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RESEARCH ARTICLE

FERMENTATION OF TIGERNUT BY LACTIC ACID BACTERIA AND TIGERNUT-MILK DRINK FERMENTATION BY LACTIC ACID BACTERIA AS A POTENTIAL PROBIOTIC PRODUCT

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ABSTRACT

In recent times several researches have been focusing on non-dairy products as alternative to dairy products for the development of potential probiotic products. Different level of successes have been achieved in that regard. Fermentation of sterilized tigernut by 3 Log_{10}CFU/g lactic acid bacteria (LAB) isolated from ogi resulted in higher LAB count (5.17-7.34 Log_{10}CFU/g) than using unsterilized tigernut (4.76-5.41 Log_{10}CFU/g). However, there was slight difference in LAB count between unsterilized and sterilized tigernut fermented by larger LAB inoculum (6 Log_{10}CFU/g). The LAB isolates identified as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus brevis* were used as mixed culture in three different combinations A, B and C to ferment tigernut-milk drink for 72hr at 45 ^{o}C. During the fermentation period (0-72hr), the pH of tigernut-milk drink Treatment A, B, C and D being the uninoculated reduced from6.40-4.36, 6.05-4.10, 6.0-4.04 and 6.34-5.40, respectively. The total culturable lactic acid bacterial count increased from 2.73-5.60 Log_{10}CFU/ml, 2.72–5.45Log_{10}CFU/ml,2.75-5.96 Log_{10}CFU/ml in tigernut-milk drink Treatment A, B and C, respectively between 0-60 hr. Between 60-72 hr, reduction in culturable lactic acid bacteria occurred in tigernut-milk drink treatment A, B and C except treatment D. No culturable LAB were isolated between 0-24 hr in Treatment D.Between 36-72hr,culturable LAB count in Treatment D increased from 2.34-3.81Log_{10}CFU/ml. Between 0-72 hr, TTA increased from 0.09-0.79 %, 0.09-0.73%,0.10-0.79 % and 0.09-0.16 % in tigernut drink Treatment A, B, C and the uninoculated, respectively. Within the same period, percentage lactic acid produced increased from 0.11-0.95 %, 0.12-0.87 %, 0.11-0.85% and 0.11-0.19 % in tigernut-milk drink Treatment A, B, C and D, respectively. The increase in culturable LAB count in all the tigernut-milk drink treatments makes it a suitable vehicle for delivering probiotic LAB.

INTRODUCTION

Tigernut is a small sized spherical tuber which looks like peanut. It develops from rhizomes that run deep into the ground (Okyere and Odamtten, 2014; Oyerinde and Olalusi, 2013). The cultivation and utilization of tigernut started with the Egyptians sometime in 5000 BC (Oyedele *et al*., 2015; Allouh *et al*., 2015). Over 6,000 years ago when utilization of tigernut was traced to Egyptians, millions of people have been using tigernut in different ways to produce different products. The three major products of tigernut tubers are tigernut flour, tigernut oil and tigernut milk (Ogundipe *et al*., 2013). The easiest means of consuming tigernut tubers is by chewing the raw tubers which has a slightly sweet and nutty flavour (Hassan, 2007). Tigernut-milk is known as tigernut drink or tigernut beverage or descriptively known as tigernut-milk drink.

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Tigernut drink, locally called 'ayah' is recommended for diabetics and people who are gluten intolerant (Bamishaiye and Bamishaiye, 2011). Ogi also known as akamu is a smooth textured food porridge that has a sour taste due to acid fermentation of cereals such as maize, sorghum and millet. *Lactobacillus* spp. ,*Bifidobacterium* spp. and few other bacteria present in ogi possess some probiotic properties and have been certified safe for direct human consumption. Some researchers had isolated lactic acid bacteria (LAB) from the surface of tigernut tubers. In fact, LAB possess adhesional adaptation which gives it ability to survive different environments such as diverse food matrices (Hayek and Ibrahim, 2013). Ndikom and Elutade (2016) in their study isolated LAB from the surface of tigernut tubers. Tigernut being slightly acidic (pH 6.34) can support the growth of lactic acid bacteria (Umerie *et al.,* 1997). Previous researches reported that tigernut is a substrate that can sustain microbial growth possibly due to the near neutral pH of tigernut tubers which favours the growth of many microorganisms. Lactic

