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Effect of Storage Temperature on the Nutritional Compositions of Lactic Acid Bacteria Fermented Spicy Tigernut- Milk Drink

N. Maduka[1*] and F. S. Ire[1]

[1]Department of Microbiology, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author NM designed this research, carried out the laboratory work, sourced the relevant literature, performed the necessary statistical analysis and drafted the first manuscript. Author FSI thoroughly made corrections in the first manuscript and suggested a reputable journal for it to be published. Both authors read and gave their approval of the final manuscript.

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ABSTRACT

The fundamental benefit for consuming edible products is based on its nutritional compositions. Several factors affect the nutritional composition of diverse edible products. This study was carried out between August-October, 2016 at Federal Institute of Industrial Research Oshodi (FIIRO), Lagos. Lactic acid bacteria (LAB) were isolated from 'ogi' using MRS agar and characterized using API 50 CHL test kit. Tigernut tubers were used to prepare tigernut-milk which was fermented by mixed culture of LAB for 12 hr at 45ºC. The LAB 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. The final products were stored at 28±2ºC and 4±2ºC for 12 wks and their nutritional composition was monitored at 4 wk interval using conventional and rapid test methods. Results obtained revealed there was reduction in carbohydrate, moisture and lipid content during storage of the tigernut-milk preparations. Importantly, the protein content in the tigernut-milk preparations increased during storage at both conditions except in the 5 % (w/v) ginger spiced tigernut-milk drink which ranged from (6.48-11.24

**Corresponding author: E-mail: maduks.mn@gmail.com;*

%) and (6.07-10.91 %) during ambient and refrigeration temperature storage, respectively. There was also reduction in Ca, K, Mg and Zn content in the tigernut-milk drink preparations during storage at 28±2ºC and 4±2ºC. Generally, the mineral content of spiced tigernut-milk drink preparations stored at 4±2ºC was higher than similar drink stored at 28±2ºC with very few exceptions. Remarkably, the high potassium content in 3 % (w/v) ginger and 3 % (w/v) garlic spiced tigernut-milk could be more effective in prevention of hypertension and artherosclerosis compared with 5 % (w/v) ginger spiced and 5 % (w/v) garlic spiced tigernut-milk drink. Therefore, this study projects tigernut-milk drink fermented by LAB and separately spiced 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. 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] and [5] compared the proximate composition as well as physicochemical properties of Peak milk® with tigernut milk. Furthermore, proximate composition of pasteurized lactic fermented varieties of tigernut milk was determined [6]. Tigernut-milk dink is nutritious because tigernut tubers are rich in carbohydrate, fats (oils), protein as well as minerals [7]. Tigernut contains digestible carbohydrate (mono, di and polysaccharides) and high nutritious fat content. Fatty acid compositions of tigernut is 689.20-732.90 g/Kg oleic acid, 125.50-141.20 g/Kg palmitic acid and 99.6-154.60 g/Kg linoleic acid [8]. Tigernut is a rich source of flavonoids [9]. [10] reported in detail the fatty acid and amino acid compositions including the essential amino acid compositions of fermented and non-fermented tigernut.

Apart from being a nutritious and tasty beverage, tigernut-milk drink is recommended for diabetics because it does not contain sodium, lactose, casein, gluten and cholesterol [5]. Tigernut milk has ability to reduce body cholesterol by lowering amount of low density lipoprotein (LDL) and increasing amount of high density lipoprotein (HDL) [11].

According to [12] tigernut contains between 17.4-20.0% sucrose as well as 21% glucose. Consumption of tigernut milk helps to control body weight [13]. 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 [14,7].

The shelf life of tigernut-milk drink can be extended at ambient (28±2 ^{o}C) and refrigeration (4±2 ^{o}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 [3, 6,15-18].

Therefore, this study is aimed at determining the effect of ambient and refrigeration temperature storage on the nutritional composition of lactic fermented tigernut-milk drink separately spiced with ginger and garlic.

