

Nutritional Composition of Ginger and Garlic Flavoured Lactic Acid Bacterial Tigernut-milk Drink Stored at Ambient and Refrigeration Temperature

ABSTRACT:

The effect of ambient (28 ± 2 °C) and refrigeration temperature (4 ± 2 °C) storage in the nutritional composition of tigernut-milk drink fermented by indigenous lactic acid bacteria (LAB) which was separately flavoured with ginger and garlic was determined. This study was carried out between August-October, 2016 at Federal Institute of Industrial Research Oshodi (FIIRO), Lagos. Tigernut tubers were used to prepare tigernut-milk. The drink was fermented by mixed culture of LAB for 12 hr at 45 °C. The LAB were isolated from 'ogi' using MRS agar and characterized using API 50 CHL test kit. The LAB fermented tigernut-milk drink was separately flavoured with 3 % (w/v) ginger, 5 % (w/v) ginger, 3 % (w/v) garlic and 5 % (w/v) garlic. The final products were stored at 28 ± 2 °C and 4 ± 2 °C for 12 wks and its nutritional composition monitored at 4 wk interval using conventional and rapid test methods. Results obtained from this study revealed that there was reduction in carbohydrate, moisture and lipid content during storage of the tigernut-milk drink preparations. Importantly, there was increase in protein content in the tigernut-milk drink preparations during storage at both conditions except in the 5 % (w/v) ginger flavoured tigernut-milk drink. Our result showed that between Wk 0-12, reduction in protein content during ambient and refrigeration temperature storage ranged from (6.48-11.24 %) and (6.07-10.91 %), respectively. There was also reduction in Ca, K, Mg and Zn content in the tigernut-milk drink preparations during ambient and refrigeration temperature storage. Remarkably, the high potassium content in 3 % (w/v) ginger and 3 % (w/v) garlic flavoured tigernut-milk could be more effective in prevention of some diseases in human body compared with lower potassium content in 5 % (w/v) ginger flavoured and 5 % (w/v) garlic flavoured tigernut-milk drink. Therefore, this study projects tigernut-milk fermented by LAB and separately flavoured with 3 % (w/v) ginger and 3 % (w/v) garlic as a nutritious drink to be preferably stored at refrigeration temperature.

Keywords: Tigernut-milk, Lactic acid bacteria, Nutritional composition, Flavouring agent, Fermented

1.0 INTRODUCTION

Tigernut-milk drink called 'Chufa de horchata', 'Atadwe', 'Kunun aya' in Spain, Ghana and Nigeria, respectively is a popular imitation milk (non-dairy) locally prepared using tigernut tubers (*Cyperus esculentus* Lativum) [1, 2]. It is a non-alcoholic beverage rich in nutrients [3, 4]. [5] in their study compared the proximate composition and physicochemical properties of Peak milk® and tigernut milk. Furthermore, proximate composition of pasteurized starter fermented varieties of tigernut milk was determined by [6]. Tigernut-milk drink is nutritious because tigernut tubers are rich in carbohydrate, fats (oils), protein as well as minerals [7].

Apart from being a nutritious and tasty beverage, tigernut-milk drink is recommended for diabetics because it does not contain sodium, lactose sugar, casein protein, gluten and cholesterol [5]. Consumption of tigernut milk helps to control body weight [8]. Due to increasing cost of cow milk in developing countries and growing demand to meet the requirement of her population, attention is gradually shifting to non-dairy milk such as tigernut-milk drink which is a cheaper product [7, 9].

The shelf life of tigernut-milk drink can be extended through addition of garlic and ginger, ~~storage at ambient temperature (28 ± 2 °C) and refrigeration temperature (4 ± 2 °C)~~. Pasteurization and irradiation can also prolong its shelf life. Garlic and ginger are natural flavouring agents. They are also natural tropical preservatives. The storage conditions adopted to preserve tigernut-derived products usually affect its nutritional composition. The fundamental benefit for consuming edible products is based on its nutritional composition. Several factors affect the nutritional composition of diverse edible products [3, 10, 11, 12, 13].

Therefore, this study is aimed at determining the effect of ambient and refrigeration temperature storage in the nutritional composition of lactic acid bacteria fermented tigernut-milk drink separately flavoured with ginger and garlic. The result obtained from this study will form the basis to recommend the tigernut-milk drink preparation and

appropriate storage condition that will preserve or improve its nutritional composition as a strategy to promote human healthiness.

2.0 MATERIALS AND METHODS

Fresh yellow variety tigernut tubers, dried ginger, dried garlic and freshly prepared *ogi* (akamu) used in this study were purchased from retailers in Oshodi Market Lagos State.

