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Evaluation of Microbial and Chemical Composition of Fermented Beverages Produced from Tiger nut (*Cyperus esculentus*) and Beetroot (*Beta vulgaris*)

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Abstract
Consumption of fruits, vegetables, and nuts is essential for human health because of their minerals, vitamins, antioxidants and dietary fibre contents. Two types of beverages were prepared (tiger nut beverage and tiger nut/beetroot beverage). 200 g tiger nut was homogenized in 1 L of sterile water to prepare tiger nut beverage while 200 g of tiger nut with 20 g of beetroot to prepare tiger nut/beetroot beverage were homogenized with 1 L of sterile water. The prepared products were pasteurized at 80 °C for 5 min and left to ferment at room temperature (28±2 °C) for 4 days. The products were analyzed for chemical composition and microbial load. Results obtained showed no significant differences in the moisture, ash and protein content products. However, significant differences existed in fibre, fat and carbohydrate contents. *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Streptococcus lactis*, *Saccharomyces cerevisiae*, *Saccharomyces exiguus*, *Saccharomyces stellatus* and *Candida pseudotropicalis* were isolated from products. The number of bacteria in Tiger nut beverages increased from 0.85×10^3 to 1.83×10^7 and 0.97×10^3 to 1.78×10^7 in the Tiger/beetroot beverage. Mould and yeast counts in the tiger nut beverage increased from 0.32×10^2 to 1.06×10^4 while in the mixed product, the increase was from 0.30×10^2 to 1.18×10^4. There were no significant differences in pH and titratable acidity throughout days of fermentation. The tiger nut/beetroot beverage has high overall acceptability. The beverages could serve as a good material for the production of quality yoghurt in place of animal milk that is mostly used for yoghurt production.

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Introduction
Different raw materials are traditionally used for the production of beverage basically according to tradition all over the world (Akharaiyi & Omoya, 2008). Therefore, the variety of beverages could emerge and consume according to location and ingredients in many parts of the world (Goldstein, 2000). In most African countries, native fermented food, alcoholic and non-alcoholic beverages are basically produced from cereals. The recognition and general acceptance of the beverages depend on their roles in health care, religious and social values (Marcellin *et al.*, 2009; Nwachukwu *et al.*, 2010). In

fact, spontaneous fermentations increase nutritional value of beverages that significantly contribute to the health of consumers and also sensory quality that is very important in beverages acceptance.

Consumption of fruits and vegetal to protect diseases as their health benefits historically have been due to their high content in phytochemicals, minerals and vitamins (Roselló-Soto *et al.*, 2019); and also for their good source of dietary fibre (Chandrasekara & Josheph Kumar, 2016; Jeske *et al.*, 2018). Several edible plants like pawpaw, water melon, pineapple, apple etc. have been used for beverage production.

Tiger nut (*Cyperus esculentus*) is majorly planted in Spain, Senegal, Ghana, Nigeria as well as Chile. Economically, tiger nut is an underutilized crop in Africa but with potential for development. The nutritional compositions of tiger nut such vitamin C and B_6, iron, zinc, magnesium, potassium and calcium revealed it beneficially for health care as it prevents heart attacks, cancer of the colon, blood circulation and thrombosis because of the high soluble glucose content. It has the potential for diabetes and can also lower cholesterol because of its high content of vitamin E (Bazine & Arslanoğlu, 2020).

Beetroot is a vegetable of the *Chenopodiaceace* family (Langer & Hill, 1991). It is widely consumed in traditional western cooking but rarely used in West Africa (Grubben & Denton, 2004). It has health-promoting properties, hepatoprotective, anti-oxidant (Georgiev *et al.*, 2010), anti-diabetic and anti -inflammatory effects (Domínguez *et al.*, 2017; Sõukand *et al.*, 2015). The purpose of this study was to prepare non-alcoholic beverage from tiger nut; and a mixture of tiger nut and beetroot by fermentation and evaluate for nutritional quality to validate for recommendation to the already available herbal drinks.