2. 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.

Plate 1. Fresh big yellow tigernut tubers

2.1 Preparation of Lactic Fermented Tigernut-milk Drink Spiced 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 [19]. *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and

Lactobacillus brevis were identified. Mixed culture of 2 % (w/v) LAB species was inoculated into 500 ml pasteurized (72 ^{o}C for 15 min.) tigernut-milk inside Erlenmeyer bottles and allowed to ferment at 45 ^{o}C for 12 hr. Flavouring agent of different concentration comprising 3 %, 5 % ginger; 3 %, 5 % garlic was separately added to the pasteurized lactic fermented tigernut-milk drink [20,3].

2.2 Storage of Spiced Lactic Fermented Tigernut-milk Drink

The spiced tigernut-milk drink preparations were separately stored at ambient (28±2 ^{o}C) and refrigeration temperature (4±2 ^{o}C) for 12 weeks.

2.3 Nutritional Composition of Spiced Lactic 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 [21] and the fat content was determined using the method described by [7]. MS-70 moisture analyzer was used to determine moisture content. The carbohydrate content was determined using Cleg Anthrone method [22,23]. The magnesium, potassium, calcium and zinc content of the tigernut-milk drink was determined by dry ash extraction method [7].

3. RESULTS AND DISCUSSION

The lactic acid bacteria isolated from ogi identified using API CHL 50 test kit is listed in Table 1. The carbohydrate profiling of each LAB isolate and the significant taxa is shown in Table 2. API well nos.1-49 for each significant taxa shows the carbohydrates fermented by each LAB isolate after 48 hr incubation.

Fig. 1 and 2 shows the carbohydrate content in spicy 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 spicy tigernut-milk drink during storage at ambient and refrigeration temperature except at Wk 4 during storage of 5 % (w/v) garlic spiced tigernut-milk at ambient temperature. [24] reported that reduction in carbohydrate content occurred during storage of a similar fermented beverage at ambient temperature. The reduction in carbohydrate content in the spiced tigernut-milk drink could be as a result of probiotic lactic acid bacteria being able to decrease the quantity of non-digestible carbohydrates (poly- and oligosaccharides) [25]. As a result of natural lactic fermentation of tigernut-milk drink which can involve the use of LAB as starter culture, the drink is reported not to contain lactose based on relevant literature [5] possibly as a result of LAB using up lactose as its main carbon source for energy and growth. This happens after lactose had been hydrolysed by lactase into galactose and glucose. Afterwards, the glucose is then converted into lactic acid through the glycolytic pathway [26,27]. Increase in 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 separately spiced with 5 % (w/v) ginger and 5 % (w/v) garlic compared with minimal carbohydrate reduction in the tigernut-milk drink spiced 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 spicy tigernut-milk drink. [28] reported that carbohydrate content in ginger and garlic is 72.84 % and 73.03 %, respectively. It could be that during ambient and refrigeration temperature storage, fermentation still occurred which resulted in the breakdown of carbohydrates into simple sugars such as sucrose and glucose. This could be the cause of the reduction in carbohydrate content in the tigernut-milk preparations during ambient and refrigeration temperature storage. The reduction in carbohydrate could be aided by some enzymes produced by the fermenting LAB in the tigernut-milk drink which breaks down carbohydrate into simple sugars [29].

Table 1. Lactic acid bacteria identified using API CHL 50

Lactic acid bacteria significant taxa	% accuracy	Remarks	Isolate code
Streptococcus thermophilus	87.5	Very good identification	B113
Lactobacillus acidophilus	90.50	Very good identification	C114
Lactobacillus brevis 3	99.6	Excellent identification	F009
Lactobacillus plantarum 1	99.9	Excellent identification	E009

Table 2. API 50 carbohydrate fermentation profiling of lactic acid bacteria isolated from ogi

