA of the control was significantly ($P<0.05$) the least. This implies that there was the presence of other organic acids with the inclusion of beetroot juice which resulted in greater total titratable acidity than the control sample. The rate of increase in TTA was higher in the control samples as there may have been no much interferences with the activities of the fermenting microorganism. This was also evidenced in the higher total soluble solids of the control sample that was higher than those of the test samples. Similar decrease in pH and increases in TTA was reported by Akharaiyi *et al*. [34] and Obinna-Echem and Torporo [35]. The level of acidity of the milk is also an indication of a good quality beverage, as lactic acid is usually produced from the activities of microorganism where milk sugar is converted to lactic acid [35].

Table 2. Effect of Beetroot Juice Concentration and Time on the pH of Hydrolysed Tigernut-Beetroot Beverage

Sample	Time (h)						
	0	4	8	12	16	20	24
HTN_{100}	6.54^{a1}±0.02	6.42^{ab12}±0.02	6.30^{b1}±0.01	6.28^{b1}±0.03	6.19^{b1}±0.13	5.40^{c3}±0.11	5.05^{d3}±0.11
$HTNB_{10}$	6.52^{a1}±0.03	6.52^{a1}±0.04	6.32^{b1}±0.01	6.24^{b1}±0.09	6.07^{c1}±0.30	5.56^{cd2}±0.13	5.38^{d2}±0.06
$HTNB_{20}$	6.52^{a1}±0.01	6.36^{b2}±0.05	6.22^{b2}±0.10	6.18^{b12}±0.00	6.04^{c1}±0.71	5.76^{cd12}±0.16	5.46^{d12}±0.04
$HTNB_{30}$	6.46^{a2}±0.04	6.48^{a12}±0.01	6.29^{b1}±0.01	6.14^{bc2}±0.08	6.02^{c1}±0.08	5.95^{c1}±0.04	5.66^{d1}±0.11

Values are mean ± standard deviation of triplicate samples.
Means with different numeric superscript (^{12}) along each column differ significantly ($P<0.05$) indicating the effect of beetroot concentration.
Means with different alphabetic superscript (^{ab}) along each row differ significantly ($P<0.05$) indicating the effect of time.
HTN_{100}= 100% hydrolysed tigernut beverage,
$HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice
$HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice
$HTNB_{30}$ = 70% hydrolysed tigernut beverage and 30% Beetroot juice.

Table 3. Effect of Beetroot Juice Concentration and Time on the Total Titratable Acidity (%Lactic acid) of Hydrolysed Tigernut-Beetroot Beverage

Sample	Time (h)						
	0	4	8	12	16	20	24
HTN_{100}	0.19^{e2}±0.01	0.36^{d2}±0.01	0.43^{c2}±0.00	0.52^{b3}±0.04	0.58^{b1}±0.01	0.63^{ab1}±0.01	0.67^{a1}±0.01
$HTNB_{10}$	0.30^{c1}±0.01	0.39^{bc2}±0.01	0.46^{b1}±0.01	0.47^{b12}±0.04	0.61^{a1}±0.02	0.64^{a1}±0.01	0.66^{a1}±0.01
$HTNB_{20}$	0.31^{d1}±0.01	0.44^{cd1}±0.01	0.48^{bc1}±0.01	0.43^{c1}±0.00	0.51^{b2}±0.01	0.67^{a1}±0.01	0.69^{a1}±0.01
$HTNB_{30}$	0.31^{c1}±0.01	0.31^{c3}±0.01	0.33^{bc3}±0.01	0.31^{bc23}±0.00	0.35^{b3}±0.01	0.33^{bc2}±0.01	0.42^{a2}±0.02

Values are mean ± standard deviation of triplicate samples.
Means with different numeric superscript (^{12}) along each column differ significantly ($P<0.05$) indicating the effect of beetroot concentration.
Means with different alphabetic superscript (^{ab}) along each row differ significantly ($P<0.05$) indicating the effect of time.
HTN_{100}= 100% hydrolysed tigernut beverage,
$HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice
$HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice
$HTNB_{30}$ = 70% hydrolysed tigernut beverage and 30% Beetroot juice

Table 4. Effect of Beetroot Juice Concentration and Time on the Total Soluble Solids of Hydrolysed Tigernut-Beetroot Beverage