acid bacteria have the ability to dominate other bacteria involved in natural fermentation. Tigernut beverages could further be developed into a potential probiotic product. According to studies carried out by Wakil *et al*. (2014), lactic acid bacteria were involved in natural fermentation of tigernut-milk drink. Several studies that involved microbial analysis of tigernut tubers and their derived products have been carried out. Little studies had been carried out on the effect of using sterilized and unsterilized tigernut for the growth of LAB as a step towards incorporating lactic acid bacteria into tigernut-milk drink as a potential probiotic product. According to WHO/FAO, a probiotic can be defined as live microorganisms which in adequate quantity can bring about health benefits to its host. Although products such as yoghurt and other fermented milk products contain probiotic bacteria, majority of the products in the market do not maintain adequate amount of the probiotic bacteria. Therefore, the population of probiotic bacteria in a product is an important requirement a product labeled a probiotic must meet (Irkin and Guldas, 2011). The aim of this study is to determine the effect of sterilized and unsterilized tigernut as a substrate for LAB growth and suitability of using tigernut-milk drink as a vehicle for delivering probiotic LAB culture isolated from ogi in adequate amount into the human body in the form of a potential probiotic product.

MATERIALS AND METHODS

Big yellow variety of tigernut tubers and ogi was purchased from Oshodi market Lagos State.

Sample Preparation

The fresh big yellow variety of tigernut tubers were handpicked to separate foreign materials mixed with the tubers. A slightly modified method described by Wakil *et al.* (2014) was used to prepare tigernut-milk drink.

Isolation of Lactic Acid Bacteria from Ogi

Ten gram ogi paste was transferred into 250 ml beaker and 90 ml distilled water poured into the beaker. The mixture was homogenized and serially diluted. Appropriate dilution was plated in deMan, Rogosa and Sharpe (MRS) agar plates prepared according to manufacturer's instruction and incubated anaerobically for 48hrs at 45^{o}C. Discrete colonies were subcultured into fresh MRS agar plates.

Sterilized and Unsterilized Tigernut as Substrate to Grow Lactic Acid Bacteria

One hundred and fifty gram (150 g) fresh yellow variety tigernut tubers were crushed using manual grinder and autoclaved at 121 ^{o}C at 15 psi for 15 minutes. Twenty five gram crushed sterilized tigernut labeled T_s in duplicate were inoculated with 3 Log_{10}CFU/ml LAB and another duplicate 25 g crushed sterilized tigernut also labeled T_s was inoculated with 6 log_{10}CFU/ml LAB. The LAB inoculated sterilized tigernut were allowed to ferment for 4 days at ambient temperature (28±2 ^{o}C). At day 0, 2 and 4, one gram sample of the LAB fermenting tigernut were collected, serially diluted using normal saline and pour plated into freshly prepared de, Man Rogosa and Sharpe agar plates. The inoculated MRS plates were incubated anaerobically for 36-48 hrs at 45^{o}C. The colony counts on the Petri dishes were noted. Using similar procedure for sterilized tigernut T_s for the growth of LAB, unsterilized tigernut T_u was also used as substrate to grow lactic acid bacteria.

Characterization of Lactic Acid Bacteria Isolates

The morphology of the colonies, Gram staining and catalase test was carried out. Using the procedure of Oluwajoba *et al.* (2013), the isolated species were confirmed with the help of a standard commercial identification known as API 50 CHL (Biomerieux®, France) carbohydrate profiling in accordance with instructions stated by the manufacturer.

