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Research Article

A Probiotic Beverage Made from Tiger-nut Extract and Milk Permeate

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Abstract

Background and Objective: Tiger-nut has long been recognized for its generous health benefits. Milk permeate as a by-product, contains lactose, soluble vitamins and salts. Probiotics are live micro-organisms that confer a benefit on the host. The aim of this work was to use a combination of tiger-nut aqueous extract (TNAE) and UF-milk permeate, fortified with probiotic bacteria, to produce a functional dairy beverage. **Materials and Methods:** Milk-permeate (65%), TNAE (30%) and sugar (5%) were the best portions used to produce permeate-tiger nut beverage. Three probiotic bacteria mixture including, *L. plantarum* and *L. acidophilus* culture (1:1), *L. plantarum* and *B. breve* culture (1:1) and *L. plantarum* with both *L. acidophilus* and *B. breve* culture (1:1:1) were added to create 3 permeate-tiger nut beverages namely T₁, T₂ and T₃, respectively. The follow up of their bacteriological, physical and chemical characteristics/changes during a storage period of 10 days was evaluated. **Results:** No changes in the survival of the probiotics bacteria were observed throughout the storage period (10 days). Meanwhile, T₃ has low pH value and acetaldehyde content but has high diacetyl content and antioxidant activity followed by T₂ and T₁. Permeate-tiger nut beverage fortified with probiotic bacteria exhibited higher lightness and lower redness and structure viscosity than the control from day 5 onwards. Also, T₃ was less sensory acceptable compared to the others. **Conclusion:** A mixture of UF-milk permeate (65%), tiger-nut aqueous extract (30%) and 5% sugar, fortified with 1% mixture of probiotic cultures produced a healthy stable beverage.

Key words: Tiger-nut aqueous extract, milk permeate, probiotic beverage, chemical, physical, bacteriological changes

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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 an underutilized tuber of family Cyperaceae, which produces rhizomes¹. It was cultivated in the region of chufa between Sudan and Egypt on the borders of the Nile river. Tiger nut has long been recognized for its health benefits as it is rich in fiber, protein, natural sugars, minerals and vitamins such as E and C². It contributes to the reduction of cholesterol, reduces the risk of coronary heart disease, arteriosclerosis and is recommended for those who have heavy digestion, flatulence and dysentery and cancer especially of the colon. It is also beneficial to diabetics and those seeking to reduce cholesterol or lose weight. The tiger nut yield more milk upon extraction contains lower fat and higher protein and less anti-nutritional factors especially polyphenols³. This milk was classified as medicinal drink due to its highly energetic and diuretic and rich in mineral, predominantly phosphorus and potassium as well as vitamins². It has a pleasant nutty flavor and consumed as a kind of snakes food and could be used in food technology. These numerous advantages and health benefits associated with tiger-nut makes it more attractive in some dairy products. Ultrafiltration (UF) of milk produces a large quantity of permeate as a by-product. It contains lactose as the major constituent in addition to soluble vitamins and salts. Therefore, permeate can be considered as a solution of nutritious significance⁴. A huge amount of UF permeate is produced annually and drained as waste. Utilization of UF permeate in the food industry will reduce environmental pollution and consider an added value. Probiotics are defined as live micro-organisms (bacteria or yeast) which when administered in adequate amounts confer a benefit on the host⁵. The health benefit of functional foods may be further enhanced by supplementation of certain lactic acid bacteria which consider the most used probiotic cultures with established human health in dairy products and beverages⁶. In this study, it developed a probiotic beverage composed of tiger-nut aqueous extract, UF milk-permeate and probiotic bacteria as a nutritive/healthy stable functional beverage food. Study of the physical, chemical, bacteriological and sensory properties of the produced beverage, during a storage time of 10 days was another goal.

MATERIALS AND METHODS

Materials: Tiger-Nut (*Cyperus esculentus*) and sugar were purchased from local market, Cairo, Egypt. Ultrafiltration (UF)

milk permeate was obtained from Animal Production Research Centre, Ministry of Agriculture. Cairo, Egypt. The probiotic bacterial strains, including *Lactobacillus acidophilus* La-5 (DVS), *Lactobacillus plantarum* and *Bifidobacterium breve* Bb-12 (DVS) were obtained from Chr-Hansen's Lab., Denmark.