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
GLY	ERY	DARA	LARA	RIB	DXYL	LXYL	ADO	MDX	GAL	Significant taxa
-	-	-	+	+	-	-	-	-	-	*Lactobacillus plantarum 1*
-	-	-	+	+	+	-	-	-	+	*Lactobacillus brevis 3*
+	-	-	+	+	+	-	-	-	-	*Lactobacillus acidophilus*
+	-	-	+	+	+	-	-	-	-	*Streptococcus thermophilus*
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	
GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN	SOR	MDM	Significant taxa
+	+	+	-	+	-	-	+	+	+	*Lactobacillus plantarum 1*
+	+	-	-	-	-	-	-	-	-	*Lactobacillus brevis 3*
+	+	+	-	-	-	+	+	+	-	*Lactobacillus acidophilus*
+	+	+	-	-	-	+	+	+	-	*Streptococcus thermophilus*
21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	
MDG	NAG	AMY	ARB	ESC	SAL	CEL	MAL	LAC	MEL	Significant taxa
+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum 1*
+	+	-	-	-	-	-	+	-	+	*Lactobacillus brevis 3*
+	-	+	+	+	+	+	+	-	-	*Lactobacillus acidophilus*
+	-	+	+	+	+	+	+	-	-	*Streptococcus thermophilus*
31	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	
SAC	TRE	INU	MLZ	RAF	AMD	GLYG	XLT	GEN	TUR	Significant taxa
+	+	-	+	+	-	-	-	+	+	*Lactobacillus plantarum 1*
-	-	-	-	-	-	-	-	-	-	*Lactobacillus brevis 3*
+	+	-	-	-	-	-	-	-	-	*Lactobacillus acidophilus*
+	+	-	-	-	-	-	-	-	-	*Streptococcus thermophilus*
41	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**		
LYX	TAG	DFUC	LFUC	DAPL	LARL	GNT	2KG	5KG		Significant taxa
-	-	-	-	+	-	+	-	-		*Lactobacillus plantarum 1*
-	-	-	-	-	-	+	-	+		*Lactobacillus brevis 3*
-	-	-	-	-	-	-	-	-		*Lactobacillus acidophilus*
-	-	-	-	-	-	-	-	-		*Streptococcus thermophilus*

Nos. 1-49 represent carbohydrates

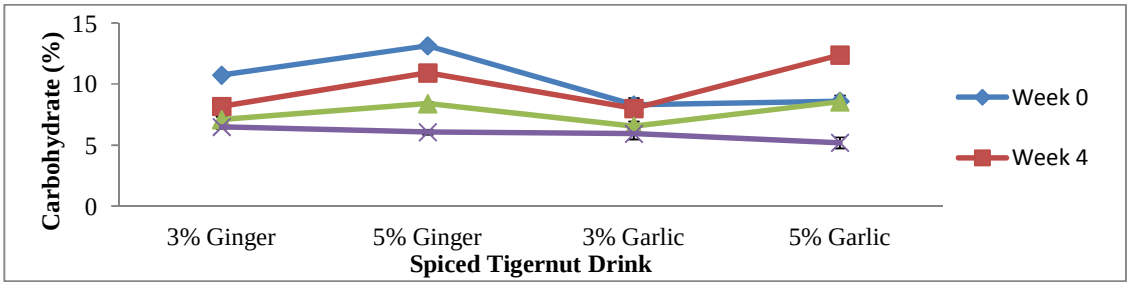

Fig. 1. Carbohydrate content of spiced tigernut-milk drink during storage at ambient temperature