2.1 Preparation of lactic acid bacteria fermented tigernut-milk drink flavoured with ginger and garlic

Tigernut-milk drink was prepared using fresh yellow variety tigernut tubers [6]. LAB was isolated from *ogi* using deMan, Rogosa and Sharpe agar (MRS) and API 50 CHL (Biomerieux®, France) was used to identify the lactic acid bacteria (LAB) isolates [14]. *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus brevis* were identified. Mixed culture 2 % (w/v) LAB species was inoculated into 500 ml pasteurized (72 °C for 15 min.) tigernut-milk inside Erlenmeyer bottles. Flavouring agent of different concentration comprising 3 % (w/v) ginger, 5 % (w/v) ginger; 3 % (w/v) garlic and 5 % (w/v) garlic was separately added to the pasteurized tigernut-milk drink [3, 15].

2.2 Storage of flavoured lactic acid bacteria fermented tigernut-milk drink

The flavoured tigernut-milk drink preparations were separately stored at ambient (28±2 °C) and refrigeration temperature (4±2 °C) or 12 weeks.

2.3 Nutritional composition of flavoured lactic acid bacteria fermented tigernut-milk drink

The nutritional composition of the tigernut-milk drink was monitored at 4 weeks interval for 12 weeks. The crude protein content was determined using Micro-Kjeldahl method [16] and the fat content was determined using the method described by [7]. AND MS-70 moisture analyzer was used to determine moisture content. The carbohydrate content was determined using Cleg Anthrone method [17, 18, 19]. The Mg, K, Ca and Zn content of the tigernut-milk drink was determined by dry ash extraction method [7].

3. RESULTS AND DISCUSSION

Fig. 1 and 2 shows the carbohydrate content in flavoured tigernut-milk drink fermented by indigenous LAB during storage at ambient and refrigeration temperature, respectively for 12 Wks. The result indicated that there was reduction in carbohydrate content in the flavoured tigernut-milk drink during storage at ambient and refrigeration temperature except at Wk 4 during storage of 5 % (w/v) garlic flavoured tigernut-milk at ambient temperature. [20] reported reduction in carbohydrate content during storage of a similar fermented beverage.

The activities of LAB in the tigernut-milk drink which utilized available nutrients and transformed it into energy required for growth and other cellular activities could be responsible for the reduction in carbohydrate content. Higher concentration of ginger and garlic from 3 to 5 % (w/v) could have provided the bacterial culture larger quantity of carbohydrate to utilize. This could be the reason for significant carbohydrate reduction in the tigernut-milk drink flavoured with 5 % (w/v) ginger or 5 % (w/v) garlic compared to minimal carbohydrate reduction in the tigernut-milk drink flavoured with 3 % (w/v) ginger or 3 % (w/v) garlic.

The carbohydrate content in ginger and garlic may not be largely responsible for differences in carbohydrate content of the flavoured tigernut-milk drink. [21] reported that the carbohydrate content in ginger and garlic is 72.84 % and 73.03 %, respectively. It could be that during storage, fermentation still occurred which resulted in the conversion of lactose to lactic acid. This could cause reduction in carbohydrate content during storage of the tigernut-milk preparations. The reduction in carbohydrate could also be as a result of enzymes produced by the fermenting LAB in the tigernut-milk drink which breaks down carbohydrate into simple sugars [22].

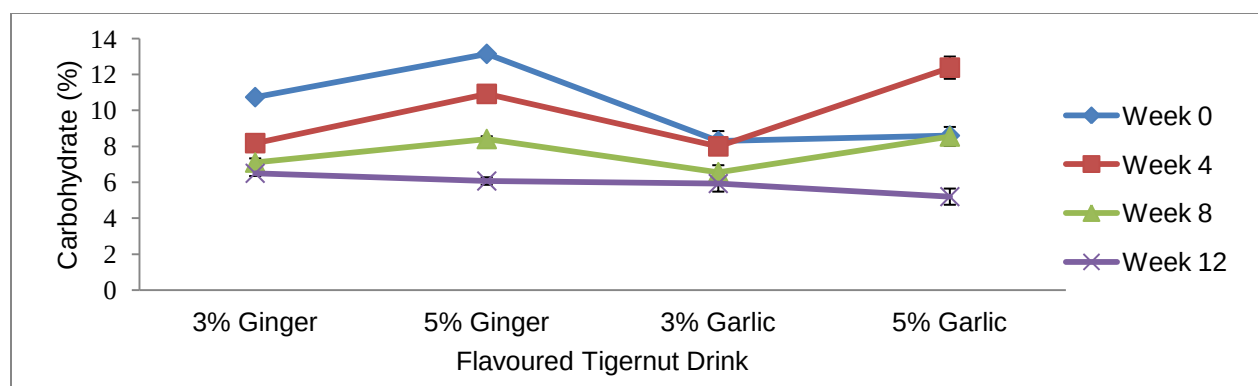


Fig. 1. Carbohydrate content of flavoured tigernut-milk drink during storage at ambient temperature