Materials and methods

Preparation of beverages

Fresh beetroot and tiger nut were purchased from Auchi market, Edo state, Nigeria and washed with water. The beetroot samples was peeled, cut into small pieces and rinsed with distilled water. Both materials were separated into two parts using two separate containers, one contained only tiger nut while the other contained both beetroot and tiger nut. 200 g tiger nut was homogenized in 1 L of sterile water to prepare tiger nut beverage while 200 g of tiger nut with 20 g of beetroot to prepare tiger nut/beetroot beverage were homogenized with 1 L of sterile water using the Malex blender (model M-002nv) to produce tiger nut/beetroot beverage. These mixtures were kept in sterile bottles and pasteurized at 80 °C for 5 min in a water bath. After cooling, they were left to ferment at room temperature (28±2 °C) for 4 days.

Isolation, characterization and identification of microorganisms

Before pasteurizing the products, 1 mL of the beverage was diluted serially to 10^{-5}. 1 mL of 10^{-4} dilution of samples was each pour plated on plate count agar for microbial enumeration, nutrient agar for bacteria cultivation and potato dextrose agar plate for the cultivation of fungi from the beverages. The bacteria culture plates were incubated at 37 °C for 24 h and fungi cultivation plates at room temperature (28±2 °C) for 72 h. This process was carried out on days 0, 1, 2, 3 and 4.

Identification of bacteria

The colonies obtained for bacteria were counted using a colony counter and each distinct colony from each of the plates were purified by sub-culturing on nutrient agar plates to obtain pure cultures. The purified cultures were then transferred to nutrient agar slant and stored at 4 °C for later use. The isolates were identified morphologically and biochemically (catalase, coagulase, motility, citrate, oxidase, indole, urease, methyl red, voges-proskauer carbohydrate utilization) according to Bergey's of Determinative Bacteriology (Bergey, 1994).

Identification of mould/yeast

2 drops of lacto phenol in cotton blue was place on a clean grease free slide. Using a sterile inoculating loop a mycelial mat was transferred on the fluid to mix up, covered with a cover slip and observed under medium power objectives of microscope for detailed structures. Identification was done by the criteria of Barnett *et al.* (2000).

Sensory evaluation of local beverages

Sensory evaluation of the prepared beverages was assessed using 15 member panelists who regularly consume the beverage. Suggested parameters for their scores are: aroma, consistency, taste, appearance and overall acceptability on a 4 point hedonic scale. The ratings were (1= dislike extremely, 2= like moderately, 3= like very much and 4= like extremely) (Granato *et al.*, 2012).

Titratable acidity

20 mL of sample was measured in a conical flask and titrated against 0.1 N sodium hydroxide with 2 drops of phenolphthalein. Calculation of total titratable acidity was with the formula:

$$Total\ titratable\ acidity = \frac{Volume\ of\ 0.1\ NaOH \times Normality}{Volume\ of\ sample} \quad (1)$$

pH determination

The pH of the beverages was determined using a pH meter after standardization with pH 4, 10 and 7 buffers. All analyses were performed in duplicate.

Analysis for chemicals

Fat, ash, protein, carbohydrates, fibre and moisture contents in the prepared beverages were determined by the method AOAC (2007).

Statistical analysis

Results obtained were expressed as the mean±SD of triplicates. SPSS version 16.0 for windows software package and Student's t-test for statistical analyses was used. Values were considered to be statistically significant at (P<0.05).

Results and discussion

The bacteria isolated and identified from the beverages through conventional and biochemical means includes: *Leuconostoc mesenteriodes*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Streptococcus lactis*. Among the bacteria species identified, *Lactobacillus plantarum* was the most frequent. These species of *Lactobacillus* occurred in the fermentation from day 2 to day four of fermentation. Other species were isolated from the fermented beverages from day one of fermentation. The yeast isolated and identified from the samples were *Saccharomyces cerevisiae*, *Candida stellata*, *Candida pseudotropicalis* and *Saccharomyces exigiuus*. *Saccharomyces cerevisiae* was the most occurring yeast species. It was isolated from the beverages right from day one to the end of fermentation.