Preparation of Mixed Culture Lactic Acid Bacteria Inoculum

A similar method as described by Ire *et al.* (2017) was adopted. Four pure bacterial isolates from ogi identified as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus brevis* were subcultured into freshly prepared MRS broth with 2 % glucose and incubated anaerobically at 30^{o}C for 48 hrs. At the end of incubation period, the microbial cells were harvested by centrifugation at 1000 rpm. Thereafter, the microbial cells were washed using sterile distilled waterand centrifuged at 1000 rpm. The wet weight of the microbial cells was determined by the difference between the weight of the centrifuge tubes and weight of centrifuge tubes and washed cells. This procedure was repeated for each of the pure cultures. Each mixed culture LAB comprise of equal weight of each pure culture.

Mixed Culture Lactic Acid Bacteria Fermentation of Tigernut-milk Drink

A similar method used by Wakil *et al*. (2014) was adopted to produce tigernut-milk drink which was pasteurized at 72^{o}C for 15 minutes. To 1000 ml tigernut drink contained in four separate Erlenmeyer bottles labeled A, B, C and D, 2 % (w/v) combinations of pure lactic acid bacteria were inoculated into bottle A-C and bottle D uninoculated is the control. The content of the bottles were allowed to ferment at 45 ^{o}C for 72 hr.

RESULTS

The result in Figure 1 shows that unsterilized tigernut inoculated with 3 Log_{10}CFU/ml LAB resulted in increase lactic acid bacterial count (4.76-5.41Log_{10}CFU/g). However, sterilized tigernut fermented with 3 Log_{10}CFU/ml LAB resulted in higher LAB count (5.17-7.34Log_{10}CFU/g). The reason for higher LAB count in sterilized tigernut inoculated with LAB compared to unsterilized tigernut given the same treatment could be as a result of little or no competition from other microorganisms for limited nutrients in the sterilized tigernut.In a related study, Nwachukwu *et al.* (2010) reported increase in total viable count in sterilized millet inoculated with *Lactobacillus plantarum*, *Lactobacillus cellobiosus* and *Pediococcus pentosaceus* during fermentation. Increase in LAB inoculum size to 6 Log_{10}CFU/ml resulted in increase LAB count in sterilized tigernut (7.29-7.45 Log_{10}CFU/g) and unsterilized tigernut (7.41-7.47 Log_{10}CFU/g), respectively. There was wider difference in lactic acid bacterial count in

sterilized tigernut fermented by lactic acid bacteria between Day 0 and 4 compared to unsterilized tigernut given the same treatment. Sterilized and unsterilized tigernut fermented by 6 Log_{10}CFU/ml LAB inoculum resulted in higher LAB count than sterilized and unsterilized tigernut fermented by 3 Log_{10}CFU/ml. The LAB count in sterilized and unsterilized tigernut between Day 0 and 4 inoculated with 6 Log_{10}CFU/ml had very narrower range compared with sterilized and unsterilized tigernut inoculated with 3 Log_{10}CFU/ml. The narrower range LAB count in unsterilized tigernut (4.76-5.41 Log_{10}CFU/g) and sterilized tigernut (7.29-7.45 Log_{10}CFU/g) fermented by 6 Log_{10}CFU/ml LAB within the fermentation period could be as a result of larger LAB inoculum size (6 Log_{10}CFU/ml) being able to rapidly utilize available nutrients in the tigernut and consequently entered the stationary growth phase.

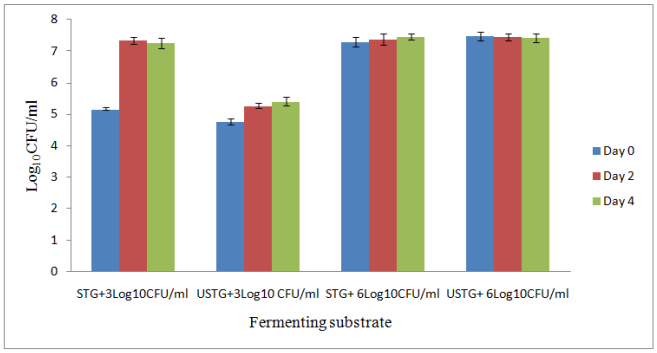

Fig. 1. Lactic Acid Bacterial Count in Sterilized and Unsterilized Tigernut Inoculated with Lactic Acid Bacteria During 4 Day Fermentation

