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Molecular Characterization of Non-lactic Bacteria in Lactic Fermented Tigernut-milk Drink and Effect of Ambient and Refrigeration Temperature Storage on Sensory Properties of the Drink Spiced with Ginger and Garlic

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Globally, non-dairy products are fast gaining acceptability comparable to dairy products in order to satisfy certain individuals with dietary limitations. Natural food additives such as ginger and garlic are preferable to chemically synthesized food additives and flavour enhancers. Sensory acceptability of non-dairy products is influenced by fermentation. Tigernut-milk drink was fermented using lactic starter culture isolated from 'ogi' for 72 h at 45℃. Surviving non-lactic bacteria in the lactic fermented drink which might not function as a probiotic in humans was identified using molecular characterization. The lactic fermented tigernut-milk drink was separately spiced with 3%, 5% ginger; 3%, 5% garlic. The products were stored at ambient (28±2℃) and refrigeration (4±2℃) temperature for 8 weeks based on a predetermined shelf life. *Bacillus amyloliquefaciens* strain D203 (SUB2424304 MA KY630545), *Bacillus methylotrophicus* strain XJAJ2 (SUB2424304 MG KY630549), *Bacillus amyloliquefaciens* strain BA-07 (SUB2424304 MB KY630546), *Bacillus amyloliquefaciens* strain WS-8 (SUB2424304 MD KY630547) and *Acinetobacter* sp. strain NC 41

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(SUB2424304 MF KY630548) are non-lactic bacteria in the tigernut-milk drink. Based on sensory score, the 5% ginger spiced lactic fermented tigernut-milk drink was the most preferred drink. In this study, non-lactic bacteria was identified as being present in lactic fermented tigernut-milk drink of which storage conditions and natural spices incorporated into lactic fermented tigernut-milk drinks influenced the sensory properties of the drinks.

Keywords: Molecular characterization; tigernut-milk; sensory property; lactic acid bacteria; flavour enhancer.

1. INTRODUCTION

The increasing cost of cow milk in developing countries has resulted in more attention being given to non-dairy milk products such as tigernut-milk drink [1]. As a result of this trend, increasing number of research is being directed towards improvement of non-dairy products in order to overcome dietary limitations of certain group of people as well as those who have religious beliefs and moral principles which influences their choice of products. Tigernut-milk drink is imitation milk. It is a very nutritious and popular drink prepared using tigernut tubers [2,3].

According to [4,5], lactic fermentation of tigernut-milk drink resulted in products that have lower sensory scores for appearance, sourness, texture and overall acceptability compared with composite milk product comprising cow milk and coconut milk. A natural energizing drink called tigernut juice is produced when tigernut tubers are crushed and the slurry is mixed with cinnamon, sugar and vanilla. This drink gives consumers refreshing feeling [6,7].

Tigernut milk is beneficial to human health [8]. Many products commercially available in the market contain chemical food additives. Due to health concerns, increasing number of consumers prefer food products which does not contain chemical food additives [9,10]. Therefore, food industries are being forced indirectly to gradually remove many of these chemical preservatives and replace them with natural preservatives. Ginger (*Zingiber officinale* Rosc.) and garlic (*Allium sativum* L.) are popular spices used as natural food additives, preservatives and flavour enhancers that go well with fermented products [11,12]. The advantage of natural food preservatives is that they are safe and usually do not cause side effect when used as flavour enhancer [10,13].

Freshly prepared tigernut-milk drink can undergo natural fermentation. Wakil [14] identified non-lactic bacteria as part of the microbial consortium involved in natural fermentation of tigernut-milk drink using conventional bacterial identification method. Majority of the non-lactic bacteria isolated from the natural fermented tigernut-milk drink have little or no importance to fermentation but an indication of product contamination which could be pathogenic to humans. The non-lactic acid bacteria isolated are *Bacillus* sp. *Escherichia coli* and *Proteus* sp. The overall quality of fermented tigernut-milk drink depends on its sensory characteristics, microorganisms present in the drink among other requirements. The use of molecular identification methods to distinctly identify bacteria present in such products could be necessary for product improvement.

This study is aimed at identifying non-lactic bacteria in lactic fermented tigernut-milk drink using molecular methods, then evaluate sensory characteristics of the freshly prepared ginger and garlic spiced lactic fermented tigernut-milk drinks and finally reevaluate the sensory properties of the drinks towards the end of its shelf life.