The proximate composition of the prepared beverage samples is shown in Table (1).

Table 1. Proximate composition of the beverages (%)

	TNB	TNBR+Beetroot
Fat	0.36±0.30[b]	9.20±0.10[a]
Ash	0.42±0.10[a]	0.51±0.30[b]
Moisture	87.56±0.40[a]	88.63±0.30[a]
Protein	2.13±0.60[a]	1.84±0.20[b]
Carbohydrate	9.60±0.10[b]	7.00±0.30[a]
Fibre	0.53±0.30[a]	0.81±0.20[b]

TNB= Tiger nut beverage, TNBR+Beaatroot= Tiger nut beverage mixed with beetroot

The tiger nut prepared beverage proximate composition was 0.36±0.30% (fat), 0.40±0.10% (ash), 87.36% (moisture), 2.13±0.60% (protein), 9.60±0.10% (carbohydrate) and 0.53±0.30% (fibre). In the tiger nut mixed with beetroot, compositions were fat (0.24±0.40%), ash (0.51±0.50%), moisture (88.63±0.30%), protein (1.84±0.20%), fibre (0.81±0.20%) and carbohydrate (7.60±0.40%). From the proximate perspective, combination of tiger nut and beetroot could be a unique quality non-alcoholic beverage with a difference. For example, where beetroot is having less protein than tiger nut and fat in beetroot than tiger nut; and several other proximate compositions of the samples.

The microbial load from the fermented products increased along with days of fermentation. Bacterial load in the tiger nut prepared beverage was 0.86×10^3 CFU/mL in day zero and subsequently increased to 1.83×10^5 CFU/mL in day 4 of fermentation. In the prepared tiger nut mixed with beetroot, microbial load of 0.97×10^3 on day zero also, subsequently increased to 1.78×10^5 at the 4^{th} day of fermentation. Likewise, increase in mould/yeast counts was as well observed where the count of 32×10^2 spore/mL at day 0 increased to 1.06×10^4 at the 4^{th} day of fermentation in the tiger nut prepared beverage (Table 2).

The increase in microbial population along with days of fermentation could be related to the high content of carbohydrate and moisture in the raw material used for the beverage preparation. Microbial properties continued to increase gradually in the beverages at the 2^{nd} day of fermentation until the 4^{th} day. The observed subsequent increase in microbial population in the period of fermentation could be resulted from the increase in pH. The increase in pH and microbial population at earlier fermentation periods is evidence that fermentable sugars present in the beverages have not been totally fermented. At this stage, the prepared products were characteristically non-alcoholic beverages. The decrease in microbial population at later fermentation period which resulted in decrease in pH values, suggested that fermentable sugars in the products were completely exhausted by lactic acid bacteria (Akharaiyi & Omoya, 2008) thus the sour taste attained in the beverages. Similar result was reported by El-Shenawy *et al.* (2019). The understanding is that, sweet and mellow taste of the products can only be maintained for 24 h at room temperature. The microbes majorly isolated at this point were yeast and lactic acid bacteria species. The growth of lactic acid bacteria in the beverages could initiate inhibitory potentials that made it possible for other bacteria species not to proliferate. For this reason, it is certain that the products could have a longer shelf life as to also keep the nutritional constituents for human intake. Due to the antimicrobial effect of lactic acid bacteria, pathogenic and food spoilage bacteria were inhibited from the products during fermentation process but with yeasts growth in the medium. Sõukand *et al.* (2015) has reported that beverages produced locally possess their own microbial ecosystem in the midst of diverse lactic acid bacteria which are associated with yeasts often. For the beverage samples to be high in bacterial counts of lactic acid species during fermentation, means that it could be a good product of probiotic food. Probiotic foods helps in weight loss and decrease belly fat. It could also lower risks of heart diseases and bad cholesterol.