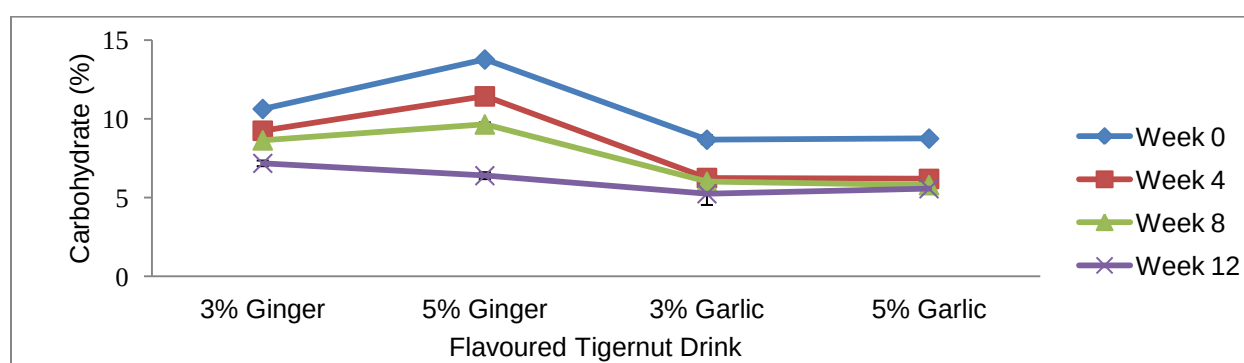


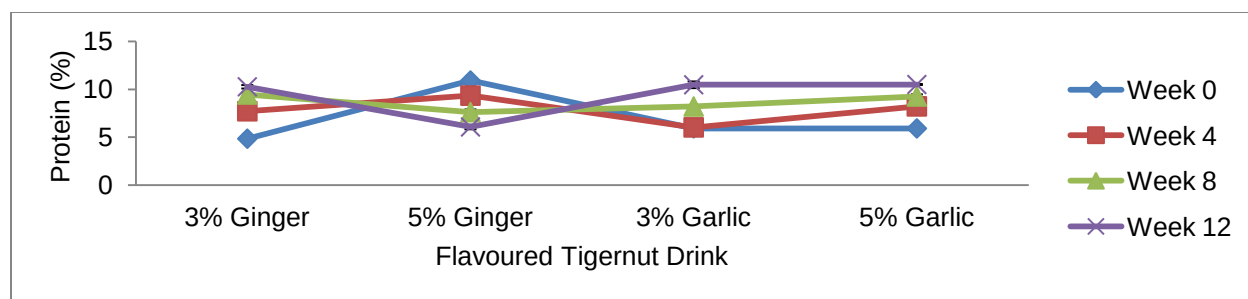
Fig. 2. Carbohydrate content of flavoured tigernut-milk drink during storage at refrigeration temperature

The protein content in flavoured tigernut-milk drink stored at ambient and refrigeration temperature, respectively for 12 wks are depicted in Fig. 3 and Fig. 4, respectively. During both storage conditions, there was increase in protein content in the tigernut-milk preparations except in 5 % (w/v) ginger flavoured tigernut-milk drink which had its protein content reduced at Week 0 (10.91 %) – Week 12 (6.07 %). It could be that concentration of ginger in the tigernut-milk drink inhibited proteolytic activity of bacteria present in the tigernut- milk drink. Studies have demonstrated that different varieties of ginger depending on the site of cultivation, method of extraction and processing methods can result in variations in its chemical composition including protein content [23].

