

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Growth Behavior of Probiotic *Lactobacillus casei* 01 in Tiger Nut Milk Prepared with Milk By-Products

¹Hassan M. Sobhy, ²Mohammed A. El-Nawawy, ⁴Nagla A. Hegazi, ³Samia M. El-Dieb and ⁴Fatma H. Abd-Elrazik

¹Department of Natural Resources, Faculty of African postgraduate Studies, Cairo University, Giza, Egypt

²Department of Food Science, Faculty of Agriculture, Ain-Shams University, Cairo, Egypt

³Department of Dairy Science, Faculty of Agriculture, Cairo University, Giza, Egypt

⁴Dairy Technology Section, Animal Production Research Institute, Dokki, Giza, Egypt

Abstract

Background and Objective: Different researches have been achieved on non-dairy products as an alternative to dairy products. The interest in tiger nut tubers has considerably increase in recent years due to its nutritional and health benefits. Fermented drinks of non-dairy origin play an important role in diets worldwide. The aim of this study was to investigate the growth behavior and viability of *L. casei*-01 in tiger nut milk made with milk permeate or cheese whey as an extraction medium. **Materials and Methods:** Tiger nut milks were prepared using tiger nut tubers at ratios 1 to 3 (w/v) of water, milk permeate or cheese whey as extract media. Tiger nut milks and Skimmed milk were inoculated with *L. casei*-01 at 2%. The Titratable Acidity (TA) and *L. casei*-01 were determined during fermentation at 37°C for 8 hrs as well as during cold storage at 4°C for 20 days. **Results:** Results showed that the substitutions of water with permeate or whey led to the change of chemical composition of tiger nut milk. Fermented permeate or whey-tiger nut milk significantly had higher rate of titratable acidity development during fermentation or during cold storage as compared with fermented water-tiger nut milk or skimmed milk. The total viable counts of *L. casei*-01 were the highest in fermented whey-tiger nut milk after 10 days. **Conclusion:** *Lactobacillus casei*-01 can grow with high viability in permeate or whey-tiger milk.

Key words: Tiger-nut milk, *L. casei*-01

Citation: Sobhy, H.M., M.A. El-Nawawy, N.A. Hegazi, S.M. El-Dieb and F.H. Abd-Elrazik, 2021. Growth behavior of probiotic *Lactobacillus casei* 01 in tiger nut milk prepared with milk by-products. Pak. J. Biol. Sci., 24: 984-988.

Corresponding Author: Fatma H. Abd-Elrazik, Dairy Technology Section, Animal Production Research Institute, Dokki, Giza, Egypt

Copyright: © 2021 Hassan M. Sobhy *et al*. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tiger nut (*Cyperus esculentus*) is edible, sweet almond-like tubers of tropical and Mediterranean regions. Tiger nut has many names, such as chufa in a Spanish and Sudan, whereas Hab-El-Aziz in Egypt^{1,2} and it is available throughout the year. Tubers of tiger nut can be consumed raw or inverted into flour, oil and milk^{3,4}. Its nutritional constituents reflect a high content of lipid and dietary fiber, starch, P, Ca and phenolic compounds, which contribute to their antioxidant activity⁴. In Europe and United States, the beverage derived from tiger nut tubers are highly appreciated as an alternative to milk and for gluten-free diets.

Lactobacillus casei is an aciduric and belongs to the featured class of thermophilic LAB. Further the *L. casei* group can be divided into three species: *L. rhamnosus*, *L. zeae* and *L. casei* including the strains of *L. paracasei* and *L. casei*. *Lactobacillus casei* group is widely used in the food industry due to its biotechnological significance of heat resistance and ability to survive adverse conditions during food preparation^{5,6}. Tiger nut beverage can be fermented with lactic acid bacteria and give sweet-sour, lactose-free fermented products that could act as a substantial source of food nutrients⁷. Fermentation of tiger nut beverages by probiotic cultures has proven the ability of lactic acid bacteria to acidify the beverages⁴.