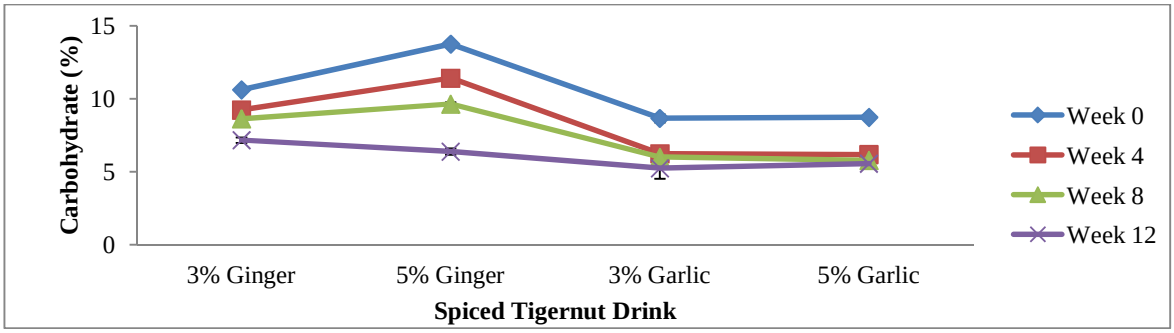

Fig. 2. Carbohydrate content of spiced tigernut-milk drink during storage at refrigeration temperature

The protein content in spiced tigernut-milk drink stored at ambient and refrigeration temperature, respectively for 12 wks are depicted in Fig. 3 and Fig. 4, respectively. During the period spiced tigernut-milk drink preparations were stored at ambient and refrigeration temperature, their protein content increased except, in 5 % (w/v) ginger spiced tigernut-milk drink which had its protein content reduced from 10.91 % (Wk 0) to 6.07 % (Wk 12). Reduction in protein content during storage of tigernut-milk drink preparation is in agreement with a similar study carried out by [3]. The reduction in protein content could be as a result of proteolysis which cause increase in peptides and amino acids. The LAB requires the amino acids for growth [30]. 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]. However, the increase in protein content in 3 % (w/v) ginger spiced, 3 % (w/v) garlic spiced and 5 % (w/v) garlic spiced tigernut-milk drink during storage at ambient and refrigeration temperature could be as a result of increase in microbial cells in the tigernut-milk preparations. The improvement in protein content of lactic fermented tigernut-milk might be due to some anabolic processes leading to polymer build up. The increase in protein content in the tigernut-milk drink could be associated with fermenting LAB. This process in addition to hydrolysis of proteins by the fermenting microorganisms usually does not result in changes in the quantity of amino acids present in the tigernut milk unless there is an external source. Therefore, the increase in protein content in the tigernut drink could be linked to the reduction in carbohydrate and volatile compounds in the spiced lactic fermented tigernut-milk drink [31]. In a related study, [6] observed that protein content of lactic fermented tigernut milk was higher than unfermented tigernut milk. They reported that increase in protein content could be as a result of constituent concentration which resulted in moisture loss in the tigernut-milk drink during pasteurization. They further suggested that destruction/inhibition of some protease inhibitors which form complexes with protein prevents them from being hydrolysed. 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 digestible protein content increased during storage at ambient and refrigeration temperature

[32]. According to [6], starter culture developed tigernut-milk is a good protein source. Studies have shown that pasteurization and fermentation improves the nutritional quality of tigernut milk [3]. The higher protein content in garlic spiced tigernut-milk drink compared with ginger spiced 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 [33]. In a similar study, [28] reported that protein content of garlic and ginger is 17.35 % and 8.58 %, respectively. A related study carried out by [34] reported increase in protein content in garlic based yoghurt.

Fig. 5 and 6 shows the moisture content in different preparations of ginger and garlic spiced tigernut-milk drink fermented by LAB during storage at ambient and refrigeration temperature, respectively for 12 wks. The result shows there were slight changes in moisture content in 3 % (w/v) and 5 % (w/v) ginger spiced tigernut-milk drink during storage at ambient and refrigeration temperature. The reduction in moisture content could be as a result of increase in dry matter attributed to increase in population of viable LAB. [35] reported that increase in dry matter resulted in the decrease in moisture content in raffia sap subjected to fermentation. A study carried out by [33] reported that dry matter of garlic and 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 separately added to the tigernut-milk drink fermented by LAB influenced the reduction in moisture content. High moisture content in any liquid beverage makes it a refreshing and thirst-quenching product. However, during ambient and refrigeration temperature storage, slim reduction in moisture content in the tigernut-milk drink preparations is an indication that its shelf life was slowly reducing [21].