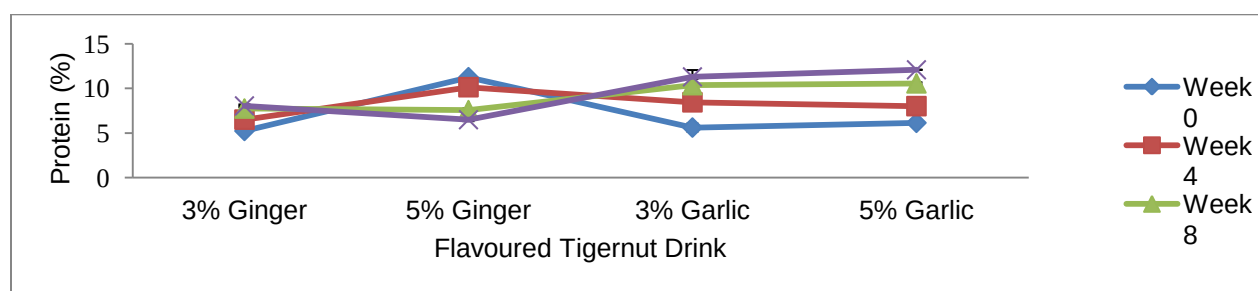
The increase in protein content could also be as a result of increase in microbial cells in the tigernut-milk drink. Furthermore, it could be that intensified activity of proteolytic enzymes produced by LAB during fermentation of tigernut-milk drink breaks down proteins into peptides and amino acids which could have contributed to the increase in protein content. Increase in population of viable probiotic LAB culture is not only beneficial to consumers because of its health benefits but it is also nutritionally advantageous because its protein content increased during storage [20].

Reduction in protein content during storage of tigernut-milk drink preparation is in agreement with similar studies carried out by [3]. According to [6], starter developed tigernut-milk is a good protein source. Furthermore, pasteurization and fermentation improves the nutritional quality of tigernut milk. The higher protein content in garlic flavoured tigernut-milk drink compared to the ginger flavoured tigernut-milk drink could be as a result of higher crude protein content in garlic compared with ginger. The protein content in garlic and ginger is 27.4 % and 7.8 %, respectively [24]. In a similar study, [21] reported that protein content of garlic and ginger is 17.35 % and 8.58 %, respectively. A related study carried out by [25] reported increase in protein content in garlic based yoghurt which could be attributed to proteolytic activity of LAB which hydrolysed the protein into peptides and amino acids. The proteolytic enzyme complement in LAB probably has ability to split most types of peptide bonds. It is suggested that

133 proteolytic enzyme hydrolysed the protein in tigernut-milk drink, freeing amino acids which combined to form
 134 peptide bond and was transformed into protein.

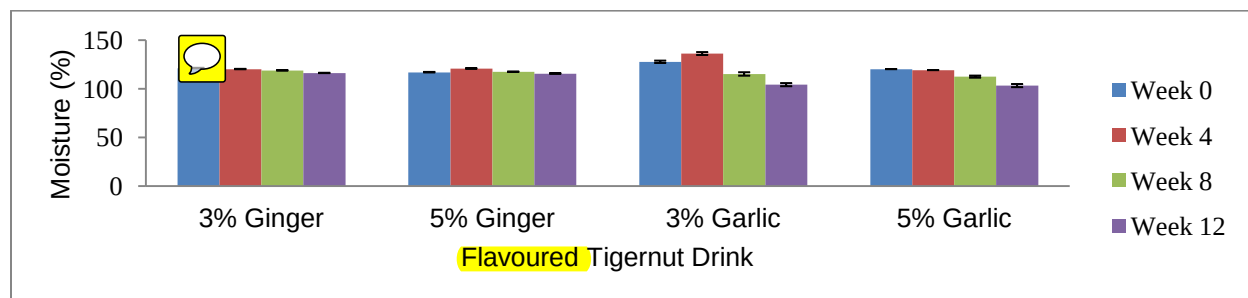


135
 136 **Fig. 3. Protein content of flavoured tigernut-milk drink during storage at ambient**
 137 **temperature**
 138



139
 140 **Fig. 4. Protein content of flavoured tigernut-milk drink during storage at refrigeration**
 141 **temperature**
 142

143 Fig. 5 and 6 shows the moisture content in different preparations of ginger and garlic flavoured tigernut-milk drink
 144 fermented by LAB which were stored at ambient and refrigeration temperature, respectively for 12 wks. The result
 145 shows there was very slight changes in moisture content of 3 % (w/v) and 5 % (w/v) ginger flavoured tigernut-milk
 146 drink during storage at ambient temperature. The reduction in moisture content could be as a result of increase in dry
 147 matter attributed to increase in population of viable LAB. According to [26], increase in dry matter usually result to
 148 decrease in moisture content of raffia sap subjected to fermentation. [24] reported that the dry matter of garlic and
 149 ginger is 90.30 g/100 g and 92.90 g/100 g, respectively. It could be that dry matter as a result of ginger and garlic
 150 separately added to the tigernut-milk drink fermented by LAB influenced the reduction in moisture content. High
 151 moisture content in any liquid beverage makes it a refreshing and thirst-quenching product. However, during
 152 ambient and refrigeration temperature storage, slim reduction in moisture content in the tigernut-milk drink
 153 preparations is an indication that its shelf life was slowly reducing [16].