Table 2. Microbial load of prepared beverages

Day	Bacterial counts (CFU/mL)		Mould counts (Spore/mL)	
	TNB	TNB+BR	TNB	TNB+BR
0	0.85×10^{3b}	0.97×10^{3a}	32×10^{2a}	30×10^{2a}
1	1.38×10^{7a}	0.86×10^{3b}	56×10^{2b}	67×10^{2a}
2	1.51×10^{7b}	1.74×10^{7a}	68×10^{2b}	76×10^{2a}
3	1.64×10^{7b}	1.70×10^{7a}	94×10^{2b}	1.12×10^{4a}
4	1.83×10^{7a}	1.78×10^{7b}	1.06×10^{4b}	1.18×10^{4a}

TNB= Tiger nut beverage, TNB+BR= Tiger nut beverage mixed with beetroot

Though the process of the beverages production did not involve ethyl alcohol fermentation hence the media were not mono-culturally fermented with yeast strains, does not rule out traces of alcohol content in the beverages. Evidence proving this was the isolation of *Candida stellata* at the initial stage of fermentation to the 2nd day but was eliminated from the 3rd day of fermentation. The reason for this is that this particular yeast and some other yeast species cannot tolerate alcohol above 2% (van der Walt & Yarrow, 1984). Also supporting the proof of alcohol presence in the beverages was the later isolation of *Saccharomyces cerevisiae* which became the dominant yeast specie in the fermented products, and are known to tolerate alcohol content of above 20% (van der Walt & Yarrow, 1984). At the 1st day of fermentation, the prepared beverages were of sweet and mellow taste. These characteristics emphasized that fermentation was yet to be established thus the carbohydrate and starch contents in the beverages still intact. If however bacteria and mould/yeast species were present in the processing beverages, at this point in time will only be negotiating for their means of either to grow or not to grow based on their growth requirement in the fermenting media. The physicochemical properties of the prepared beverages are shown in Fig. (1).

Decrease in pH was observed in the prepared beverage samples. The pH of tiger nut beverages at day zero of preparation was 6.65±0.04 and thereafter decreased to 4.57±0.01. In the tiger nut mixed with beetroot beverage, pH was 6.68±0.03 on day 0 and 5.06±0.05 on day 4 of fermentation. The pH recorded for the tiger nut mixed with beetroot had higher pH value than the beverage prepared only from tiger nut (Fig. 1). Increase in titratable acidity (TTA) was observed in the prepared beverages. In the tiger nut beverage, it was 0.10±0.06 at day 0 and subsequently increased to 0.22±0.11 on day 4 of fermentation. In the tiger nut mixed with beetroot beverage, increase in TTA from 0.15±0.11 in day 0 to 0.25±0.06 in day 4 of fermentation was observed (Fig. 1). The neutral pH recorded at day 0 from the products could contribute immensely for the growth of microbes to initiate the fermentation. The pH recorded in this research was higher than the pH reported for beetroot juice products by Gamage *et al.* (2016); and similar to what was reported on tiger nut extract by El-Shenawy *et al.* (2019). However, the decrease in pH alongside days of fermentation, could be associated with the production of organic acid and other acids metabolic end products which have culminated to have some effects on the pH reduction (Gabriel & Akharaiyi, 2007).