2. MATERIALS AND METHODS

2.1 Preparation of Lactic Fermented Plain Tigernut-milk Drink

Tigernut-milk drink was prepared using big yellow variety tigernut tubers. The drink was pasteurized at 72℃ for 15 min. The procedure adopted by [15] was used to isolate LAB from 'ogi' and then characterized using API CHL test kit. The method described by [16] which involved using 2% (w/v) mixed culture of LAB as a starter culture which comprised of *Streptococcus thermophilus, Lactobacillus plantarum, L. acidophilus* and *L. brevis* was adopted.

2.2 Isolation of Surviving Bacteria in Lactic Fermented Plain Tigernut-milk Drink

Pour plate method was used to inoculate 1 ml fermented tigernut-milk drink on freshly prepared

de Man, Rogosa and Sharpe agar plates. Distinct bacterial colonies were picked from the culture plate and were subjected to Gram staining and catalase test. Subsequently, the isolates were inoculated into Luria-Bertani (LB) broth and incubated at 37℃ overnight followed by the process of DNA extraction (crude method).

2.3 Molecular Characterization of Non-Lactic Bacteria in Lactic Fermented Tigernut-milk Drink

The steps described by [17] for molecular identification of DNA of pure isolates was adopted in identifying bacterial isolates from the lactic fermented tigernut-milk drink. The steps involved are extraction of DNA, quantification of the extracted DNA, amplification of the 16S rRNA, amplification of internal transcribed space (ITS), sequencing and phylogenetic tree analysis [18]. The nucleotide sequences obtained for each strain were edited using bioinformatics algorithm trace edit after which similar sequences were downloaded from NCBI database using BLASTIN. The nucleotide sequences obtained were aligned using ClustalX and Neighbor-Joining method described by [19] were used to deduce evoluntionary history of the isolates using [20] as a guide. Computing of evolutionary distances was carried out using the Jukes-Cantor method stated in [21].

2.4 Preparation of Spicy Lactic Fermented Tigernut-milk Drink

The freshly prepared lactic fermented tigernut-milk drink was separately spiced with 3% (w/v) ginger, 5% (w/v) ginger, 3% (w/v) garlic and 5% (w/v) garlic in line with the method described by [16].

2.5 Storage of Spicy Lactic Fermented Tigernut-milk Drink

The freshly prepared ginger and garlic spiced lactic fermented tigernut-milk drinks were stored at ambient (28±2℃) and refrigeration (4±2℃) temperature for 8 weeks in line with a previous study carried out by [22] which reported shelf life of the products as 8 weeks based on safe dairy products requirements by Codex Alimentarius Commission.

2.6 Sensory Evaluation of Spicy Lactic Fermented Tigernut-milk Drink

A blind organoleptic test for sensory qualities (appearance, colour, aroma, flavour, taste, aftertaste and overall acceptability) of freshly prepared 3%, 5% garlic spiced; 3%, 5% ginger spiced lactic fermented tigernut-milk drinks were evaluated by untrained 20 sensory panelists using a similar procedure described by [14,23]. At week 8, sensory evaluation of the ginger and garlic spiced lactic fermented tigernut-milk drinks stored at ambient and refrigeration temperature was repeated based on 8 weeks shelf life of the drinks previously determined by [16].

3. RESULTS AND DISCUSSION

DNA of five non-lactic bacterial isolates from lactic fermented tigernut-milk drink placed on agarose gel inside an electrophoresis tank connected to a high voltage power supply formed distinct bands showing amplified 16S rRNA gene is depicted in Fig. 1. The evolutionary relatedness of the bacterial isolates is shown in Fig. 2. The isolates were identified as *Bacillus amyloliquefaciens* strain D203 (SUB2424304 MA KY630545), *Bacillus methylotrophicus* strain XJAJ2 (SUB2424304 MG KY630549), *Bacillus amyloliquefaciens* strain BA-07 (SUB2424304 MB KY630546), *Bacillus amyloliquefaciens* strain WS-8 (SUB2424304 MD KY630547) and *Acinetobacter* sp. strain NC 41 (SUB2424304 MF KY630548).