The lipid content in ginger and garlic spiced tigernut-milk drink fermented by LAB stored at ambient and refrigeration temperature for 12 wks is presented in Fig. 7 and 8, respectively. During ambient and refrigeration temperature storage, there was reduction in lipid content in the spiced tigernut-milk drink preparations. The reduction in lipid content during storage of spiced tigernut-milk drink could be as a result of lipases produced by contaminating psychrotrophic

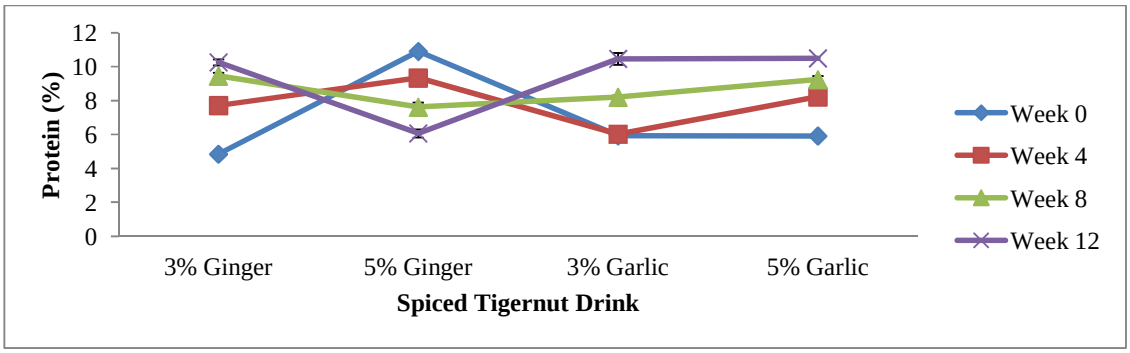

Fig. 3. Protein content of spiced tigernut-milk drink during storage at ambient temperature

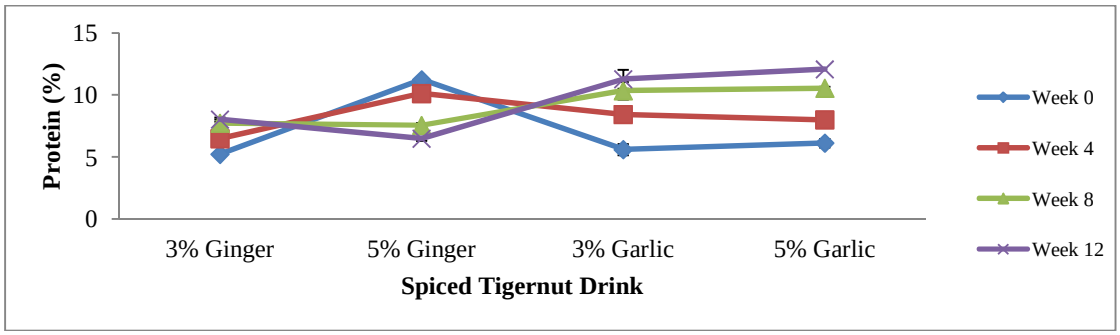

Fig. 4. Protein content of spiced tigernut-milk drink during storage at refrigeration temperature