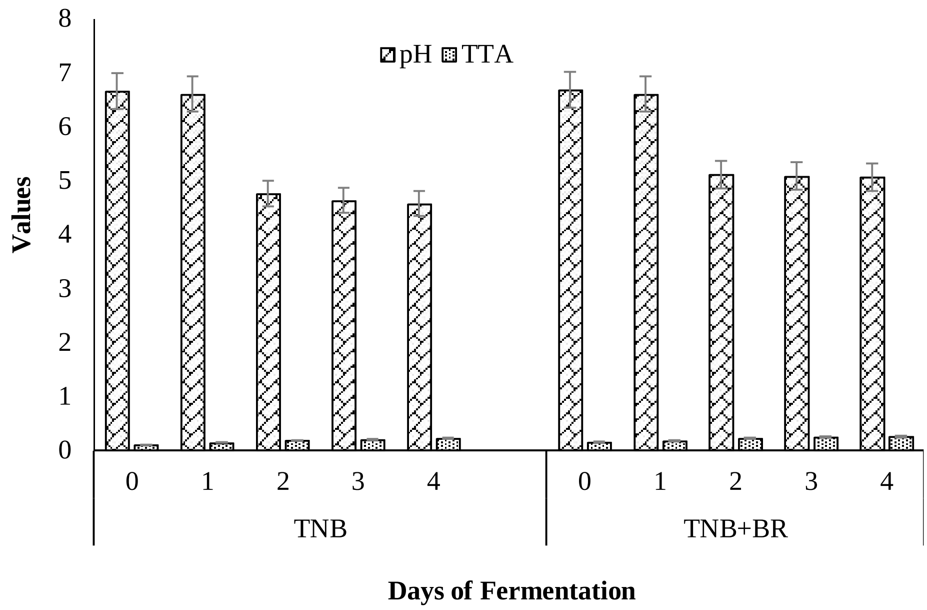

Fig. 1. Physicochemical properties of prepared beverages

Sensory evaluation of the prepared beverages is presented in Table (3). The tiger nut aroma was rated 8.00±0.66, consistency as 7.00±0.45, taste as 7.00±0.65, appearance as 7.00±0.66 and overall acceptability as 7.86±0.73. Tiger nut mixed with beetroot has an aroma that was rated 8.00±0.54, consistency as 8.00±0.36, taste as 8.00±0.06, appearance as 8.00±0.54 and overall acceptability as 8.00±0.68. The fermented tiger nut beverage has a rating of aroma as 7.00±0.32, consistency as 7.00±0.25, taste as 7.00±0.66, appearance as 7.00±0.52 and overall acceptability as 7.00±0.27. The fermented tiger nut mixed with beetroot was rated higher, where in aroma it was 7.00±0.42, consistency as 7.00±0.42, taste as 7.00±0.65, appearance as 7.00±0.54 and overall acceptability as 7.00±0.58. There was no significant difference (P>0.05) observed between the samples in terms of aroma and taste. The panelists commented that the beverages were good but the tiger nut mixed with beetroot was the best. Tiger nut beverage has no beany flavor and throat catching sensations. Significant (P<0.05) difference was observed only in color and overall acceptability between the two prepared beverages. Despite the significant differences in sensory quality in the beverages, the samples were generally acceptable to the panelists. Such related acceptability has led to the commercialization of tiger nut products in Spain (Traders, 2009), and could as well lead to the general populace of Nigerians acceptance if this products are popularized based on taste, the general good quality, and characteristics the beverages possessed. The sensory quality of all samples were rated alike in almost all the sensory attributes evaluated, indicating the minimal effect the varying proportion had on the organoleptic property of the beverages.

Table 3. Sensory evaluation of the prepared beverages

Samples	Aroma	Consistency	Taste	Appearance	Overall acceptance
TNU	8.00±0.66[a]	7.00±0.45[b]	7.00±0.65[b]	7.00±0.66[b]	7.00±0.73[b]
TNBF	7.00±0.32[d]	7.00±0.25[d]	7.00±0.66[b]	7.00±0.52[c]	7.00±0.27[d]
TNB+BR U	8.00±0.54[b]	8.00±0.36[a]	8.00±0.06[a]	8.00±0.54[a]	8.00±0.68[a]
TNB+BR F	7.00±0.42[c]	7.00±0.42[c]	7.00±0.65[b]	7.00±0.54[c]	7.00±0.58[c]

TNBU= Tiger nut beverage unfermented, TNBF= Tiger nut beverage fermented, TNB+BR U= Tiger nut beverage mixed with beetroot unfermented, TNB+BR F= Tiger nut beverage mixed with beetroot fermented

Conclusions

Sweetness in the prepared beverages only lasted for a day and after which it turned to sour taste as a result of fermentation. Though it has milk product, microorganisms associated were low in population unlike animal milk that is highly populated with microorganisms. The fermented products have a quality taste not different from yoghurt prepared from milk therefore proposing it as good material for quality yoghurt production. Based on the quality of the prepared beverages, it could serve as a potential good nutritious weaning food for babies when not fermented and can be done either in whole or in mixture with liquid pap or any other weaning food for babies. The beverages are so unique that they can stand out as a significant item in both local and international trade and could serve for entertainment drink in any ceremony.