Bacillus species are generally spore formers while some of them produce lactic acid as one of its fermentation end product. Few researchers have studied probiotic potential of spore forming lactic acid bacteria (SFLAB). This group of bacteria share common characteristics with *Bacillus* genera (motile and spore formers) and *Lactobacillus* genera (produce lactic acid, microaerophilic). Based on available literature, the *Bacillus* species isolated from lactic fermenting tigernut-milk drink is yet to be categorized as SFLAB. However, its probiotic potentials have been studied by very few researchers [24].

Bacillus amyloliquefaciens is a non-pathogenic rod-shaped aerobic bacterium. It is Gram positive and catalase positive. *Bacillus amyloliquefaciens* exhibit high resistance to environmental changes. This could be the reason *Bacillus* sp.

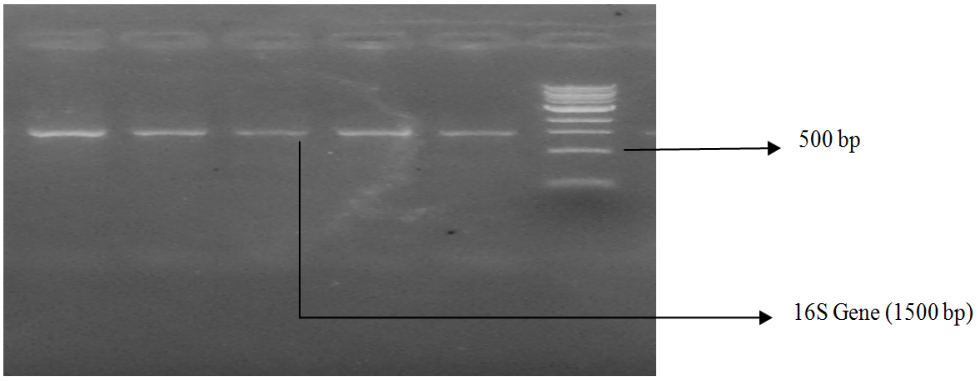

Fig. 1. Agarose electrophoresis of amplified 16S rRNA Gene of bacterial Isolates
Key: Lane 1-5 represents the isolates while lane M is the 1kb Quick-Load DNA molecular ladder

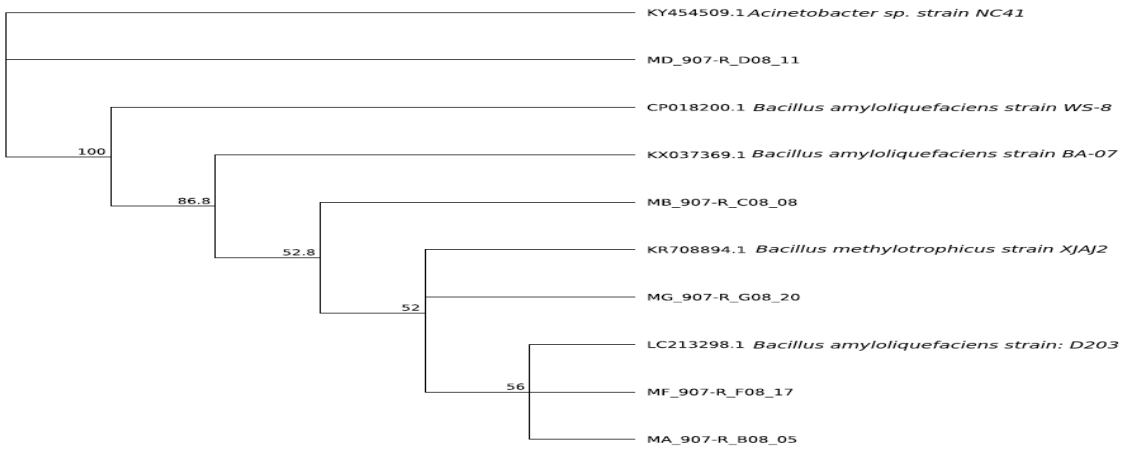

Fig. 2. Phylogenetic tree of evolutionary relationship between bacterial isolates

