

## 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 litre 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 litre of sterile water. The prepared products were pasteurized at 80 °C for 5 minutes 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*, *L. acidophilus*, *Streptococcus lactis*, *Saccharomyces cerevisiae*, *S. exiguus*, *Candida stellate* and *C. pseudotropicalis* were isolated from products. The number of bacteria in Tiger nut beverages increased from  $0.85 \times 10^3$  to  $1.83 \times 10^7$  and  $0.97 \times 10^3$  to  $1.78 \times 10^7$  in the Tiger/beetroot beverage. Mould and yeast counts in the tiger nut beverage increased from  $0.32 \times 10^2$  to  $1.06 \times 10^4$  while in the mixed product, the increase was from  $0.30 \times 10^2$  to  $1.18 \times 10^4$ . There were no significant differences in pH and titratable acidity throughout days of fermentation. The tiger nut/beetroot beverage have 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.

Received: 2021.09.03

Accepted: 2022.01.11

### Keywords

Beverage

Chemical

Fermentation

Microorganisms

Plants

### Introduction

Different raw materials are traditionally used for the production of wine basically according to tradition all over the world (Akharaiyi and Omoya, (2008). Therefore,

the variety of beverages could emerge and consume according to location and ingredients in many parts of the world

It is of great pleasure and delight to announce that the paper has been approved by the reviewers of the journal and currently passing the final procedure to be published. Therefore, the paper should be referenced mentioning DOI.

(Block and Glen, 2000). In most Africa 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 (Dje *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 (Elena *et al.*, 2019); and also for their good source of dietary fibre (Chandrasekara and Joseph, 2016; Jeske *et al.*, 2018). Wine can be alcoholic when fruits or high carbohydrate fruits are fermented or non-alcoholic beverages that require no fermentation. Several edible plants like pawpaw, water melon, pineapple, apple etc have been used for wine 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 B6, iron, zinc, mannesium, 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 and Arslanoğlu, 2020).

Beetroot is a vegetable of the *chenopodiaceae* family (Hill and Langer, 1991). It is widely consumed in traditional western cooking but rarely used in West Africa (Grubben and Denton, 2004). It has health-promoting properties, hepatoprotective, anti-oxidant (Georgiev *et al.*, 2010), anti-diabetic and anti-

inflammatory effects (Clifford *et al.*, 2015; Domínguez *et al.*, 2017). 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 Beet root and Tiger nut were purchased from Auchi market, Edo state, Nigeria and washed with water. The beet root 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 litre 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 litre of sterile water using the Malex blender (model M-002nv) to produce tiger/beetroot beverage. These mixtures were kept in sterile bottles and pasteurized at 80 °C for 5 minutes 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<sup>-5</sup>. 1ml of 10<sup>-4</sup> 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 hours and fungi cultivation plates at room temperature (28±2 °C) for 72 hours. 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 (Holt *et al.*, 1994).

### Identification of mould/yeast

Two 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 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)

### Titrateable acidity

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

$$\frac{\text{Volume of 0.1 NaOH} \times \text{Normality}}{\text{Volume of sample}}$$

$$\text{Volume of 0.1 NaOH} = \text{Initial} = \text{Final}$$

### 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  $\pm$  SD of triplicates. SPSS version 16.0 for window 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 discussions

The bacteria isolated and identified from the beverages through conventional and biochemical means includes: *Leuconostoc mesenteroides*, *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 two 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 exiguius*. *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±0.3 <sup>b</sup>	9.20±0.1 <sup>a</sup>
Ash	0.42±0.1 <sup>a</sup>	0.51±0.3 <sup>b</sup>
Moisture	87.56±0.4 <sup>a</sup>	88.63±0.3 <sup>a</sup>
Protein	2.13±0.6 <sup>a</sup>	1.84±0.2 <sup>b</sup>
Carbohydrate	9.60±0.1 <sup>b</sup>	7.0±0.3 <sup>a</sup>
Fibre	0.53±0.3 <sup>a</sup>	0.81±0.2 <sup>b</sup>

**Legend:** TNB = Tiger nut beverage, TNBR+Beetroot = Tiger nut beverage mixed with Beetroot

The Tiger nut prepared beverage proximate composition was 0.36±0.3% (fat), 0.40±0.1% (ash), 87.36% (moisture), 2.13±0.6% (protein), 9.60±0.1% (carbohydrate) and 0.53±0.3% fibre). In the Tiger nut mixed with Beetroot, compositions were fat (0.24±0.4%), ash (0.51±0.5%), moisture (88.63±0.3%), protein (1.84±0.2%), fibre (0.81±0.2%) and carbohydrate (7.60±0.4%). 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  $0.86 \times 10^3$  CFU/ml in day 0 and subsequently increased to  $1.83 \times 10^5$  CFU/ml in day four of fermentation. In the prepared Tiger nut mixed with Beetroot, microbial load of  $0.97 \times 10^3$  on day 0 also, subsequently increased to  $1.78 \times 10^5$  at the fourth day of fermentation. Likewise, increase in mould/yeast counts was as well observed where the count of  $32 \times 10^2$  spore/ml at day 0 increased to  $1.06 \times 10^4$  at the fourth day of fermentation in the tiger nut prepared beverage (Table 2.).