Methods: This study was carried out in the laboratories of the National Research Centre (Giza, Egypt) during the period April till July, 2018.

Preparation of the tiger-nut aqueous extract: The dry tiger-nuts were sorted, washed and soaked in tap water at a ratio of 1:3 (w/v) at room temperature of 30°C for 24 h to obtain the hydrated or fresh-like nuts of 45-50% moisture content. The hydrated tiger-nuts were blanched by boiling in 0.2% solution of sodium bicarbonate to eliminate the nutty flavor, which may be objectionable to some consumers and washed twice in tap water. Then, the hydrated tiger-nuts were blended with distilled water (1:3 w/v) using a kitchen blender (Philips, HR 7740, Hungary) at the maximum speed for 5 min. The homogenous slurry was filtered using a muslin cloth by squeezing until virtually no extract was recovered and centrifuged at 2500 xg for 5 min. The supernatant obtained and heated at 70°C for 20 min, cooled to 4°C and refrigerated for further processes. The average composition of the tiger-nut extract was 96.86, 0.34, 0.95, 1.65 and 0.20% for moisture, protein, fat, carbohydrates and ash, respectively.

Activation of the bacterial strains: Strains of *Bifidobacterium breve* Bb-12, *L. plantarum* and *L. acidophilus* were activated individually by three successive transfers in the modified MRS followed by three successive transfers in the sterile 10% reconstituted skim milk powder. The cultures were incubated at 37°C for 48 h under anaerobic conditions⁷ and prepared 24 h before use.

Beverage manufacture: Firstly, different concentrations of UF milk permeate fortified with 5, 10, 20, 30 and 40% tiger-nut aqueous extract (TNAE) have been done. According to the primary sensory evaluation, the mixture of 30% TNAE, 65% UF milk permeate and 5% sugar was used, heated to 90°C for 5 min and then cooling to 40°C. The mixture was divided into 4 portions and inoculated separately with 1.0% of *L. plantarum* and *L. acidophilus* culture (1:1), *L. plantarum* and *B. breve* Bb-12 culture (1:1) and *L. plantarum* with both *L. acidophilus* and *Bifidobacterium breve* Bb-12 culture

(1:1:1) to create three probiotics containing beverages (T_1 , T_2 and T_3 , respectively). The fourth portion had no probiotic bacteria and served as a control. The inoculated beverages were mixed thoroughly, dispensed in 200 mL glass bottles. Bacteriological analysis of all beverage treatments was analyzed at day 1, 3, 5, 7 and 10, while the chemical analysis was done at day 1, 5 and 10 of storage at $4 \pm 2^\circ\text{C}$. The beverage manufacturing was done three separated times.

Bacteriological analysis: All beverage samples were serially diluted and subsequently plated, in duplicate, onto the following selective media. The *B. breve* was counted according to Blanchette *et al.*⁸ using modified MRS agar supplemented with 0.05% L-cysteine-HCl. The plates were incubated at 37°C for 48 h under anaerobic conditions. The viable cell count of *L. acidophilus* was determined on MRS agar plus 0.2 g/100 mL oxgall as described by Gilliland and Walker⁹. The *L. plantarum* was determined by serial dilution in peptone water-Himedia and seeded in De Man-Rogosa-Sharpe agar (MRS-Himedia) according to De Souza *et al.*¹⁰. The plates were incubated at 37°C for 72 h under aerobic conditions.

Chemical analysis: The composition of TNAE for moisture, protein, fat, carbohydrates and ash was done according to AOAC¹¹. The changes in pH in the beverage samples during storage period were measured using a laboratory pH meter with a glass electrode (HANNA, Instrument, Portugal). The concentration of acetaldehyde and diacetyl in the beverage samples were measured using spectrophotometer method as described by Lee and Jago¹². Radical scavenging activity of permeate tiger-nut beverage was estimated using a stable DPPH radical (DPPH^{*}) assay according to Brand-Williams *et al.*¹³.