survived in lactic fermented tigernut-milk drink after pasteurization. There is a close relationship between *Bacillus amyloliquefaciens* and *Bacillus subtilis* which was previously assigned a subspecie status (*Bacillus subtilis* subspecie *amyloliquefaciens*) before further studies revealed many physiological and biochemical specificities of *Bacillus amyloliquefaciens* [25]. One notable difference between the two species is that *Bacillus amyloliquefaciens* produces more extracellular enzyme than *Bacillus subtilis* [26]. *Bacillus amyloliquefaciens* has the ability to exhibit broad spectrum antibacterial activity. It is important to note that this bacterium produce α-amylase which is an enzyme that aid the digestion of carbohydrates. This could be responsible for the reduction in carbohydrate content during storage of spicy lactic fermented tigernut-milk drink. Other enzymes produced by *Bacillus amyloliquefaciens* are cellulases, proteases and metalloproteases. A study to determine the effect of administering probiotic *Bacillus amyloliquefaciens* on growth performance and hematological variables of Nile tilapia was carried out by [27]. In a related study, [28] reported that *Bacillus amyloliquefaciens* is the predominant *Bacillus* species isolated from ready-to-eat foods.

The 16S identification of *Bacillus sp*. revealed that *Bacillus amyloliquefaciens* and *Bacillus methylotrophicus* are closely related [29]. Studies have identified *Bacillus methylotrophicus* C14 as a probiotic strain which has positive effect when it was introduced in the intestine of birds infected with *Salmonella gallinarum* [30]. Sim [31] demonstrated that *Bacillus methylotrophicus* C14 could have the potential of functioning as a probiotic. Meanwhile, [32] described in detail the nature of a novel specie of *Bacillus* which they

proposed to be named *Bacillus methylotophicus* sp. nov.

Acinetobacter species/johnsonii/baumannii/junii, *Bacillus* sp, *Lactobacillus* sp, *Streptococcus thermophilus* are part of microbiota in milk [33]. *Bacillus mycoides, Bacillus thuringiensis* and *Acinetobacter berezinae* have been identified as part of numerous microorganisms present in maize during its fermentation for the production of ogi. *Bacillus* sp. and *Acinetobacter* sp. can be referred as soil bacteria. Okeke [17] isolated *Acinetobacter* spp, *Bacillus* sp and other genera from 'kenkey' and white maize grains which they steeped for 24 h for production of 'ogi'. They pointed out that *Bacillus* sp is involved in food fermentations practiced in African and Asian countries but *Acinetobacter* sp. seems not to play any beneficial role in fermentation process.

Table 1 shows the average sensory evaluation score for sensory attributes of spicy lactic acid bacteria fermented tigernut-milk drinks. Based on overall acceptability, freshly prepared 5% ginger spiced and 3% garlic spiced lactic fermented tigernut-milk drink was assigned the highest and least average sensory evaluation score 8.15 and 6.75 interpreted as being very acceptable and slightly acceptable, respectively based on 9 point Hedonic scale. Considering the average sensory evaluation scores of freshly prepared spicy lactic fermented tigernut-milk drinks and the average sensory evaluation scores of the same drink after being subjected to ambient and refrigeration temperature storage for 8 Weeks, Table 1 revealed that 5% garlic spiced and 5% ginger spiced lactic fermented tigernut-milk drink was the least and most preferred drink, respectively.

Flavouring agents of different concentrations - 3% (w/v) garlic, 5% (w/v) garlic, 3% (w/v) ginger and 5% (w/v) garlic separately added to lactic fermenting tigernut-milk drink did not significantly influence the colour of freshly prepared lactic fermented tigernut-milk drink at Week 0. The reason the sensory panelists were not able to detect significant differences in the spicy lactic fermented tigernut-milk drink could also be that the concentration of the flavouring agents added to the lactic fermented tigernut-milk drink was little to have caused significant changes in colour/appearance of the spicy tigernut-milk drink at Wk 0.

Similarly, the sensory panelist reported no significant difference in respect to taste, aftertaste, aroma, flavour, taste and overall acceptability of 3% and 5% ginger spiced lactic fermented tigernut-milk drink. A similar result trend was also observed between 3% and 5% garlic spiced lactic fermented tigernut-milk drink. Since 3% (w/v) and 5% (w/v) concentration of ginger are close; 3% (w/v) and 5% (w/v) concentration of garlic are also close, this could be the reason the average sensory evaluation score for all the sensory attributes including overall acceptability between two lactic fermented tigernut-milk drinks separately spiced with 3% (w/v) and 5% (w/v) as well as another two lactic fermented tigernut-milk drinks separately spiced with 3% (w/v) and 5% (w/v) garlic were not significantly different. On the contrary, the same concentration of ginger and garlic separately added to lactic fermented tigernut-milk drink resulted in significant differences in all the sensory attributes due to uniqueness of their flavours. That is to say, ginger and garlic of the same concentration are unique flavouring agents which influenced the sensory attributes of lactic fermented tigernut-milk drinks evaluated except colour/appearance at Wk 0. A related study carried out by [34] revealed that ginger spiced tigernut-milk drink is a very acceptable tigernut-milk based on organoleptic assessment. Their study further revealed that garlic spiced tigernut-milk drink had low acceptability rating because of its flavour and taste. However, it is interesting to note that garlic demonstrated better effectiveness than ginger in functioning as a preservative despite being less preferable to ginger in terms of sensory attribute. Mohammed [35] reported that there were significant differences in sensory attributes of preserved and unpreserved tigernut milk. It was revealed from their study that average sensory score for taste, aroma, colour, mouth feel and overall acceptability of ginger spiced tigernut-milk drink prepared separately using brown and yellow variety tigernut tubers was higher than similar tigernut-milk drinks that was not spiced with ginger extract.