Water was the main liquid used for preparation of tiger nut milk. Utilization of milk by-products, (i.e., whey and permeate), for preparation of fermented tiger nut milk encourages the growth of starter bacteria. The valorization of milk by-products such as whey or permeate has become a major topic of research to develop the sustainability of food chain. Whey can be utilized in various food products for its nutritional and functional properties^{7,8}. Ultra-Filtration (UF) of milk produces a large quantity of permeate, which is considered as a solution of nutritious significance due to its contents of lactose and some soluble vitamins and salts. Utilization of UF permeate in food industry is considered an added value^{9,10}.

More studies are necessarily to pick out lactic acid bacterial starters which are well adapted to tiger nut beverage and result in a well-accepted beverage. Therefore, the aim of this study was to evaluate the growth behavior of probiotic *L. casei*-01 in tiger nut milk made with whey or permeate compared with skimmed milk during fermentation or during cold storage.

MATERIALS AND METHODS

Materials: Dried tiger nut tubers (*Cyperus esculentus* L.) were purchased from local market, Cairo, Egypt in December, 2020. Skimmed milk and cheese whey were obtained from the Dairy Technology Unit of Faculty of Agriculture, Cairo University, Giza, Egypt, while permeate was obtained from Animal Production Research Institutes, Cairo, Egypt. Probiotic *Lactobacillus casei*-01 was obtained from MIFAD Company, Cairo, Egypt. De Man-Rogosa-Sharpe Agar (MRS) medium was purchased from Hi-media Company, India. All chemicals were obtained from Sigma-Aldrich Cairo, Egypt.

Methods

Preparation of tiger nut milk (TNM): Water, milk permeate and cheese whey were utilized as extraction media. 100 g of dried tiger nut tubers were sorted, washed, put in boiling water for 10 min then drained and divided to 3 portions. The first portion of tubers was soaked in distilled water, the second portion was soaked in milk permeate and the third portion was soaked in cheese whey for 24 hrs. The tubers were washed, then milled with distilled water, permeate or sweet whey using blender at ratio of 1:3 (tubers/extraction medium). Each filtrate was then homogenized at two stages (1500 and 500 psi).

Growth behavior of *L. casei*-01 in TNM compared with skimmed milk: *Lactobacillus casei*-01 was active at 37°C using skimmed milk medium. The three Tiger Nut Milks (TNM) and the skimmed milk were heated at 70°C for 2 min, then cooled to 37°C and each was inoculated with 2% *L. casei*-01 and then incubated at 37°C. The titratable acidity and counts of *L. casei*-01 were determined during the fermentation period (0, 2, 4, 6 and 8 hrs).

Evaluation of *L. casei*-01 viable in TNM during cold storage: All fermented tiger nut milks were stored at 4°C for 20 days. The titratable acidity and the viability of *L. casei*-01 in all fermented tiger nut milks were followed during cold storage.

Methods of analysis

Chemical analysis: Titratable acidity, total solids, total protein, fat and ash contents were determined according to AOAC¹¹.

Bacterial analysis: *Lactobacillus casei*-01 counts were enumerated using de Man, Rogosa and Sharpe (MRS) medium, according to APHA¹².

Statistical analysis: The TNM treatments were carried out in duplicate, where each trial for each sample was done in triplicate. Data were recorded as the mean $\pm$ Standard Deviation (SD). The Mstat-C software was used to calculate the Least Significant Differences (LSD) at $p \leq 0.05$ that compare the significant differences among the means of different treatments.