Data are expressed as mean ±SD, determined from two replicates. Error bars represent (±SD)

Where: STG – Sterilizedtigernut
USTG – Unsterilizedtigernut

The result in Table 1 shows colonial appearance, Gram reaction, morphology and test for catalase of LAB isolated from ogi using de Man, Rogosa and Sharpe (MRS) agar. All the isolates were catalase negative and Gram positive. Separate study carried out by Ijabadeniyi (2007) and Okpalla and Onyeneto (2013) identified *Lactobacillus plantarum* and *Lactobacillus fermentum* as bacteria involved in secondary fermentation of ogi. Streptococcus sp is a group of bacteria classified as LAB (Chelule *et al.*, 2010). In fact, diverse microorganisms comprising of several genera of bacteria, moulds and yeast such as *Lactobacillus* spp., *Pediococcus* spp., *Leuconostoc* spp and *Weissella* spp are involved in the fermentation process but very few microbial genera determines the final quality of ogi (Oyedeji *et al*., 2013; Onwuakor *et al.*, 2014). Some of these microorganisms could be responsible for therapeutic and prophylactic effect attributed to consumption of raw ogi slurry. Majorly, *Lactobacillus* and *Bifidobacterium* species and a few other bacteria present in ogi has been certified safe for human use and therefore can be classified as probiotics (David and Famurewa, 2010). The result presented in Table 2 revealed the percentage accuracy involved in identification of each LAB isolate based on API 50 CHL fermentation profiling. Four lactic acid bacterial isolates with the highest percentage accuracy was selected in different combinations as mixed culture LAB to ferment tigernut-milk drink. The pH, culturable lactic acid bacterial count, titratable acidity and lactic acid produced during fermentation of tigernut-milk drink is presented in Table 3, 4, 5 and 6, respectively.

Table 1. Colonial Appearance of Lactic Acid Bacterial Isolate, Gram Reaction and Catalase Test

Isolate Code	Colonial Appearance	Gram Reaction	Gram Morphology Reaction	Catalase Test
A110	Long and slender rods in singles	Positive	Cream, soft smooth surface, convex round and opaque	Negative
B113	Long rods in chains	Positive	Cream-white, soft, smooth, moderate colonies, convex and circular	Negative
C114	Long rods in chains	Positive	Cream soft, smooth surface, dull surface and raised	Negative
D115	Long rods in chains and singles	Positive	Cream, soft, smooth surface, moderate colonies and entire	Negative
E009	Slender short rods in chains	Positive	Cream soft, dull surface, big colonies, entire and opaque	Negative
F009	Short rods in cluster	Positive	Creamy-white, soft, smooth, small colonies	Negative

Table 2. Lactic Acid Bacteria Isolated from Ogi Identified Using API CHL 50

Lactic acid Bacteria Significant Taxa	% Accuracy	Remarks	Isolate Code
Lactobacillus acidophilus 2	79.5	Very good identification	A110
Streptococcus thermophilus	87.5	Very good identification	B113
Lactobacillus acidophilus	90.50	Very good identification	C114
Lactobacillus acidophilus	70.5	Very good identification	D115
Lactobacillus brevis 3	99.6	Excellent identification	F009
Lactobacillus plantarum 1	99.9	Excellent identification	E009

Table 3. The pH ofTigernut-milk Drink Inoculated and Uninoculated with Lactic Acid Bacteria During Fermentation