The result obtained from sensory evaluation of spicy lactic fermented tigernut-milk drinks by the 20 member sensory panelist indicates that a larger population of consumers of this product will prefer the ginger spiced lactic fermented tigernut-milk drink to garlic spiced lactic fermented tigernut-milk drink. The characteristic flavour of garlic left even after taste may not be liked by many people. Probably, the 20 sensory panelists considered 3% (w/v) and 5% (w/v) garlic added to lactic fermented tigernut-milk drink as not being moderate. World Health

Organization (WHO) recommend daily consumption of 2-5 g of fresh garlic, 0.3-1.0 g dry extract and 0.4-1.2 g dry powdered garlic [36].

In terms of overall acceptability, spicy lactic fermented tigernut-milk drinks stored at refrigeration temperature for 8 Weeks were assigned higher sensory score than the freshly prepared drink as well as spicy lactic fermented tigernut-milk drinks stored at ambient temperature except 5% garlic spiced lactic fermented tigernut-milk drink which had the same average sensory score 6.90 assigned to the tigernut-milk drink subjected to ambient and refrigeration temperature storage at Week 8 which was equally higher than 6.85 assigned to the freshly prepared tigernut-milk drink. Rapid biochemical reactions and microbial activities that occurred in spicy lactic fermented tigernut-milk drinks during ambient temperature storage could be the reason lower average sensory score was assigned to the tigernut-milk drinks compared with tigernut-milk drinks stored at refrigeration temperature. Based on total culturable heterotrophic bacterial count in line with requirements of Codex Alimentarius Commission for classifying dairy product as safe, shelf life of the lactic fermented tigernut-milk drinks was reported as 8 weeks by [22]. Apart from microbial quality of the product, sensory property of the product is also necessary to guarantee consumer acceptability towards the end of the product shelf life. Therefore, this study firstly considered microbial quality of the drinks in carrying out sensory evaluation of the products. The next stage of this product development shall take into consideration the sensory scores and microbial quality of the products to arrive at a shelf life that will not significantly affect customers' satisfaction. Higher sensory score for the refrigerated lactic fermented tigernut-milk drinks reported in this study could be as result of refrigeration temperature storage which reduced the activities of spoilage microorganisms in the drink which could have impacted the sensory attribute of the freshly prepared spicy lactic fermented tigernut-milk drink. It could also be that the improved sensory properties of lactic fermented tigernut-milk drink were better preserved during refrigeration temperature storage compared with ambient temperature storage. Despite the differences in sensory score for overall acceptability of spicy lactic fermented tigernut-milk drinks subjected to refrigeration temperature storage for 8 Wks, it is interesting to report that the interpretation of the sensory score assigned to the tigernut-milk drinks based on 9 point Hedonic scale were the same except 3% and 5% garlic spiced lactic fermented tigernut-milk drink. In broader terms, the interpretation of sensory score for spicy lactic fermented tigernut-milk drinks stored at refrigeration temperature was generally acceptable. This is in agreement with sensory evaluation of fermented tigernut-milk drink reported by [37].