Sample	Time (h)						
	0	4	8	12	16	20	24
HTN_{100}	20.30^{a1}±0.00	20.07^{b1}±0.01	20.02^{b1}±0.01	19.80^{c1}±0.10	19.56^{d1}±0.02	19.35^{e1}±0.04	19.23^{e1}±0.03
$HTNB_{10}$	19.98^{a2}±0.00	19.84^{ab2}±0.01	19.71^{b2}±0.08	19.53^{c2}±0.01	19.43^{c12}±0.08	19.22^{d2}±0.02	19.02^{e23}±0.01
$HTNB_{20}$	19.93^{a3}±0.01	19.80^{b2}±0.01	19.62^{c2}±0.02	19.54^{c2}±0.01	19.38^{d2}±0.01	19.17^{e2}±0.01	19.04^{f2}±0.01
$HTNB_{30}$	19.89^{a3}±0.02	19.71^{b3}±0.04	19.67^{b2}±0.02	19.51^{c2}±0.01	19.47^{c12}±0.01	19.19^{d2}±0.01	18.95^{e3}±0.03

Values are mean ± standard deviation of triplicate samples.
Means with different numeric superscript (^{12}) along each column differ significantly ($P<0.05$) indicating the effect of beetroot concentration.
Means with different alphabetic superscript (^{ab}) along each row differ significantly ($P<0.05$) indicating the effect of time.
HTN_{100}= 100% hydrolysed tigernut beverage,
$HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice
$HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice
$HTNB_{30}$ = 70% hydrolysed tigernut beverage and 30% Beetroot juice.

Total soluble solid content of the samples varied significantly (P<0.05) with time and concentration of beetroot juice. TSS increased significantly (P<0.05) in the samples with time and decreased with the addition of beetroot juice. The samples had significantly (P<0.05) the least TSS after 24 h. The values ranged from 20.30 – 19.23, 19.98 – 19.02, 19.93 – 19.04 and 19.89 – 18.95% respectively, for the control (HTN100), the samples with 10, 20, and 30% of beetroot juice. The decrease in TSS was significantly (P<0.05) higher in the control sample without beetroot juice concentration. The initial TSS of the control was significantly (P<0.05) the highest and the fermenting microorganisms had more sugars to metabolize. This explains the greater decrease in pH and higher increase in TTA of the control samples.

3.3. Effect of Beetroot juice concentration and fermentation time on *L. acidophilus* count.

The growth of *L. acidophilus* in partially hydrolysed tigernut beverage with different concentrations of beetroot juice over time is shown in Table 5 while the increase in cell number at each time interval is shown in Figure 2. There was significant (P<0.05) increase in *L. acidophilus* count with time from <2.00 - 6.22, 2.81 - 6.37, 2.30 - 6.94 and 2.45 - 6.29 $\log_{10}$CFU/ml respectively, for the control (HTN_{100}), the samples with 10, 20, and 30% of beetroot juice. This is in agreement with Barber *et al*. [25] where the final counts of *L. acidophilus* and *B. bifidium* in mono and co-culture soymilk yoghurt increased greater than the 10^6 viable cells recommended for probiotic products. The concentration of beetroot juice at each given time had no significant (P<0.05) on the *L. acidophilus* count except after 1, 4 and 24 h where the counts in sample with 10 and 20% beetroot had significantly (P<0.05) the highest count. Considering the difference in the initial count, the differences in growth from each time interval as shown in Figure 2 indicated significant (P<0.05) increase in cell numbers at 4, 8 and 20 h. The control had significantly (P<0.05) the highest increase in cell numbers at 4 and 8 h, sample with 10% and 20% beetroot had significantly (P<0.05) the highest count at 12 and 24 h respectively. However, there was no significant (P>0.05) difference in the computed overall growth rate of the organism in the different samples (0.15 - 0.17) except for sample with 30% beetroot with growth rate of 0.14. This implies that the samples were all able to support adequate growth of the probiotic *L. acidophilus* count and the activation of the organisms in tigernut milk may have contributed to the proliferation of the organism in the nutrient rich hydrolyzed tigernut and beetroot beverage. This confirms the weak acidity (5.96 – 6.81) of tigernut [36] and can support the growth of lactic acid bacteria [37]. This beverage combination can be recommended for the production of a functional beverage with probiotic characteristics considering the support of the viability of the probiotic organisms which offers several health benefits [27].