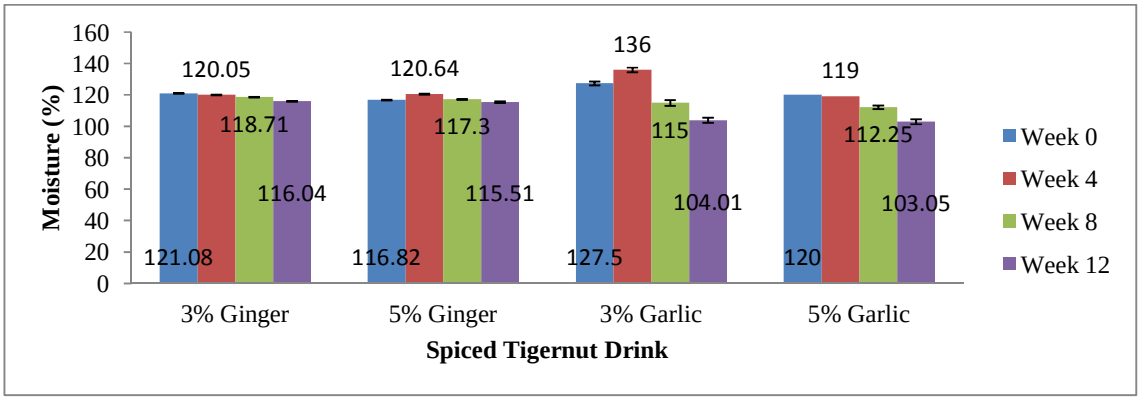

Fig. 5. Moisture content of spiced tigernut-milk drink during storage at ambient temperature

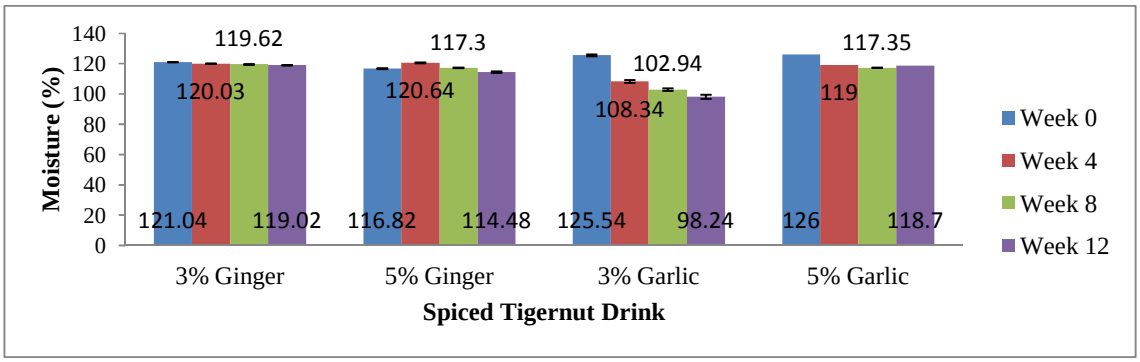

Fig. 6. Moisture content of spiced tigernut-milk drink during storage at refrigeration temperature

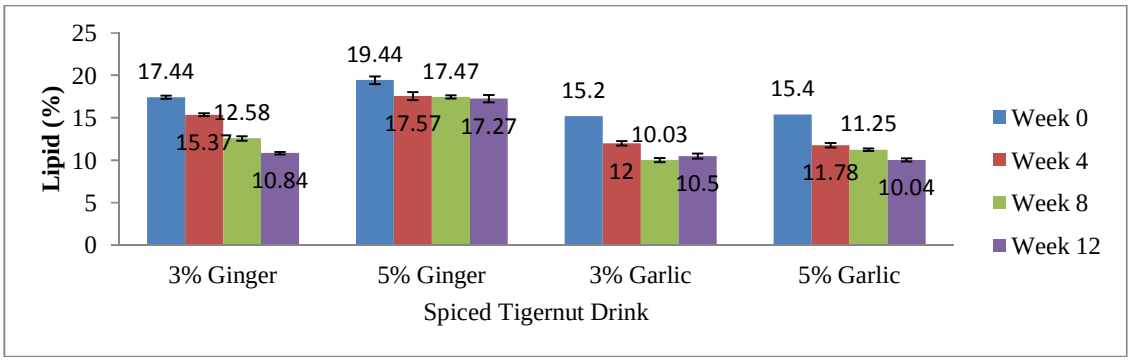

Fig. 7. Lipid content of spiced tigernut-milk drink during storage at ambient temperature