RESULTS AND DISCUSSION

Chemical composition of tiger nut milk: Chemical composition of skimmed milk and different tiger nut milks that were extracted with water, milk permeate or cheese whey were presented in Table 1. The results showed that water-tiger nut milk contained 9.28% total solids, 0.41% protein, 1.47% fat, 0.22% ash and 7.18% total carbohydrates. Compared to water-tiger nut milk, permeate or whey-tiger nut milk significantly had the highest percentages of total solids, protein and ash as shown in Table 1. Whey-tiger nut milk was the highest in fat and total carbohydrates content. In this regard, Kizzie-Hayford *et al.*¹³ found that tiger nut milk extracted using water contained 10.40% total solids, 1.02% protein, 2.23% fat, 0.28% ash and 6.87% total carbohydrate. Another study by Wakil *et al.*¹⁴ declared that milk extracted from the three different tigernut varieties of tubers contained a range of 13.44 to 9.64% total solids, 0.76-1.66% protein, 0.76-1.66% fat, 0.32-0.42% ash and 7.77-8.34% carbohydrates.

Growth behavior of *L. casei* 01 in TNM compared with skimmed milk during fermentation: Titratable acidity and total viable counts of *L. casei* in fermented TNM and in skimmed milk during fermentation were tabulated in Tables 2 and 3. Concerning the titratable acidity during fermentation for 8hrs, it ranged between 0.19-0.36 in water-tiger nut milk (T1), 0.24-0.50 in permeate-tiger nut milk (T2), 0.18-0.51 in whey-tiger nut milk (T3) and 0.17-0.35 in skimmed milk (T4). The titratable acidity significantly differed among fermented tiger nut milk treatments, where fermented permeate and whey-tiger nut milk significantly had higher titratable acidity than that of fermented water-tiger nut milk and fermented skimmed milk. This result may be attributed to that the permeate and the whey contain lactose and whey proteins which encourage the growth of *L. casei* thus increase the lactic acid. Khedkar *et al.*⁷ observed that whey has been found to be useful as a base for growth of some of the lactic acid bacteria in the preparation of whey beverage. Also, enrichment of TNM with proteins significantly increased the lactic acid content, which was higher in the fermented products with

higher protein content¹³. Wakil *et al.*¹⁴ noticed that there was an increase in lactic acid content and decrease in pH of the fermenting tigernut milk with increase in fermentation time.

The total viable counts of *L. casei* in different fermented tiger nut milk samples during fermentation were shown in Table 3. The viable counts of *L. casei* during fermentation were ranged between 6.78-7.65, 6.31-8.34, 6.58-8.33 and 6.42-8.52 (log CFU mL⁻¹) for T1, T2, T3 and T4, respectively. The results showed that the counts at the end of fermentation period increased in all treatment compared with their initial counts. At the end of fermentation at 37°C, fermented water-tiger nut milk had the lowest viable counts of *L. casei*. The growth and viability of *L. casei* was enhanced in Tiger nut milk made with permeate or whey at the end of fermentation period compared with water-tiger nut milk.

In a similar study by Fiagbor¹⁵, tiger nut milk extracted in water alone could not support the growth and viability of *L. rhamnosus*, while the addition of whey protein and pectin to the extract led to an increase of 1.37 log CFU g⁻¹ in bacterial population of the treated over the control. In different study, Wakil *et al.*¹⁴ noticed that heat treated Yellow tiger nut milk sample (90°C/15 m) had the highest lactic acid bacterial count after 24 hrs (2.0×10^8 CFU mL⁻¹) while the lowest was recorded in same sample at zero hour (2.0×10^4 CFU mL⁻¹). It could be concluded that using of permeate and whey enhance growth and viability of *L. casei*.

Evaluation of *L. casei* 01 viability in TNM during cold storage: The changes in titratable acidity and *L. casei* viable counts of different treatments of fermented tiger nut milk during storage at 4°C for 20 days were shown in Tables 4 and 5, respectively. Titratable acidity had increased during cold storage in all treatments. It was noticed that T1 had the lowest rate of increase of titratable acidity compared to other treatments during storage at 4°C. At the end of storage, T3 had the highest rate of increase of titratable acidity than T1 and T2. This could be due to the dominance of LAB in the fermentation environment which contains more carbohydrates. It was reported that the ability of a high acid production has been demanded for lactic cultures to be used as starter cultures for dairy fermentation technology¹⁶. The results were in harmony with that obtained by Kizzie-Hayford *et al.*¹⁷ who found that fortification of tiger nut milk with sodium caseinate or whey proteins significantly reduced the pH and increased titratable acidity of the fermented tiger nut milk after 15 days of cold storage. In this regard, Fiagbor¹⁵ observed that addition of whey protein and pectin to tiger nut milk extract could support the growth and viability of *L. rhamnosus*.