Table 1 shows that the 3% garlic spiced and 5% garlic spiced lactic fermented tigernut-milk drinks subjected to both storage conditions (ambient and refrigeration temperature) at Week 8 was moderately and slightly acceptable, respectively in terms of overall acceptability. On the contrary, the refrigerated 3% and 5% ginger spiced lactic fermented tigernut-milk drink at Week 8 was very acceptable whereas 3% and 5% ginger spiced lactic fermented tigernut-milk drink subjected to ambient temperature storage at Week 8 was moderately acceptable. It could be that refrigeration and ambient temperature storage had less effect in the overall acceptability of either 3% garlic spiced or 5% garlic spiced lactic fermented tigernut-milk drink compared with 3% ginger spiced or 5% ginger spiced lactic fermented tigernut-milk drink.

Considering the sensory score for most of the sensory attributes of spicy lactic fermented tigernut-milk drinks, Table 1 revealed that there was slight improvement in sensory score for spicy lactic fermented tigernut-milk drinks after they had been stored at ambient and refrigeration temperature for 8 weeks. This could be as a result of weakened pungent characteristics of garlic and ginger in the lactic fermented tigernut-milk drinks. It is interesting to observe from this study that sensory score for spicy lactic fermented tigernut-milk drinks were higher than sensory score for similar drink separately spiced with different flavouring agents reported by [37]. The improved sensory scores for spicy lactic fermented tigernut-milk drinks reported in this study could be as a result of activities of mixed culture of lactic acid bacteria in the spicy tigernut-milk drinks. Previous studies reported that lactic acid fermentation improves some sensory qualities of many fermented products [37]. Fermentation of spicy tigernut-milk drink by lactic acid bacteria possibly improved the taste and flavour of the drink which was reported by the 20 sensory panelists.

The sensory score for taste, aroma and flavour of freshly prepared 3% ginger spiced lactic fermented tigernut-milk drink was higher than

similar tigernut-milk drink stored at ambient and refrigeration temperature at Week 8. Lower sensory scores assigned to 3% ginger spiced lactic fermented tigernut-milk drink subjected to ambient and refrigeration storage condition could be as a result of pungency of ginger in the lactic fermented tigernut-milk drink weakened during the period of storage. Sensory evaluation of the spicy lactic fermented tigernut-milk drinks revealed that weakened pungency of garlic and ginger present in the drink might be the reason the sensory panelist assigned higher and lower sensory scores, respectively to the drink. The reduction in average sensory evaluation score for taste, aroma and flavour of freshly prepared 3% ginger spiced lactic fermented tigernut-milk after it was subjected to ambient and refrigeration temperature storage at Week 8 could also be as a result of biochemical reactions which occurred during the period of storage which released off-flavours into the drink. The off-flavours might have directly affected the taste, aroma and flavour of spicy lactic fermented tigernut-milk drinks during ambient and refrigeration temperature storage. Despite the differences in average sensory score of freshly prepared 3% ginger spiced lactic fermented tigernut-milk drink and a similar drink stored at ambient and refrigeration temperature for 8 weeks in terms of taste, aroma and flavour, it is interesting to observe that taste of 3% ginger spiced lactic fermented tigernut-milk drink stored for 8 Weeks at refrigeration and ambient temperature had the same average sensory score. This could be as a result of minimal effect of refrigeration and ambient temperature storage on taste of 3% ginger spiced lactic fermented tigernut-milk drink. On the contrary, the average sensory score for colour/appearance of 3% ginger spiced lactic fermented tigernut-milk drink subjected to ambient and refrigeration temperature storage was higher than the freshly prepared drink. The improvement in average sensory score could be as a result of biochemical reactions that occurred in 3% ginger spiced lactic fermented tigernut-milk drink during ambient and refrigeration temperature storage.

Table 1 shows that average sensory score of all the sensory attributes of freshly prepared 5% ginger spiced lactic fermented tigernut-milk drink was higher than average sensory score assigned to a similar drink after it had been subjected to ambient and refrigeration temperature for 8 weeks. This could be as a result of characteristic sensory attribute of ginger appreciated by the sensory panelist weakened during the period of storage. However, there was slight improvement in the sensory score for overall acceptability of the refrigerated drink compared with the freshly prepared drink and the one subjected to ambient temperature storage for 8 weeks. Similarly, there was significant improvement in sensory score for colour/appearance and overall acceptability of refrigerated 3% ginger spiced lactic fermented tigernut-milk drink at Week 8 compared with the freshly prepared drink.

It is interesting to observe that there was improvement in sensory score for all the sensory attributes of 3% garlic spiced lactic fermented tigernut-milk drink stored at refrigeration temperature compared with the freshly prepared tigernut-milk drink and the one subjected to ambient temperature storage at Week 8 except colour/appearance. This could be as a result of pungency of garlic in the tigernut-milk drink weakened during the period of storage was better appreciated by sensory panelist.