154
 155 **Fig. 5. Moisture content of flavoured tigernut-milk drink during ambient temperature**
 156 **Storage**

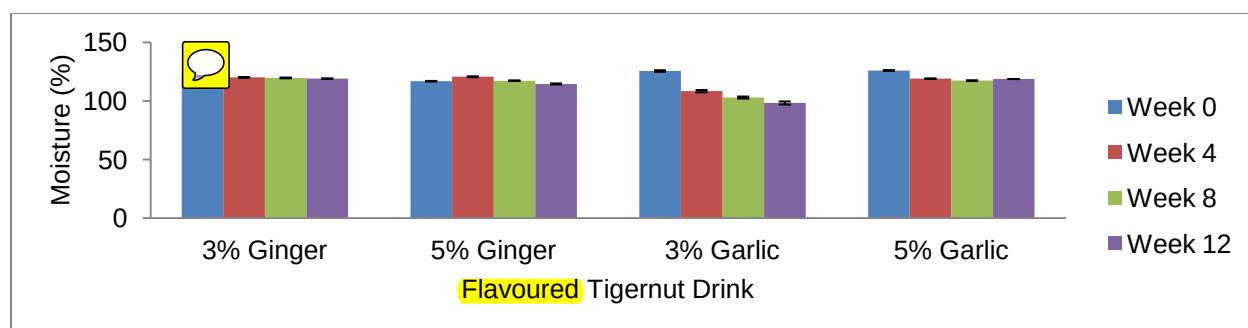


Fig. 6. Moisture content of flavoured tigernut-milk drink during refrigeration temperature storage

The lipid content of ginger and garlic flavoured tigernut-milk drink fermented by LAB stored at ambient and refrigeration temperature for 12 wks is presented in Fig. 7 and 8, respectively. During storage, there was reduction in lipid content of the flavoured tigernut-milk drink preparations. This result trend is in agreement with similar studies carried out by [3]. This could be as a result of lipolytic activity of enzymes produced by fermenting bacteria in the drink. Increase in concentration of ginger added to tigernut-milk drink from 3 to 5 % (w/v) probably increased its lipid content but similar increase in garlic resulted in slight increase in the lipid content in garlic flavoured tigernut-milk drink. Increase in concentration of ginger possibly inhibited the activities of lipolytic enzymes which resulted in more stability in lipid content compared with garlic. The ability of some microorganisms to utilize fatty acids as energy source results in fat content reduction in some food products. Reduction in carbohydrate, lipid and moisture content but increase in protein content during fermentation of tigernut-milk drink was reported by [3, 15, 20].

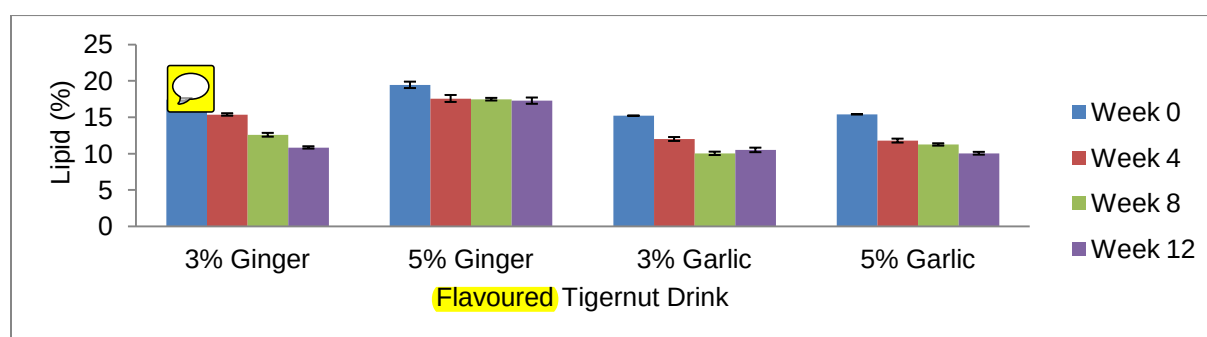


Fig. 7. Lipid content of flavoured tigernut-milk drink during ambient temperature storage