Table 1: Chemical composition of tiger nut milk made with milk by-product

Parameters (%)	Treatments			
	T1	T2	T3	T4
Total solids	9.28±0.04 ^c	11.76±0.07 ^b	13.76±0.09 ^a	8.99±0.11 ^d
Ash	0.22±0.03 ^c	0.38±0.03 ^c	0.45±0.02 ^b	0.75±0.01 ^a
Protein	0.41±0.04 ^d	0.84±0.04 ^c	1.20±0.06 ^b	3.60±0.02 ^a
Fat	1.47±0.05 ^b	1.38±0.03 ^b	2.10±0.11 ^a	0.10±0.01 ^c
Carbohydrate	7.18±0.07 ^c	9.16±0.10 ^b	10.01±0.09 ^a	4.54±0.11 ^d

Values in the same column or row for each parameter with different superscript letters differ significantly ($p \leq 0.05$). T1: Water-tiger nut milk, T2: Permeate-tiger nut milk, T3: Whey-tiger nut milk and T4: Skimmed milk (control)

Table 2: Titratable acidity values of tiger nut milk and skim milk samples during fermentation using *Lactobacillus casei*-01

Treatments	Fermentation time (hrs)				
	0	2	4	6	8
T1	0.19±0.01 ^j	0.22±0.006 ⁱ	0.26±0.01 ^g	0.32±0.029 ^{de}	0.36±0.02 ^c
T2	0.24±0.015 ^{hi}	0.27±0.01 ^f	0.32±0.02 ^d	0.41±0.017 ^b	0.50±0.01 ^a
T3	0.18±0.01 ^j	0.24±0.021 ^{gh}	0.30±0.006 ^{de}	0.41±0.012 ^b	0.51±0.021 ^a
T4	0.17±0.01 ^j	0.19±0.006 ^j	0.23±0.015 ^{hi}	0.30±0.016 ^e	0.35±0.01 ^c

Values in the same column or row for each parameter with different superscript letters differ significantly ($p \leq 0.05$). T1: Fermented water-tiger nut milk, T2: Fermented permeate-tiger nut milk, T3: Fermented whey-tiger nut milk and T4: Skimmed milk (control)

Table 3: Total viable count of *Lactobacillus casei*-01 (log CFU mL⁻¹) in tiger nut milk and skim milk samples during fermentation

Treatments	Fermentation time (hrs)				
	0	2	4	6	8
T1	6.78±0.12 ^j	7.42±0.21 ^{gh}	7.39±0.90 ^{gh}	7.43±0.10 ^{efgh}	7.65±0.144 ^{defg}
T2	6.31±0.26 ^j	7.09±0.29 ^{hi}	7.96±0.23 ^{bcd}	7.89±0.19 ^{b-g}	8.34±0.51 ^{abc}
T3	6.58±0.28 ^j	7.82±0.09 ^{d-g}	7.85±0.07 ^{c-g}	7.93±0.35 ^{b-e}	8.33±0.28 ^{abc}
T4	6.42±0.104 ^j	7.91±0.13 ^{b-f}	7.70±0.27 ^{defg}	8.36±0.12 ^{ab}	8.52±0.23 ^a

Values in the same column or row for each parameter with different superscript letters differ significantly ($p \leq 0.05$). T1: Fermented water-tiger nut milk, T2: Fermented permeate-tiger nut milk, T3: Fermented whey-tiger nut milk and T4: Skimmed milk (control)