Table 5. Effect of Beetroot Juice Concentration and Time on *L. acidophilus Count* in Hydrolysed Tigernut-Beetroot Beverage

Sample	Time (h)						
	1	4	8	12	16	20	24
$HTNB_{100}$	<2.00	3.22^{d2}±0.06	3.79^{c1}±0.09	3.91^{c1}±0.01	3.98^{c1}±0.05	6.10^{b1}±0.08	6.22^{a2}±0.02
$HTNB_{10}$	2.81^{d1}±0.05	3.36^{c2}±0.12	3.66^{b1}±0.07	3.98^{b1}±0.14	4.12^{b1}±0.00	6.20^{a1}±0.01	6.37^{a2}±0.01
$HTNB_{20}$	2.30^{c2}±0.00	3.54^{b1}±0.01	3.78^{b1}±0.12	3.94^{b1}±0.03	4.06^{b1}±0.03	6.16^{a1}±0.10	6.94^{a1}±0.71
$HTNB_{30}$	2.45^{d2}±0.21	3.41^{c12}±0.13	3.81^{b1}±0.04	3.90^{bc1}±0.13	4.01^{b2}±0.05	6.10^{a1}±0.02	6.29^{a2}±0.01

Values are mean ± standard deviation of triplicate samples.
Means with different numeric superscript (^{12}) along each column differ significantly (P<0.05) indicating the effect of beetroot concentration.
Means with different alphabetic superscript (^{ab}) along each row differ significantly (P<0.05) indicating the effect of time.
<2 = Below detection limit
HTN_{100}= 100% hydrolysed tigernut beverage,
$HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice
$HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice
$HTNB_{30}$ = 70% hydrolysed tigernut beverage and 30% Beetroot juice

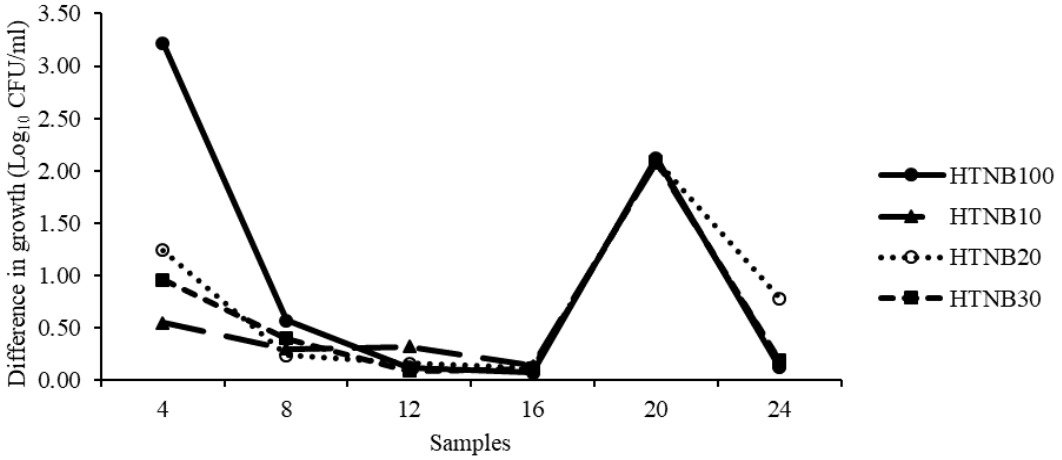

Figure 2. Increase in *L. acidophilus* Count at Each Time Interval (HTN_{100}= 100% hydrolysed tigernut beverage, $HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice, $HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice, $HTNB_{30}$ = 70% hydrolysed tigernut beverage and 30% Beetroot juice)