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Author contributions

Fred Coolborn Akharaiyi: Writing and Drafting of the manuscript, Presenting the research idea and study analysis, Data analysis, Revising and editing the manuscript, Approval of the final version; **Phebean Onosen Ozolua**: Date collection, Revising and editing the manuscript, Approval of the final version; **Christopher Johnson**: Writing and Drafting of the manuscript, Presenting the research idea and study analysis, Approval of the final version.

Conflict of interest

There is no conflict of interest based on the writers.

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ارزیابی ترکیبات میکروبی و شیمیایی نوشیدنی‌های تخمیری تولیدشده از آجیل ببر (*Cyperus esculentus*) و چغندر (*Beta vulgaris*)

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چکیده

مصرف میوه‌ها، سبزی‌ها و آجیل‌ها به‌دلیل داشتن مواد معدنی، ویتامین‌ها، آنتی‌اکسیدان‌ها و فیبر غذایی برای سلامت انسان ضروری است. دو نوع نوشیدنی تهیه شد (نوشیدنی آجیل ببر و نوشیدنی آجیل ببر/چغندر). 200 گرم آجیل ببر در 1 لیتر آب استریل برای تهیهٔ نوشیدنی آجیل ببر و 200 گرم آجیل ببر با 20 گرم چغندر برای تهیهٔ آجیل ببر/نوشیدنی چغندر با 1 لیتر آب استریل همگن شدند. محصولات تهیه‌شده به مدت 5 دقیقه در دمای 80 درجهٔ سانتی‌گراد پاستوریزه‌شده و به مدت 4 روز در دمای اتاق (2±28 درجهٔ سانتی‌گراد) تخمیر شدند. محصولات ازنظر ترکیب شیمیایی و بار میکروبی آنالیز شدند. نتایج به‌دست‌آمده تفاوت معنی‌داری را در میزان رطوبت، خاکستر و پروتئین محصولات نشان نداد. بااین‌حال، تفاوت‌های قابل‌توجهی در محتوای فیبر، چربی و کربوهیدرات وجود داشت. *لوکونوستوک مزانتروید*، *لاکتوباسیلوس پلانتاروم*، *لاکتوباسیلوس اسیدوفیلوس*، *استرپتوکوکوس لاکتیس*، *ساکارومایسس سرویزیه*، *ساکارومایسس اگزیگوس*، *ساکارومایسس استلاتوس و کاندیدا پسودوتروپیکالیس* از محصولات جداسازی شدند. تعداد باکتری‌ها در نوشیدنی‌های آجیل ببر از $0/85×10^3$ به $1/83×10^7$ و $0/97×10^3$ به $1/78×10^7$ افزایش یافت. تعداد کپک و مخمر در نوشیدنی آجیل ببر از $0/32×10^2$ به $1/06×10^4$ افزایش یافت. درحالی‌که در محصول مخلوط، این افزایش از $0/30×10^2$ به $1/18×10^4$ بود. تفاوت معنی‌داری در pH و اسیدیتهٔ قابل تیتراسیون در طول روزهای تخمیر وجود نداشت. نوشیدنی آجیل ببر/چغندر از مقبولیت کلی بالایی برخوردار است. این نوشیدنی‌ها می‌توانند به‌عنوان مادهٔ خوبی برای تولید ماست با کیفیت به‌جای شیر حیوانی که بیشتر برای تولید ماست استفاده می‌شود، عمل کنند.

واژه‌های کلیدی: تخمیر، شیمیایی، گیاهان، میکروارگانیسم‌ها، نوشیدنی