Treatment	A	B	C	Control
Time (hr)				
0	6.40 ± 0.14^{b}	$6.05\pm0.13^{ab.}$	6.00 ± 0.14^{a}	6.34 ± 0.12^{ab}
12	5.41 ± 0.15^{ab}	5.16 ± 0.13^{b}	5.68 ± 0.11^{bc}	5.98 ± 0.04^{c}
24	5.08 ± 0.04^{a}	5.07 ± 0.07^{a}	5.06 ± 0.07^{a}	6.01 ± 0.05^{b}
36	4.80 ± 0.06^{a}	4.84 ± 0.04^{a}	4.72 ± 0.17^{a}	5.81 ± 0.18^{b}
48	4.89 ± 0.13^{b}	4.50 ± 0.06^{a}	4.49 ± 0.13^{a}	5.88 ± 0.11^{c}
60	4.48 ± 0.11^{a}	4.30 ± 0.08^{a}	4.25 ± 0.06^{a}	5.84 ± 0.13^{b}
72	4.36 ± 0.06^{b}	4.10 ± 0.05^{a}	4.04 ± 0.028	5.40 ± 0.08^{c}

Value show means of duplicate analysis ± SD. Figures with different superscript across the row, are significantly different (P< 0.05).

Table 4. Culturable Lactic Acid Bacteria Count in Tigernut-milk Drink Inoculated and Uninoculated with Lactic Acid Bacteria During Fermentation

Time (hr)	A	B	C	Control
		Log_{10}Cfu/ml		
0	2.73	2.72	2.75	No Growth
12	2.91	2.74	2.86	No Growth
24	3.52	3.89	3.06	No Growth
36	4.72	4.90	4.86	2.34
48	4.88	4.84	4.63	2.61
60	5.60	5.45	5.96	2.64
72	4.92	4.88	4.89	3.81

Value show means of duplicate analysis ± SD. Figures with different superscript across the row, are significantly different (P< 0.05).
Treatment A - *Lactobacillus plantarum*+ *Streptococcus thermophilus*+*Lactobacillus brevis*
Treatment B - *Lactobacillus acidophilus* + *Streptococcus thermophilus*+ *Lactobacillus brevis*
Treatment C - *Lactobacillus plantarum*+ *Streptococcus thermophilus*+*Lactobacillus acidophilus*

Table 5. Titratable Acidity of Tigernut-milk Drink Inoculated and Uninoculatedwith Lactic Acid Bacteria During Fermentation

Treatment	A	B	C	Control
Time (hr)		%		
0	0.09 ± 0.00^{ab}	0.09 ± 0.00^{ab}	0.10 ± 0.00^{b}	0.09 ± 0.01^{a}
12	0.17 ± 0.01^{b}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
24	0.50 ± 0.00^{c}	0.70 ± 0.01^{d}	0.41 ± 0.01^{b}	0.10 ± 0.00^{a}
36	0.541 ± 0.01^{c}	0.69 ± 0.00^{d}	0.49 ± 0.01^{b}	0.11 ± 0.00^{a}
48	0.70 ± 0.00^{b}	0.69 ± 0.01^{b}	0.74 ± 0.01^{c}	0.12 ± 0.01^{a}
60	0.70 ± 0.01^{b}	0.72 ± 0.01^{c}	0.75 ± 0.01^{d}	0.15 ± 0.01^{a}
72	0.79 ± 0.00^{c}	0.73 ± 0.00^{b}	0.79 ± 0.00^{c}	0.16 ± 0.00^{a}

Value show means of duplicate analysis ± SD. Figures with different superscript across the row, are significantly different (P< 0.05).

Table 6. Lactic Acid Produced in Tigernut-milk Drink Inoculated and Uninoculated with Lactic Acid Bacteria During Fermentation

Treatment	A	B	C	Control
Time (hr)		%		
0	0.11 ± 0.00^{a}	0.12 ± 0.00^{a}	0.11 ± 0.01^{a}	0.11 ± 0.00^{a}
12	0.21 ± 0.00^{a}	0.12 ± 0.00^{b}	0.11 ± 0.02^{b}	0.12 ± 0.00^{b}
24	0.59 ± 0.01^{c}	0.83 ± 0.01^{d}	0.45 ± 0.05^{b}	0.12 ± 0.00^{a}
36	0.66 ± 0.01^{c}	0.83 ± 0.00^{d}	0.54 ± 0.08^{b}	0.14 ± 0.01^{a}
48	0.84 ± 0.01^{b}	0.84 ± 0.01^{b}	0.81 ± 0.10^{b}	0.13 ± 0.00^{a}
60	0.83 ± 0.00^{b}	0.86 ± 0.00^{b}	0.82 ± 0.10^{b}	0.17 ± 0.00^{a}
72	0.95 ± 0.00^{b}	0.87 ± 0.01^{b}	0.85 ± 0.08^{b}	0.19 ± 0.01^{a}