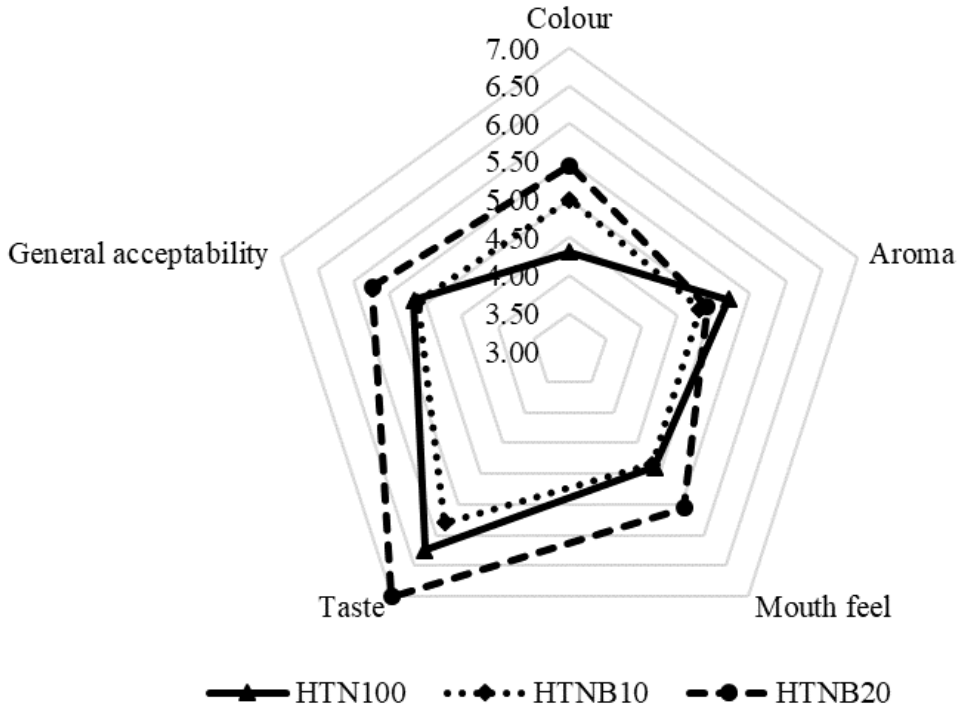

Figure 3. Sensory Properties of Probiotic *L. acidophilus* fermented Hydrolysed Tigernut and Beetroot Beverage (HTN_{100}= 100% hydrolysed tigernut beverage, $HTNB_{10}$ = 90% hydrolysed tigernut beverage and 10% Beetroot juice, $HTNB_{20}$ = 80% hydrolysed tigernut beverage and 20% Beetroot juice)

3.4. Sensory Properties of *L. acidophilus* Fermented Partially Hydrolysed Tigernut-Beetroot Beverage

The sensory attributes of the hydrolysed tigernut-beetroot beverage are shown in Figure 3. The addition of beetroot juice had significant effect on the assessor's degree of likeness of the samples. The degree of likeness varied respectively, from for 4.3 - 5.45, 4.8 - 5.2, 4.85 - 5.56, 5.8 - 7.0 and 5.11 - 5.74 colour, aroma, mouthfeel, taste and general acceptability. Sample with 20% beetroot concentration had significantly ($P<0.05$) the highest degree of likeness for all the attribute except aroma, while sample with 10% had the least except for colour. The degrees of likeness of the attributes for the 20% beetroot addition was that of neither like or dislike for colour, aroma and mouth feel while other samples were disliked slightly. The taste of the sample with 20% beetroot juice was liked moderately while others were neither liked nor dislikes. These degrees of likeness of the beverage indicates that of a new product which may need time to be appreciated by the consumers. However, the addition of 20% of beetroot juice will be recommended based on the degree of likeness.

4. Conclusion

The study revealed significant ($P<0.05$) production of sugar from the hydrolysis of the tigernut starch which is appreciated for microbial breakdown in a fermented product. pH significantly ($P<0.05$) decreased while titratable acidity (TTA) increased during the fermentation. Total soluble solid (TSS) increased significantly ($P<0.05$) in the samples with time and decreased with the addition of beetroot juice. With the initial activation of the probiotic *L. acidophilus* in tigernut milk, all the samples supported adequate growth of the probiotic *L. acidophilus* with count of 6 Log10 CFU/ml at the end of fermentation The sensory attributes of the blended fermented hyrdolysed tigernut and beetroot beverage was liked to varying degrees by the assessors but the sample with 20% beetroot addition with significantly ($P<0.05$) the highest degree of likeness would be recommended for the production of this potential probiotic beverage.

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