Sensory score for freshly prepared 5% garlic spiced lactic fermented tigernut-milk drink and similar tigernut-milk drink stored at 28±2℃ and 4±2℃ for 8 weeks is interpreted as slightly acceptable except colour/appearance and few other sensory attributes which are moderately acceptable. This could be as a result of high concentration of garlic in 5% garlic spiced lactic fermented tigernut-milk drink was less appreciated by the sensory panelist because of pungency of garlic in the drink. Reduction in the pungency of garlic in 5% garlic spiced lactic fermented tigernut-milk drink during the period of storage could be the reason for slight improvement in the sensory score of all the sensory attributes except colour/appearance of 5% garlic spiced lactic fermented tigernut-milk drink.

An overview of the sensory scores assigned to freshly prepared garlic spiced lactic fermented tigernut-milk drinks and similar tigernut-milk drinks stored at ambient and refrigeration temperature at week 8 revealed that the average sensory scores were closer compared with ginger spiced lactic fermented tigernut-milk drink. Probably, the concentration of antimicrobial substances in 3% (w/v) garlic and 5% (w/v) garlic used to spice lactic fermented tigernut-milk drink was more effective against microbial activities in the drink which produce metabolic end products that could impact on the sensory attributes of the drink compared with 3% (w/v) ginger and 5% (w/v) ginger used to spice lactic fermented tigernut-milk drink.

Table 1. Average sensory score for spicy lactic acid bacteria fermented tigernut-milk drinks

Sensory attributes	3% ginger spiced tigernut-milk drink			3% garlic spiced tigernut-milk drink			5% ginger spiced tigernut-milk drink			5% garlic spiced tigernut-milk drink		
	FR	AT	RT	FR	AT	RT	FR	AT	RT	FR	AT	RT
Colour	8.15±1.14^{a}	8.25±0.97^{b}	8.30±0.66^{c}	7.35±1.14^{a}	7.25±0.72^{a}	7.85±0.88^{ab}	8.10±0.97^{a}	7.95±1.05^{b}	7.35±0.81^{a}	7.40±1.47^{a}	7.05±0.95^{a}	7.40±0.88^{a}
Taste	8.30±0.98^{b}	7.90±0.97^{b}	7.90±0.91^{b}	6.90±0.97^{a}	7.05±0.89^{a}	7.55±0.88^{ab}	8.1±0.64^{b}	7.75±1.02^{b}	8.00±0.80^{b}	6.60±1.05^{a}	6.95±0.61^{a}	7.10±0.85^{a}
Aftertaste	7.85±0.81^{b}	7.80±0.89^{b}	7.90±0.79^{c}	6.40±1.54^{a}	7.15±0.88^{a}	7.25±0.91^{ab}	8.1±0.85^{b}	7.85±0.75^{b}	7.55±0.76^{bc}	6.25±1.25^{a}	6.8±0.89^{a}	6.85±0.93^{a}
Aroma	7.85±0.93^{b}	7.70±1.03^{b}	7.50±0.89^{b}	6.70±1.22^{a}	7.25±0.97^{ab}	7.20±0.70^{ab}	7.85±0.75^{b}	7.8±0.89^{b}	7.65±0.67^{b}	6.75±1.02^{a}	6.95±0.89^{a}	7.00±0.73^{a}
Flavour	8.40±0.68^{b}	7.95±0.76^{b}	7.80±0.70^{b}	6.75±1.16^{a}	7.3±1.03^{a}	7.20±0.70^{a}	8.30±0.66^{b}	7.6±0.94^{ab}	7.65±0.75^{b}	6.20±1.44^{a}	7.25±0.91^{a}	7.00±0.65^{a}
Overall acceptability	8.00±0.97^{b}	7.60±0.88^{b}	8.10±0.91^{c}	6.75±0.97^{a}	7.25±0.97^{ab}	7.50±0.76^{b}	8.15±0.75^{b}	7.70±1.08^{b}	8.20±0.77^{c}	6.85±1.35^{a}	6.90±0.91^{a}	6.90±0.72^{a}