Value show means of duplicate analysis ± SD. Figures with different superscript across the row, are significantly different (P< 0.05).
Treatment A - *Lactobacillus plantarum* + *Streptococcus thermophilus* + *Lactobacillus brevis*
Treatment B - *Lactobacillus acidophilus* + *Streptococcusthermophilus* + *Lactobacillus brevis*
Treatment C - *Lactobacillus plantarum* + *Streptococcus thermophilus* + *Lactobacillus acidophilus*

The result presented in Table 3 shows a gradual reduction in pH of tigernut-milk drink inoculated with mixed culture LAB and the uninoculated tigernut-milk drink from beginning of the fermentation till 72 hr. The decrease in pH could be as a result of fast LAB growth rate which broke down carbohydrate that resulted in the increase in quantity of lactic acid released into the fermenting tigernut-milk drink as fermentation progressed. The decrease in pH observed during fermentation of tigernut-milk drink by LAB is in agreement with a related study carried out by Wakil *et al.* (2014). Since LAB had been identified as predominant microorganisms that constitute total microflora of spontaneously fermenting tigernut-milk drink as reported by Wakil *et al*. (2014), this could be the reason why the pH of uninoculated tigernut-milk drink and tigernut-milk drink inoculated with mixed LAB were close. Table 4 shows that during the fermentation period, there was steady increase in LAB count in each of the three separately bottled tigernut-milk drink inoculated with three different mixed culture lactic acid bacteria species within 0 – 60 hr. However, between 60 - 72 hr, there was reduction in lactic acid bacterial count across the three separately bottled tigernut-milk drink fermented by mixed culture lactic acid bacteria except the uninoculated.

The reduction in lactic acid bacterial count at 60 hr could be as a result of fast depleting nutrients in the tigernut-milk drink. Hence, the LAB might be at the stationary phase and probably as a result of stiffer competition for limited fast depleting nutrients, the population of LAB that entered death phase started increasing which resulted in reduction in viable LAB count. It is also possible that the reduction in pH from 4.30 - 4.04 affected the population of LAB in the tigernut-milk drink. According to Mortazavian *et al.* (2012), very low pH is capable of limiting the growth and stability of probiotic bacteria in fermented foods and beverages. Food Standards Code H8 stipulates that pH $\leq$ 4.5 is capable of affecting the viability of probiotic bacteria. The reduction in LAB population could be as a result of the probiotic LAB strain being at the physiological stage. If bacteria undergo transition between the exponential phase and the stationary phase, it becomes increasingly susceptible to stress associated with storage conditions than bacteria that had maintained the stationary growth phase for a short while which could eventually lead to reduced viability of probiotic bacteria involved in the fermentation process (Marhamatizadeh *et al*., 2011). In a related study that involved lactic acid bacterial fermentation of non-dairy probiotic beverage, *Lactobacillus*