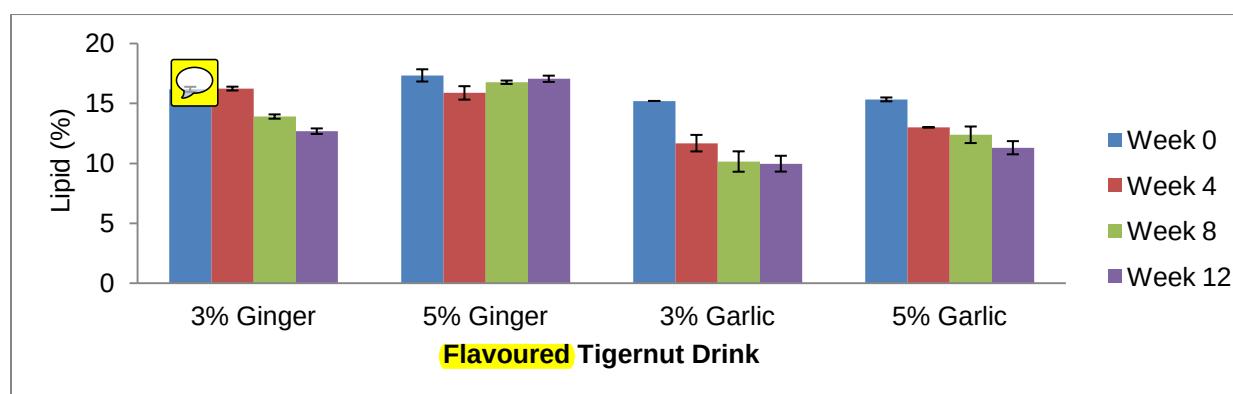


Fig. 8. Lipid content of flavoured tigernut-milk drink during refrigeration temperature storage

Table 1 shows the mineral content in ginger and garlic flavoured tigernut-milk drink fermented by LAB which were stored at ambient and refrigeration temperature for 12 wks. During ambient and refrigeration temperature storage, there was continuous reduction in calcium, potassium, magnesium and zinc content in the flavoured tigernut-milk drink preparations. [27] reported similar result trend during natural fermentation of soymilk nono. The reason for reduction in mineral content of fermenting tigernut-milk drink could be as a result of microorganisms which utilized the minerals for growth and metabolism. Although there was gradual reduction in mineral content, human deficiency in these essential minerals rarely occurs because most of the time the recommended values are usually exceeded in the diet consumed by human beings. Calcium is needed in the human body for blood clotting, mineralization of bone and teeth, hormone secretion and nerve transmission. Zinc is useful in human cell division. It plays a vital role in functionality of the enzyme associated with energy generation from carbohydrate, fat and protein. Zinc deficiency makes the process of healing of wounds slow, causes reduced appetite and suppression of immune system [28, 29]. Potassium is one of the essential nutrients required for the maintenance of total body fluid value as well as acid and electrolyte balance. It is also required for normal cell function [30]. Magnesium is needed during protein synthesis, production of energy and contraction of muscles. In fact, regular consumption of edible products that contains very little quantity of magnesium will increase the risk of individuals to suffer coronary heart disease and Type 2 diabetes [31]. It is interesting to note that tigernut-milk drink contains more quantity of magnesium than cow milk [5].

Table 1. Mineral content of 3 % (w/v) ginger flavoured tigernut-milk stored at ambient and refrigeration temperature

Wk	Ca (mg/L)		K (mg/L)		Mg (mg/L)		Zn (mg/L)	
	AT	RT	AT	RT	AT	RT	AT	RT
0	61.98±0.64 ^d	61.03±0.20 ^d	2119.07±0.24 ^d	2095.05±0.20 ^d	58.46±0.18 ^d	61.06±0.22 ^d	4.74±0.17 ^d	4.52±0.23 ^a
4	60.48±0.47 ^c	60.28±0.35 ^c	2006.15±0.21 ^c	2008.11±0.22 ^c	58.08±0.26 ^c	60.60±0.35 ^c	4.03±0.21 ^c	4.58±0.17 ^b
8	55.53±0.27 ^b	59.83±0.17 ^b	2001.41±0.33 ^b	2006.39±0.26 ^b	50.76±0.16 ^b	58.58±0.33 ^b	3.71±0.16 ^b	4.56±0.18 ^b
12	51.62±0.17 ^a	58.77±0.15 ^a	1986.06±0.26 ^a	2004.52±0.30 ^a	42.75±0.22 ^a	55.08±0.22 ^a	3.56±0.17 ^a	4.56±0.18 ^a

Values show means of triplicate analysis ±SD. Figures with different superscript down the column, are significantly different (P<0.05). 'AT' denote ambient temperature; 'RT' denote refrigeration temperature.