Table 4: Titratable acidity values of tiger nut milk treatments during storage at 4±1 °C

Treatments	Storage periods (days)		
	1	10	20
T1	0.36±0.006 ^b	0.46±0.006 ⁱ	0.50±0.006 ^e
T2	0.50±0.015 ^a	0.54±0.012 ^d	0.72±0.015 ^b
T3	0.51±0.015 ^a	0.65±0.021 ^c	0.75±0.015 ^a

Values in the same column or row for each parameter with different superscript letters differ significantly ($p \leq 0.05$). T1: Fermented water-tiger nut milk, T2: Fermented permeate-tiger nut milk, T3: Fermented whey-tiger nut milk

Table 5: Total viable count of *Lactobacillus casei*-01 (log CFU mL⁻¹) in tiger nut milk treatments during storage at 4±1 °C

Treatments	Storage periods (days)		
	1	10	20
T1	8.29±0.12 ^{bc}	7.75±0.13 ^c	6.85±0.22 ^d
T2	8.88±0.12 ^{ab}	8.83±0.04 ^{ab}	6.70±0.61 ^d
T3	8.57±0.15 ^{ab}	8.98±0.10 ^a	8.51±0.10 ^{ab}

Values in the same column or row for each parameter with different superscript letters differ significantly ($p \leq 0.05$). T1: Fermented water-tiger nut milk, T2: Fermented permeate-tiger nut milk, T3: Fermented whey-tiger nut milk

The growth of *L. casei* differed in all fermented tiger nut milk treatments with diverse extents. The *L. casei* viable counts on the day 1 of storage were as follows log 8.29, 8.88 and 8.57 (CFU mL⁻¹) for T1, T2 and T3, respectively. However, after 10 days of storage at refrigeration (4 °C), the numbers significantly decreased in fermented water-tiger nut milk while significantly increased in fermented whey-tiger nut milk. After 20 days of storage, *L. casei* count exhibited a drastic decline in fermented water and permeate-tiger nut milk compared to that of fermented whey-tiger nut milk. These results may be attributed to that whey contains lactose and whey proteins which encourage the growth of *L. casei*. Also, Yeboah⁶ mentioned that the growth of *L. casei* depended upon its ability to utilize the nutrients available in the growth medium for its survival. In addition according to Yeboah⁶ lactobacilli to survive in any media aside from the fermentable carbohydrate, there is the need for amino acids, vitamins and related growth factors.

Concerning this field, Kizzie-Hayford *et al.*¹⁷ reported that the enrichment of fermented tiger nut milk extracted in water with whey proteins or sodium caseinate led to

increase the viable counts of yogurt starter. In this respect, El-Shenawy *et al.*¹⁰ observed that the viable counts of *L. plantarum*, *L. acidophilus* and *Bifidobacterium breve* in fermented permeate-tiger nut beverages slightly increased until day 3 of storage and reduced thereafter. In another study, the viable counts of *S. thermophilus*, *L. bulgaricus* and *Bifidobacterium bifidum* of yogurt prepared with tiger nut extract and skimmed buffalo milk slightly increased until day 3 of storage and reduced there after¹⁸.

CONCLUSION

From the obtained results, Milk by-products (whey and permeate) were preferable in preparation of tiger nut milk than water. It was noticed that using Permeate or whey as extraction media in preparing tiger nut milk enhance the growth and viable counts of *L. casei*. Therefore, it's successful to offer fermented tiger nut milk to consumer as a wholesome food for different age groups.