bacteria present in the spiced tigernut-milk drink. According to [36, 37] lipases produced by LAB were found very difficult to hydrolyse lipids because of high molecular weight triglycerides. In fact, the lipase present in *Streptococcus thermophilus* have minimal effect on the free fatty acid content of fermented dairy products [38]. 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 spiced tigernut-milk drink. Increase in concentration of ginger added to tigernut-milk drink possibly caused more inhibition to the activities of lipolytic enzymes which resulted in more significant increase in lipid content compared with garlic spiced tigernut-milk drink. Reduction in carbohydrate, lipid and moisture content but increase in protein content during fermentation of tigernut-milk drink was reported by [3,20,24].

Table 3 shows the mineral content in ginger and garlic spiced 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 spiced tigernut-milk drink preparations. The result in Table 1 shows that tigernut-milk drink spiced with 3 % (w/v) ginger stored at refrigeration temperature contain higher quantity of minerals than similar tigernut-milk preparation stored at ambient temperature with few exceptions. [39] reported similar result trend during natural fermentation of soymilk which involved *Lactobacillus acidophilus, Streptococcus cremoris, Micrococcus aureus* and *Streptococcus lactis*. In Nigeria, fermented dairy product is known as 'nono'. The reason for reduction in mineral content of fermenting tigernut-milk drink could be as a result of microorganisms activities which utilized available minerals in the tigernut-milk drink preparations for growth and metabolism. Lactic acid bacteria are fastidious and therefore rely on available mineral content in a medium for growth. Although there was gradual reduction in mineral content in the spiced tigernut-milk drink preparations during refrigeration and ambient temperature storage, 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, cause reduced appetite and suppress the immune system [40, 41]. Potassium is one of the essential nutrients required for the maintenance of total body fluid value. It also help in maintaining acid and electrolyte balance. Potassium is required for normal cell function [42]. Magnesium is needed during protein synthesis, production of energy and contraction of muscles. In fact, regular consumption of edible products that contain very little quantity of magnesium will increase the risk of such individuals to suffer coronary heart disease and Type 2 diabetes sometime in the future [43]. It is interesting to state that tigernut-milk drink contains higher quantity of magnesium than cow milk [5].

Table 4 shows the mineral content in 5 % (w/v) ginger spiced 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 spiced tigernut-milk drink stored at refrigeration temperature was higher than similar tigernut-milk drink stored at ambient temperature except magnesium content in the tigernut-milk drink at Week 0. Our result shows there is a wide difference between potassium content in 3 % (w/v) ginger spiced tigernut-milk drink and 5 % (w/v) ginger spiced tigernut-milk drink during storage at refrigeration and ambient temperature. A similar relationship was observed during ambient and refrigeration temperature storage of 3 % (w/v) and 5 % (w/v) garlic spiced tigernut-milk drink presented in Table 5 and 6. The high quantity of potassium in 3 % (w/v) ginger spiced and 3 % (w/v) garlic spiced 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 of the tigernut-milk separately spiced with 3 % (w/v) ginger and 3 % (w/v) garlic [39]. In a related study, [44] reported a drastic reduction in potassium content in fermented sorghum-Irish potato gruel attributed to the effect of starter culture which comprise of *Lactobacillus acidophilus*, *L. fermentum*, *L. plantarum*, *Geotrichum candidum* and *Saccharomyces cerevisiae*. There is no convincing report about adverse effect of high intake of potassium to the human body [45]. 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, artherosclerosis, osteoporosis, Alzheimer's disease, kidney stone and arthritis [46]. The Food and Nutrition Board of the Institute of Medicine recommend 4700 mg/day potassium intake. On their part, potassium intake recommended by the World Health Organization/Food and Agriculture Organization Expert Consultation is 4700 mg/day [44]. The reduction in mineral content during storage of spiced tigernut-milk preparations reported in this study corroborates the findings from a related research study conducted by [47] which involved development of low-fat symbiotic yoghurt using water buffalo milk.