Table 2 shows the mineral content in 5 % (w/v) ginger flavoured tigernut-milk drink stored at ambient and refrigeration temperature for 12 weeks. During the storage period, there was continuous reduction in Ca, K, Mg and Zn content in the tigernut-milk drink preparations. The mineral content in 5 % (w/v) ginger flavoured tigernut-milk drink stored at refrigeration temperature was higher than similar tigernut-milk drink stored at ambient temperature. There is a wide difference between potassium content in 3 % (w/v) ginger flavoured and 5 % (w/v) ginger flavoured tigernut-milk drink during storage at both refrigeration and ambient temperature. A similar relationship was observed during ambient and refrigeration temperature storage of 3 % (w/v) garlic flavoured and 5 % (w/v) garlic flavoured tigernut-milk drink presented in Table 3 and 4. The high quantity of potassium in 3 % (w/v) ginger flavoured and 3 % (w/v) garlic flavoured tigernut-milk drink could be as a result of low concentration of ginger and garlic which favoured the release of chelated complex compounds as a result of microbial activities involved in fermentation [27].

In a related study, [32] reported a drastic reduction in potassium content of a fermented sorghum-Irish potato gruel attributed to the effect of starter culture which comprise *Lactobacillus acidophilus*, *L. fermentum*, *L. plantarum*, *Geotrichum candidum* and *Saccharomyces cerevisiae*. There is no convincing report about adverse effect of high intake of potassium [33]. Rather, the consumption of diets or liquid beverages low in potassium content over a long period predisposes the body to disease conditions such as hypertension, arteriosclerosis, osteoporosis, Alzheimer's disease, kidney stone and arthritis [34]. The Food and Nutrition Board of the Institute of Medicine recommend 4700 mg/day potassium intake. On their part, potassium intake recommended by the WHO/Food and Agriculture Organization Expert Consultation is 4700 mg/day [33]. The reduction in mineral content during storage of flavoured tigernut-milk drink corroborates a related research study by [35] which involved development of low-fat symbiotic yoghurt using water buffalo milk.

227 **Table 2. Mineral content of 5 % (w/v) ginger flavoured tigernut-milk stored at ambient**
 228 **and refrigeration temperature**

Wk	Ca (mg/L)		K (mg/L)		Mg (mg/L)		Zn (mg/L)	
	AT	RT	AT	RT	AT	RT	AT	RT
0	71.66±0.36 ^d	74.75±0.48 ^d	14.43±0.59 ^d	14.72±0.26 ^c	60.35±0.60 ^c	60.15±0.27 ^b	5.17±0.20 ^c	5.08±0.17 ^d
4	66.32±0.56 ^c	72.74±0.45 ^c	12.11±0.32 ^c	13.40±0.12 ^b	56.49±1.42 ^b	60.04±0.23 ^b	5.06±0.27 ^c	5.15±0.27 ^c
8	65.13±0.25 ^b	71.61±0.15 ^b	11.47±0.17 ^b	13.40±0.21 ^b	55.16±0.18 ^{ab}	59.71±0.28 ^{ab}	4.69±0.24 ^b	5.05±0.19 ^b
12	64.46±0.59 ^a	70.25±0.48 ^a	10.51±0.26 ^a	12.78±0.33 ^a	52.46±1.11 ^a	58.15±1.21 ^a	4.26±0.31 ^a	5.22±0.36 ^a

229 Values show means of triplicate analysis ±SD. Figures with different superscript down the column, are significantly different
 230 (P<0.05). 'AT' denote ambient temperature; 'RT' denote refrigeration temperature.

231 The result presented in Table 3 shows the mineral content of 3 % (w/v) garlic flavoured tigernut-milk
 232 drink stored at ambient and refrigeration temperature for 12 weeks. There was variation in mineral
 233 content of the tigernut-milk drink flavoured with 3 % (w/v) garlic stored at ambient temperature and
 234 refrigeration temperature. Table 3 shows that the calcium, magnesium, potassium and zinc content in 3 %
 235 (w/v) garlic flavoured tigernut-milk drink stored at refrigeration temperature was higher than similar
 236 tigernut-milk drink stored at ambient temperature. During ambient and refrigeration temperature storage,
 237 there was continuous reduction in calcium, potassium, magnesium and zinc content in 3 % (w/v) garlic
 238 flavoured tigernut-milk drink.