Structure viscosity: Structure viscosity of the beverage samples was measured at 7°C using a Brookfield digital viscometer (Model DV-II, Canada) fitted with spindle-00. The beverage samples were subjected to selected shear rates ranging from 3.0-60.0 S^{-1} for an upward curve. Structure viscosity was expressed as mPa sec.

Color parameters: Hunter L, a and b parameters of beverage samples were measured using a spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale

(Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode. Where, L: Darkness from black (0) to white (100), a: Colour red (+) to green (-), b: Colour yellow (+) to blue (-).

Sensory evaluation: Expert judges (males and females) were selected from staff member of Department of Dairy Science, National Research Center, Egypt, to evaluate the taste (10 points), flavor (10 points), color (10 points) and consistency (10 points) of the beverage samples. The beverage samples were coded with three-digit random numbers. Water and non-salted crackers were provided to clean their palates between tasting.

Statistical analysis: Statistical analysis was performed using the GLM procedure with SAS¹⁴ software. The differences between treatments, storage and their interactions were identified using two-way independent factorial analysis of variance (ANOVA). Duncan's multiple comparison procedure was used to compare the means at significance level of 0.05.

RESULTS AND DISCUSSION

Tiger-nut extracts percentage: The sensory evaluations of the different tiger-nut's aqueous extract (TNAE) levels added to UF-milk permeate to select the best addition level are shown in Table 1. The score values of all sensory items (consistency, flavor, taste and color) increased as the concentration of TNAE increased. The increase was more pronounced in the UF-milk permeate fortified with 30 and 40% TNAE than that fortified with 5, 10 and 20% TNAE ($p < 0.05$). Therefore, taking into consideration the production cost, 30% TNAE was selected to produce the probiotic permeate tiger-nut beverage.

Probiotic permeate tiger-nut beverage

Viable counts of probiotic bacteria: Table 2 exhibited the changes in viable counts ($\Delta \log_{10} \text{CFU mL}^{-1}$) of the probiotic

Table1: Sensory evaluations of UF-milk permeate fortified with different levels of tiger-nut aqueous extract

Tiger-nut aqueous extract (%)	Consistency	Flavor	Taste	Color
5	5.18 ^c	6.27 ^b	5.72 ^b	5.27 ^b
10	5.45 ^c	6.27 ^b	6.27 ^b	5.64 ^b
20	6.91 ^b	7.09 ^{ba}	7.55 ^a	7.36 ^{ba}
30	8.18 ^a	7.91 ^a	8.00 ^a	8.36 ^a
40	9.00 ^a	8.18 ^a	8.27 ^a	8.82 ^a

Means with the same letters are not significantly different ($p \leq 0.05$)

Table 2: Viable counts (\log_{10} CFU mL⁻¹) of probiotic bacteria in permeate-tiger nut beverage during storage period at $4 \pm 2^\circ\text{C}$ for 10 days

Organisms	Storage period (day)	Beverage treatments (\log_{10} CFU mL ⁻¹)			
		Control	T ₁	T ₂	T ₃
<i>Lactobacillus acidophilus</i>	1	-	7.90	-	7.93
	3	-	8.64	-	8.58
	5	-	8.34	-	8.66
	7	-	7.92	-	8.37
	10	-	7.94	-	7.93
<i>Bifidobacterium breve</i> Bb-12 (DVS)	1	-	-	8.60	8.13
	3	-	-	8.68	8.86
	5	-	-	8.80	8.69
	7	-	-	8.68	8.44
	10	-	-	8.57	8.13
<i>Lactobacillus plantarum</i>	1	-	8.35	7.90	7.80
	3	-	8.91	8.74	8.81
	5	-	8.80	8.54	8.49
	7	-	8.61	7.89	8.25
	10	-	8.35	7.81	7.75

Control: UF-milk permeate with 30% TNE and 5% sugar; T₁: Control beverage with 1% *L. plantarum* and 1% *L. acidophilus*; T₂: Control beverage with 1% *Bifidobacterium breve* and 1% *L. plantarum*; T₃: Control beverage with 1% *L. plantarum*, *Bifidobacterium breve* and 1% *L. acidophilus*