Table 3. Mineral content of 3 % (w/v) ginger spiced tigernut-milk during storage 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 4. Mineral content of 5 % (w/v) ginger spiced tigernut-milk during storage 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	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}

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

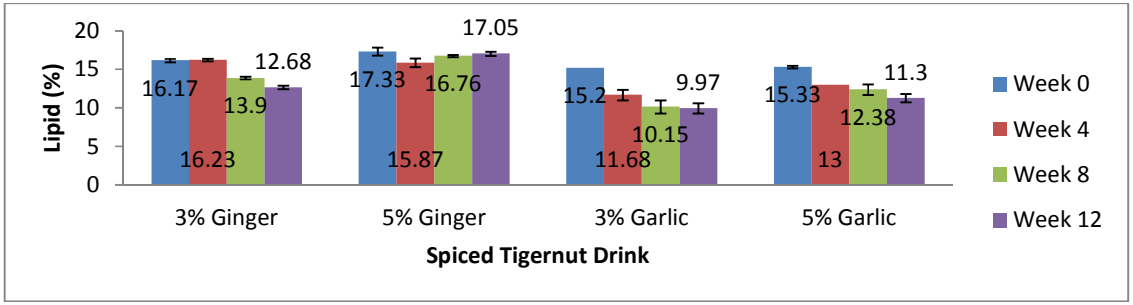

Fig. 8. Lipid content of spiced tigernut-milk drink during storage at refrigeration temperature

Table 5. Mineral content of 3 % (w/v) garlic spiced tigernut-milk during storage 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	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}

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

Table 6. Mineral content of 5 % (w/v) garlic spiced tigernut-milk during storage 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	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}

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.

The result presented in Table 5 shows the mineral content of 3 % (w/v) garlic spiced tigernut-milk drink stored at ambient and refrigeration temperature for 12 weeks. There was variation in mineral content in the tigernut-milk drink spiced with 3 % (w/v) garlic stored at ambient temperature and refrigeration temperature. Our study shows that during ambient and refrigeration temperature storage of 3 % (w/v) garlic spiced tigernut-milk drink for 12 weeks, there was continuous reduction in calcium, potassium, magnesium and zinc content in the tigernut-milk drink. The reduction in mineral content in spiced tigernut-milk drink could be as a result of LAB utilizing some quantity of mineral. According to [38], bioavailability of some minerals could be influenced as a result of changes in pH that occured during fermentation. However, the mineral content in the 3 % (w/v) garlic spiced tigernut-milk drink stored at refrigeration temperature was higher than similar tigernut-milk drink stored at ambient temperature with few exceptions at Wk 0.

Table 6 shows the mineral content of 5 % (w/v) garlic spiced tigernut-milk drink stored at ambient and refrigeration temperature for 12 weeks. The result presented in Table 4 shows that calcium, potassium, magnesium and zinc content in tigernut-milk drink spiced with 5 % (w/v) garlic continuously reduced as the period of ambient and refrigeration temperature storage increased. It was observed that 5 % (w/v) garlic spiced tigernut-milk drink stored at refrigeration temperature contained higher quantity of minerals than similar tigernut-milk drink stored at ambient temperature with few exceptions at Week 0. It is the recommendation of [6] that tigernut milk should be stored at refrigeration temperature in order to preserve the quality of the drink if it is not served immediately after preparation.

4. CONCLUSION

This study demonstrated that lactic fermented tigernut-milk drink separately spiced with 3 % (w/v) garlic, 5 % (w/v) garlic, 3 % (w/v) ginger and 5 % (w/v) ginger stored at refrigeration temperature contained higher quantity of calcium, magnesium, potassium and zinc compared with similar tigernut-milk drink preparation stored at ambient temperature with few exceptions. The protein content of tigernut-milk drink preparations increased during ambient and refrigeration temperature storage except the 5 % (w/v) ginger spiced 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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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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