240 **Table 3. Mineral content of 3 % (w/v) garlic flavoured tigernut-milk stored at ambient and**
 241 **refrigeration temperature**

Wk	Ca (mg/l)		K (mg/l)		Mg (mg/l)		Zn (mg/l)	
	AT	RT	AT	RT	AT	RT	AT	RT
0	66.92 ±0.17 ^d	66.45±0.21 ^c	2006.10±0.00 ^c	2098.25±0.50 ^d	56.60±0.95 ^c	59.00±0.00 ^b	6.53±0.57 ^c	6.28±0.07 ^b
4	60.00±0.00 ^c	62.07±0.00 ^b	2006.10±0.00 ^c	2008.07±0.27 ^c	41.00±1.00 ^b	54.13±1.44 ^a	5.83±0.16 ^b	6.00±0.00 ^a
8	54.19±0.15 ^b	59.15±0.36 ^c	1976.35±0.30 ^b	2001.25±0.14 ^b	39.08±0.92 ^a	52.65±1.20 ^a	5.48±0.18 ^{ab}	5.95±0.14 ^a
12	50.12±0.16 ^a	57.46±0.59 ^a	1828.73±1.13 ^a	1995.31±0.56 ^a	38.5 ^b ±0.71 ^b	50.14±0.37 ^a	5.11±0.17 ^a	5.83±0.27 ^a

242 Values show means of triplicate analysis ±SD. Figures with different superscript down the column, are significantly different
 243 (P<0.05). 'AR' denote ambient temperature; 'RT' denote refrigeration temperature.

244 Table 4 below shows the mineral content of 5 % (w/v) garlic flavoured tigernut-milk drink stored at ambient and
 245 refrigeration temperature for 12 weeks. The result presented in Table 4 shows that calcium, potassium, magnesium
 246 and zinc content in tigernut-milk drink flavoured with 5 % (w/v) garlic continuously reduced as the period of
 247 ambient and refrigeration temperature storage increased. It was observed that 5 % (w/v) garlic flavoured tigernut-
 248 milk drink stored at refrigeration temperature contained higher quantity of calcium, potassium, magnesium and zinc
 249 than similar tigernut-milk drink stored at ambient temperature. Comparison between Table 3 and 4 revealed that
 250 tigernut-milk drink flavoured with either 3 % (w/v) garlic or 5 % (w/v) garlic stored at refrigeration temperature
 251 contain a higher quantity of calcium, potassium, magnesium and zinc than similar tigernut-milk preparation stored at
 252 ambient temperature.

253 **Table 4: Mineral content of 5 % (w/v) garlic flavoured tigernut-milk stored at ambient and**
 254 **refrigeration temperature**

Wk	Ca (mg/l)		K (mg/l)		Mg (mg/l)		Zn (mg/l)	
	AT	RT	AT	RT	AT	RT	AT	RT
0	89.50 ±0.00 ^d	89.86 ±0.60 ^d	16.38±0.61 ^c	19.10±1.82 ^a	73.00±1.67 ^c	70.40±0.63 ^d	7.30±0.32 ^b	7.27±0.10 ^c
4	77.45±1.43 ^c	86.45±0.00 ^c	17.28±0.57 ^c	20.04±0.20 ^a	62.59±0.34 ^b	63.50±0.00 ^c	6.55±0.51 ^a	7.05±0.21 ^c
8	65.81±0.55 ^b	82.26±0.23 ^b	14.52±0.11 ^b	18.51±0.23 ^a	59.56±0.48 ^b	62.19±0.20 ^b	6.23±0.07 ^a	6.79±0.16 ^b
12	60.60±0.78 ^a	80.96±0.14 ^a	10.75±0.00 ^a	15.24±0.98 ^a	55.52±0.66 ^a	60.24±0.34 ^a	6.08±0.10 ^a	6.52±0.14 ^a

255 Values show means of duplicate analysis ±SD. Figures with different superscript down the column, are significantly different
 256 (P<0.05). 'AT' denote ambient temperature; 'RT' denote refrigeration temperature.

257 4. CONCLUSION

258 This study demonstrated that LAB fermented tigernut-milk drink separately flavoured with 3 % (w/v) garlic, 5 %
 259 (w/v) garlic, 3 % (w/v) ginger and 5 % (w/v) ginger stored at refrigeration temperature contained higher quantity of
 260 calcium, magnesium, potassium and zinc compared with similar tigernut-milk drink preparation stored at ambient

temperature. The protein content of tigernut-milk drink preparations increased during ambient and refrigeration temperature storage except the 5 % (w/v) ginger flavoured tigernut-milk drink. Therefore, the tigernut-milk preparations preferably stored at refrigeration temperature are nutritious non-dairy products to be recommended to everyone.

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