REFERENCES

- Sanchez-Zapata, E., J. Fernandez-Lopez and J.A. Perez-Alvarez, 2012. Tiger nut (*Cyperus esculentus*) commercialization: Health aspects, composition, properties and food applications. *Compr. Rev. Food Sci. Food Safety*, 11: 366-377.
- Maduka, N. and F. Ire, 2018. Tigernut plant and useful application of tigernut tubers (*Cyperus esculentus*)-a review. *Curr. J. Appl. Sci. Technol.*, 30: 1-23.
- Gambo, A. and A. Da'u, 2014. Tiger nut (*Cyperus esculentus*): Composition, products, uses and health benefits-a review. *Bayero J. Pure Applied Sci.*, 7: 56-61.
- Roselló-Soto, E., C. Garcia, A. Fessard, F.J. Barba, P.E.S. Munekata, J.M. Lorenzo and F. Remize, 2019. Nutritional and microbiological quality of tiger nut tubers (*Cyperus esculentus*), derived plant-based and lactic fermented beverages. *Fermentation*, Vol. 5, No. 1, 10.3390/fermentation5010003.
- Nezhad, M.H., M.A. Hussain and M.L. Britz, 2015. Stress responses in probiotic *Lactobacillus casei*. *Crit. Rev. Food Sci. Nutr.*, 55: 740-749.
- Yeboah, S., 2020. Physicochemical and nutritional profiling of fermented tiger nut-cereal-based synbiotic dairy drink. Masters Thesis, University of Ghana. <http://197.255.68.205/handle/123456789/35307>.
- Khedkar, J.N., D.M. Choudhari, B.K. Pawar and V.S. Kadam, 2014. Use of milk by-product (whey) for preparation of beverages. *Asian J. Anim. Sci.*, 9: 193-197.
- Mudgil, D. and S. Barak, 2019. Dairy-Based Functional Beverages, In: *Milk-Based Beverages*, Grumezescu, A.M. and A.M. Holban (Eds.), Elsevier, Amsterdam, Netherlands, ISBN-13: 978-0-12-815504-2, pp: 67-93.
- Rizk, A.E., 2016. Study of production functional beverages of milk permeate fortified with fruit and herbs. *Middle East J. Appl. Sci.*, 6: 155-161.
- El-Shenawy, M., M.T. Fouad, L.K. Hassan, F.L. Seleet and M.A. El-Aziz, 2019. A probiotic beverage made from tiger-nut extract and milk permeate. *Pak. J. Biol. Sci.*, 22: 180-187.
- AOAC., 2012. Association of Official Analytical Chemists, AOAC International Gaithersburg, MD.
- APHA, 2004. Standard Method of Examination of Dairy Products. 17th Edn., American Public Health Association, Washington, DC., USA, ISBN-13: 978-0-87553-264-6.
- Kizzie-Hayford, N., D. Jaros, S. Zahn and H. Rohm, 2016. Effects of protein enrichment on the microbiological, physicochemical and sensory properties of fermented tiger nut milk. *LWT*, 74: 319-324.
- Wakil, S.M., O.T. Ayenuro and K.A. Oyinola, 2014. Microbiological and nutritional assessment of starter-developed fermented tigernut milk. *Food Nutr. Sci.*, 5: 495-506.
- Fiagbor, R., 2017. Development of probiotic milk with tiger nut (*Cyperus esculentus*) milk extract. Masters Thesis, North Carolina A&T State University. <https://www.proquest.com/openview/38eae8316519fe09d91fbd5e36ef758e/1?pq-origsite=gscholar&cbl=18750>
- Buchenhushkes, H.J., 1993. Selection criteria for lactic acid bacteria to be used as starter cultures in various food commodities. *FEMS Microbiol. Rev.*, 12: 253-272.
- Kizzie-Hayford, N., D. Jaros and H. Rohm, 2017. Enrichment of tiger nut milk with microbial transglutaminase cross-linked protein improves the physico-chemical properties of the fermented system. *LWT Food Sci. Technol.*, 81: 226-232.
- El-Shenawy, M., M.A. El-Aziz, W.I. El-Kholy and M.T. Fouad, 2012. Probiotic yoghurt manufactured with tiger-nut extract (*Cyperus esculentus*) as a functional dairy food. *J. Agric. Nat. Resour.*, 1: 20-31.