Table 3: Chemical properties and antioxidant activity of probiotic permeate-tiger nut beverage during storage period at $4 \pm 2^\circ\text{C}$ for 10 days

Items	Storage period (day)	Beverage treatments			
		Control	T ₁	T ₂	T ₃
pH	1	6.27 ^a	6.23 ^a	6.05 ^a	5.76 ^{ab}
	5	5.98 ^a	5.87 ^{ab}	5.63 ^{ab}	5.05 ^c
	10	5.90 ^a	5.24 ^b	4.85 ^{dc}	4.53 ^d
Acetaldehyde ($\mu\text{mol}/100\text{ g}$)	1	6.02 ^{ab}	6.40 ^{ab}	6.08 ^{ab}	5.68 ^{ab}
	5	5.08 ^b	6.00 ^{ab}	8.56 ^a	3.96 ^b
	10	4.92 ^{ab}	5.92 ^{ab}	5.12 ^{ab}	2.56 ^b
Diacetyl ($\mu\text{mol}/100\text{ g}$)	1	0.84 ^e	0.92 ^e	3.88 ^c	3.60 ^c
	5	1.84 ^{ed}	1.88 ^{ed}	4.56 ^{cb}	6.08 ^b
	10	2.24 ^d	4.04 ^c	4.52 ^{bc}	9.04 ^a
Antioxidant activity (%)	1	4.19 ^d	8.68 ^{ab}	7.21 ^{bc}	6.78 ^{cd}
	5	4.41 ^d	8.90 ^{ab}	9.34 ^{ab}	10.22 ^a
	10	4.71 ^{cd}	8.43 ^{ab}	7.44 ^{bc}	10.22 ^a

Control: UF-milk permeate with 30% TNE and 5% sugar; T₁: Control beverage with 1% *L. plantarum* and 1% *L. acidophilus*; T₂: Control beverage with 1% *Bifidobacterium breve* and 1% *L. plantarum*; T₃: Control beverage with 1% *L. plantarum*, *Bifidobacterium breve* and 1% *L. acidophilus*

bacteria (*L. plantarum*, *B. breve* and *L. acidophilus*) during storage of the permeate-tiger nut beverage at $4 \pm 2^\circ\text{C}$ for 10 days. In general, all probiotic bacteria slightly increased until day 3 ($p > 0.05$). In general, all probiotic bacteria slightly increased until day 3 ($p > 0.05$) and slightly decreased thereafter ($p > 0.05$). The decrease was not less than the initial count (day 1). Similar trends were observed in the production of probiotic yoghurt supplemented with tiger-nut extract¹⁵, date palm and yoghurt syrups¹⁶ and fermented soy milk¹⁷ where no significant difference ($p > 0.05$) in the viable counts of the fortified lactic acid bacteria starters were observed.

Chemical properties: Chemical properties of permeate-tiger nut beverage during storage period at $4 \pm 2^\circ\text{C}$ for 10 days are

presented in Table 3. On day 1, pH value of control beverage and T₁ exhibited higher pH value than T₂ and T₃; the difference was not significant ($p > 0.05$). During the storage period, pH value decreased as the time of storage increased. The decrease in pH value was significant at day 5 and 10 for T₃ ($p < 0.05$), whereas was significant only at day 10 for T₂ ($p < 0.05$). The low pH value in T₃ followed by T₂ may be related to the presence of *L. acidophilus*, the primary function of which is to produce lactic acid¹⁸. However, the decrease during storage in pH value for control beverage and T₁ was not significant ($p > 0.05$).