Values show means of 20 member sensory panelist score ± SD. Figures with different superscript across the row for 'FR' spicy tigernut-milk drinks, 'AT' storage of spicy tigernut-milk drinks at Week 8 and 'RT' storage of spicy tigernut-milk drinks, respectively are significantly different (P<0.05). 'FR' depict Fresh; 'AT' depict Ambient Temperature (28±2℃); 'RT' depict Refrigeration Tempe rature (4 ±2℃)

Table 2. Ranking of spicy lactic fermented tigernut-milk drinks

Panelist	3% ginger spiced drink			5% ginger spiced drink			3% garlic spiced drink			5% garlic spiced drink		
	FR	AT	RT	FR	AT	RT	FR	AT	RT	FR	AT	RT
1	2	2	2	1	1	1	4	3	3	3	4	4
2	2	2	1	1	1	2	3	4	3	4	3	4
3	1	2	2	2	1	1	4	4	3	3	3	4
4	1	2	1	2	1	2	3	3	3	4	4	4
5	2	2	2	1	1	1	3	3	4	4	4	3
6	2	3	2	1	4	1	4	1	4	3	2	3
7	2	4	1	1	3	2	3	2	3	4	1	4
8	1	2	2	2	1	1	4	3	3	3	4	4
9	2	2	1	1	1	2	4	3	3	3	4	4
10	1	2	1	2	1	2	3	4	3	4	3	4
11	4	3	2	3	4	1	2	1	3	1	2	4
12	2	2	1	1	1	2	3	3	3	4	4	4
13	3	4	1	4	3	2	2	2	3	1	1	4
14	2	2	2	1	1	1	3	3	3	4	4	4
15	1	2	2	2	1	1	3	4	3	4	3	4
16	4	3	3	1	1	4	2	4	2	3	2	1
17	1	2	1	2	1	4	3	3	2	4	4	3
18	1	2	2	2	1	1	3	4	4	4	3	3
19	2	3	3	1	4	1	3	1	2	4	2	4
20	4	2	2	3	1	1	2	3	3	1	4	4
Sum (Column total) T_P	40	48	34	34	33	33	61	58	60	65	61	73

Key: 'FR' depict fresh; 'AT' depict ambient temperature (28±2℃); 'RT' depict refrigeration temperatu re (4±2℃)

Table 2 shows the product ranking of freshly prepared spicy lactic fermented tigernut-milk drinks together with a similar drink subjected to ambient and refrigeration temperature storage at Wk 8. Friedman rank test at a significant level of 5% (p=0.05) revealed that there was no significant difference in ranking freshly prepared 3% ginger spiced lactic fermented tigernut-milk and a similar drink separately stored at 28±2℃ and 4±2℃ at Wk 8 by the 20 sensory panelist. Similarly, there was no significant difference in the ranking of freshly prepared 5 % ginger spiced lactic fermented tigernut-milk drink and a similar drink stored at 28±2℃ and 4±2℃ at Wk 8 by the 20 member sensory panelist. Ranking of freshly prepared 3% garlic spiced lactic fermented tigernut-milk drink and a similar drink after 8 Wks of storage at ambient and refrigeration temperature had no significant difference based on product ranking by the 20 sensory panelist. The ranking of freshly prepared 5% garlic spiced lactic fermented tigernut-milk alongside similar drink separately stored at 4±2℃ and 28±2℃ for 8 Wks had no significant difference based on Friedman rank test of the product ranking done by 20 sensory panelists. This shows consistency in the ranking of freshly prepared 3%, 5% ginger spiced; 3%, 5% garlic spiced lactic fermented tigernut-milk drinks together with similar tigernut-milk drinks subjected to refrigeration and ambient temperature storage at Wk 8 by the 20 member sensory panelist. Among the spicy lactic fermented tigernut-milk drinks, the sum of ranking down the column shown in Table 2 assigned to 5% ginger spiced and 5% garlic spiced lactic fermented tigernut-milk drink was the least and highest, respectively. This study has shown that 5% ginger spiced and 5% garlic spiced lactic fermented tigernut-milk drink as the most and least preferred tigernut-milk drink, respectively.

4. CONCLUSION

Ginger and garlic spiced lactic fermented tigernut-milk drinks are generally acceptable based on their sensory attributes. This study demonstrated that these flavouring agents had significant effect in the sensory attributes of spicy lactic fermented tigernut-milk drinks at ambient and refrigeration temperature storage. Non-pathogenic *Bacillus* sp. were identified in lactic fermenting tigernut-milk drink.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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