acidophilus and *Lactobacillus plantarum* present in the beverage resulted in pH reduction from 6.3 to less than 4.5 after 48 hr fermentation (Eksiri *et al*., 2015). In this study, it was observed that culturable LAB was not detected between 0-24 hr in the tigernut-milk drink uninoculated with LAB which is the control. It could be that LAB present in the uninoculated tigernut-milk drink was unculturable and spore forming LAB might have survived pasteurization of tigernut-milk drink. Molecular identification methods can be used to identify LAB involved in fermentation of tigernut-milk drink that was not part of LAB inoculum incorporated into the drink. During fermentation, reduction in pH from 6.34 to 6.01between 0–24hr is an indication that some level of LAB activity was going on in the uninoculated tigernut-milk drink. Between 24-72 hr, there was steady increase in heterotrophic bacterial count in the uninoculated tigernut-milk drink. Throughout the fermentation period, LAB count in the uninoculated tigernut-milk drink was lower than what was recorded in tigernut-milk drink inoculated with LAB as a probiotic culture. The lower LAB count in uninoculated tigernut-milk drink could be as a result of LAB inoculum not incorporated into the tigernut-milk drink at the start of the fermentation process. It is recommended that products containing 6 Log $_{10}$ CFU/ml per serving of probiotic microorganisms should be consumed regularly to ensure that human body derive health benefits (Nyanzi and Jooste, 2012). This requirement was not met by the mixed culture lactic acid bacteria namely *Lactobacillus plantarum, Lactobacillus lactis* and *Lactobacillus thermophilus* during fermentation of tigernut-milk drink (Wakil *et al*., 2014). In this study, the highest LAB count 5.96 Log $_{10}$CFU/ml observed in Treatment C did not meet the recommended dose of probiotic bacteria in a product that will ensure the body derives health benefits. However, Treatment C especially and Treatment A and B could still be useful in providing some health benefits to the human body if is consumed regularly and in large quantity.

The result presented in Table 5 shows that there was gradual increase in titratable acidity (TTA) of tigernut-milk drink fermented by mixed culture lactic acid bacteria as fermentation period increased. A similar increase in TTA occurred in the tigernut-milk drink uninoculated with LAB. Increase in TTA in the fermenting tigernut-milk drink is a very strong indication that LAB species in the fermenting tigernut-milk drink were significantly increasing in population. Wakil *et al.* (2014) in a related study reported a similar result during natural fermentation of tigernut-milk drink. The quantity of lactic acid produced by mixed culture LAB in the fermenting tigernut-milk drink was determined using standard titration procedure for TTA. The result in Table 6 depict that there was steady increase in amount of lactic acid produced by the mixed culture LAB inoculated into tigernut-milk drink as fermentation period increased. A similar trend occurred in the tigernut-milk drink uninoculated with LAB except a slight reduction in percentage lactic acid produced from 0.14-0.13% which occurred between 36-48 hr. A similar observation occurred at the later stage during spontaneous fermentation of cereals for the production of ogi which Nwachukwu*et al.* (2010) suggested could be as a result of yeast utilizing some quantity of lactic acid produced during fermentation. The fermentative pathway for different strains of LAB present in the tigernut-milk drink might have played an important role in the differences that occur in lactic acid production and change in pH during LAB fermentation. Other end products of heterofermenting LAB are acetate and ethanol (Hayek and Ibrahim, 2013). Wakil *et al*. (2014) in a related study reported that there was increase in total yeast count during fermentation of unsterilized and sterilized tigernut milk. In this study, it was observed that percentage lactic acid produced in the uninoculated tigernut-milk drink subjected to natural fermentation was far lower than what was produced in tigernut-milk drink inoculated with mixed culture lactic acid. It could be that high amount of lactic acid produced in the LAB inoculated tigernut-milk drink was as a result of mixed culture LAB used as starter culture which resulted in larger quantity of lactic acid being released in the fermenting tigernut-milk drink.

Conclusion

Generally, different microorganisms require different range of optimal pH to favour their growth. Also, competition for limited nutrients from numerous microorganisms that have diverse nutrient requirements significantly influences the population of beneficial microorganisms particularly in fermented foods and beverages. Tigernut-milk drink is usually produced domestically for household consumption and can be sold to the public. *Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus acidophilus* and *Lactobacillus acidophilus* isolated from ogi were able to utilize tigernut-milk drink which resulted in population increase of lactic acid bacteria but it did not meet the minimum of biovalue (MBV) which is one of the requirements of a probiotic product to function effectively in the body. This study has demonstrated that inoculating LAB into sterilized tigernut resulted in higher LAB count than unsterilized tigernut. Therefore, pasteurization of tigernut-milk drink before inoculating LAB into it is most likely to result in higher LAB count than using locally prepared tigernut-milk drink which had not undergone pasteurization or any form of heating.

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