**Table 2.** Microbial load of prepared beverages

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

**Legend:** TNB = Tiger nut beverage, TNB+BR = Tiger nut beverage mixed with Beetroot

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 increase continued gradually in the beverages at the second day of fermentation until the fourth 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 an evidence that fermentable sugars present in the beverages have not been totally fermented. At this stage, the prepared products were characteristically

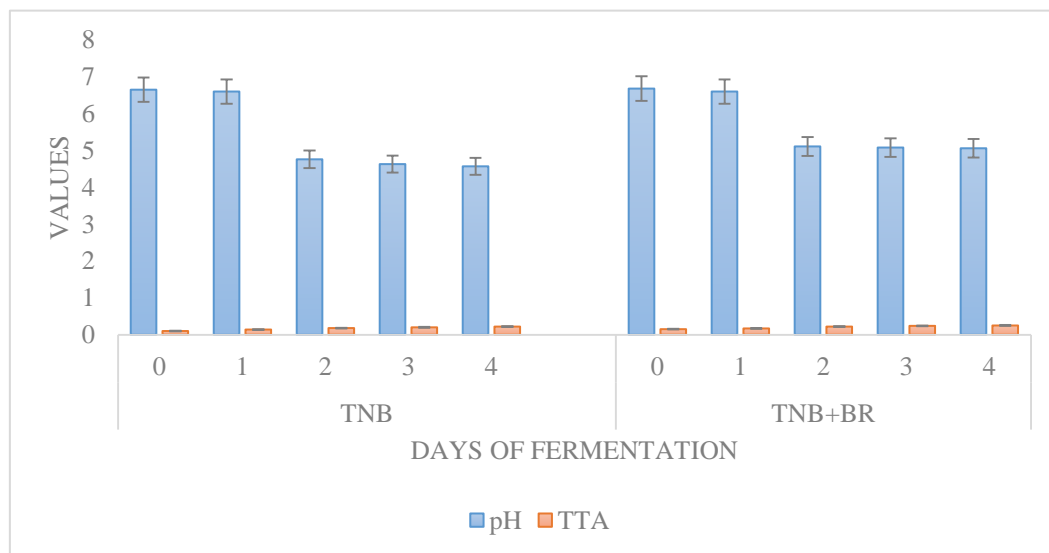
non-alcoholic beverages. The decrease in microbial population at later fermentation period which resulted in decrease in pH values, suggested that fermentable sugars in the products were completely exhausted by lactic acid bacteria (Akharaiyi and Omoya, 2008) thus the sour taste attained in the beverages. Similar result was reported by Moustafa *et al.* (2019). The understanding is that, sweet and mellow taste of the products can only be maintained successfully under 24 hours 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. Soukand *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.

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 second day but was eliminated from the third day of fermentation. The reason for this is that this particular yeast and some other yeast species cannot tolerate alcohol above 2% (Kreger van Rig. 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% (Kreger van Rig. 1984). At the first 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 is shown in Fig. 1.



**Figure 1.** Physicochemical properties of prepared beverages

Decrease in pH was observed in the prepared beverage samples. The pH of tiger nut beverages at day 0 of preparation was  $6.65 \pm 0.04$  and thereafter decreased to

$4.57 \pm 0.01$ . In the Tiger nut mixed with Beetroot beverage, pH was  $6.68 \pm 0.03$  on day 0 and  $5.06 \pm 0.05$  on day four 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  $0.10 \pm 0.06$  at day 0 and subsequently increased to  $0.22 \pm 0.11$  on day four of fermentation. In the Tiger nut mixed with Beetroot beverage, increase in TTA from  $0.15 \pm 0.11$  in day 0 to  $0.25 \pm 0.06$  in day four of fermentation was observed. (Figure 1). The neutral pH recorded at day 0 from the products could contribute immensely for the growth of microbes to initiate fermentation of desired product. 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 Moustafa *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 affect on the pH reduction (Gabriel and Akharaiyi, 2007).

Sensory evaluation of the prepared beverages is presented in table 3. The Tiger nut aroma was rated  $8 \pm 0.66$ , consistency as  $7 \pm 0.45$ , taste as  $7.0 \pm 0.65$ , appearance as  $7 \pm 0.66$  and overall acceptability as  $7.86 \pm 0.73$ . Tiger nut mixed with Beetroot has an aroma that was rated  $8 \pm 0.54$ , consistency as  $8 \pm 0.36$ , taste as  $8 \pm 0.06$ , appearance as  $8 \pm 0.54$  and overall acceptability as  $8 \pm 0.68$ . The fermented Tiger nut beverage has a rating

of aroma as  $7 \pm 0.32$ , consistency as  $7 \pm 0.25$ , taste as  $7 \pm 0.66$ , appearance as  $7 \pm 0.52$  and overall acceptability as  $7 \pm 0.27$ . The fermented Tiger nut mixed with Beetroot was rated higher, where in aroma it was  $7 \pm 0.42$ , consistency as  $7 \pm 0.42$ , taste as  $7 \pm 0.65$ , appearance as  $7 \pm 0.54$  and overall acceptability as  $7 \pm 0.58$ . 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 flavour and throat catching sensations. Significant ( $p < 0.05$ ) difference was observed only in colour 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 (Tigernuts and 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	$8 \pm 0.66^a$	$7 \pm 0.45^b$	$7 \pm 0.65^b$	$7 \pm 0.66^b$	$7 \pm 0.73^b$
TNBF	$7 \pm 0.32^d$	$7 \pm 0.25^d$	$7 \pm 0.66^b$	$7 \pm 0.52^c$	$7 \pm 0.27^d$
TNB+BR U	$8 \pm 0.54^b$	$8 \pm 0.36^a$	$8 \pm 0.06^a$	$8 \pm 0.54^a$	$8 \pm 0.68^a$
TNB+BR F	$7 \pm 0.42^c$	$7 \pm 0.42^c$	$7 \pm 0.65^b$	$7 \pm 0.54^c$	$7 \pm 0.58^c$

**Legend:** 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.

### Conclusion

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.

#### Acknowledgements

Mr. Aigboje J. I and Mr. Omabe, O. N are highly appreciated in this research for their assistance in microbiological and chemical analysis.

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