Acetaldehyde and diacetyl are the major aroma compounds in cultured dairy products^{19,20}. On day 1, there was no significant difference ($p > 0.05$) in the concentration of acetaldehyde produced in probiotics containing

Table 4: Color properties of probiotic permeate-tiger nut beverage during storage period at 4±2°C for 10 days

Color properties	Storage period (day)	Beverage treatments			
		Control	T ₁	T ₂	T ₃
L	1	71.38	70.81	72.06	70.68
	5	66.59	69.35	72.01	72.86
	10	67.53	70.08	72.68	71.94
a	1	-0.02	0.03	-0.12	0.33
	5	0.27	0.25	0.15	-0.55
	10	0.56	-0.23	-0.66	0.07
b	1	16.83	16.33	16.17	16.83
	5	17.07	16.74	16.63	15.86
	10	17.6	16.9	17.23	17.44

Control: UF-milk permeate with 30% TNE and 5% sugar; T₁: Control beverage with 1% *L. plantarum* and 1% *L. acidophilus*, T₂: Control beverage with 1% *Bifidobacterium breve* and 1% *L. plantarum*, T₃: Control beverage with 1% *L. plantarum*, *Bifidobacterium breve* and 1% *L. acidophilus*, L: Darkness from black (0) to white (100), a: Color red (+) to green (-), b: Color yellow (+) to blue (-)

beverages compared with the control beverage (Table 3). As the storage period increased, acetaldehyde content decreased and diacetyl content increased. Acetaldehyde content was the highest in T₂ and the lowest in T₃ at day 5 and 10 (p<0.05), respectively. The content of acetaldehyde may be positively related with pH value, decrease as a pH value decrease. Inversely, the higher concentration of diacetyl content was found in T₃ at day 5 and 10. In mixed culture, diacetyl production is enhanced by the rapid drop in pH²¹. Such result has been reported by Hassan *et al.*²² in yoghurt containing cress seed mucilage and guar gum.

As shown in Table 3, the antioxidant activity of permeate-tiger nut beverage without probiotic bacteria (control beverage) was the lowest as compared to all probiotics containing beverages (p<0.05) at all storage periods. That means fortification of beverage with probiotic bacteria enhances the antioxidant activity. Both Shen *et al.*²³ and Persichetti *et al.*²⁴ showed that probiotic bacteria exhibit significant antioxidant abilities both *in vivo* and *in vitro*. Lin and Yen²⁵ have reported that intracellular cell-free extract of all *L. acidophilus*, *L. bulgaricus*, *S. thermophilus* and *B. longum* demonstrated antioxidative activity with inhibition rates of ascorbate autoxidation in the range of 7-12%. However, no significant difference was found in the antioxidant activity among all probiotic beverage treatments (p<0.05), even if the antioxidant activity was numerically higher in T₃ at day 5 and 10.

Physical properties

Color parameters: As shown in Table 4, there was no much difference in whiteness, redness and yellowish degree of all probiotic permeate-tiger nut beverage compared with control beverage. On day 1, the whiteness, redness and

yellowish degree were ranged from 70.68-72.06, -0.12 to 0.33 and 16.17- 16.83, respectively. As the time of storage increased, the whiteness degree reduced and redness increased in control beverage (without probiotic bacteria), while were more stable in all probiotics containing beverages. Stability of whiteness degree with the time storage may be associated with antioxidant activity²⁶, which was higher in probiotics containing beverages compared to control beverage (Table 3). Inversely, the yellowish degree slightly increased as the time increased in all beverage treatments.

Structure viscosity: Structure viscosity of permeate-tiger nut beverage during storage period at 4±2°C for 10 days is shown in Fig 1. In general, the structure viscosity of all permeate-tiger nut beverages was gradually decreased along with the increase of the shear rate, which reflected that all the beverage treatments show shear-thinning behavior. On day 1, there was no much difference in structure viscosity among all beverage treatments at different shear rates. The structure viscosity of control beverage was higher than that of probiotics containing beverages at day 5 and 10. Similar observations were found by El-Shenawy *et al.*¹⁵. The change in pH value which affect the ionic bonds and hydrogen bonds and the probiotic bacteria which able to produce amylase enzyme, decrease the molecular association between starch chains and hydrolyze the starch chains and hence decrease the viscosity. However, T₁ exhibited the lower viscosity compared with other probiotics containing beverages.

Sensory properties: Sensory properties of permeate-tiger nut beverages during storage period at 4±2°C for 10 days are presented in Table 5. The results revealed that there was no significant difference in consistency and color attributes

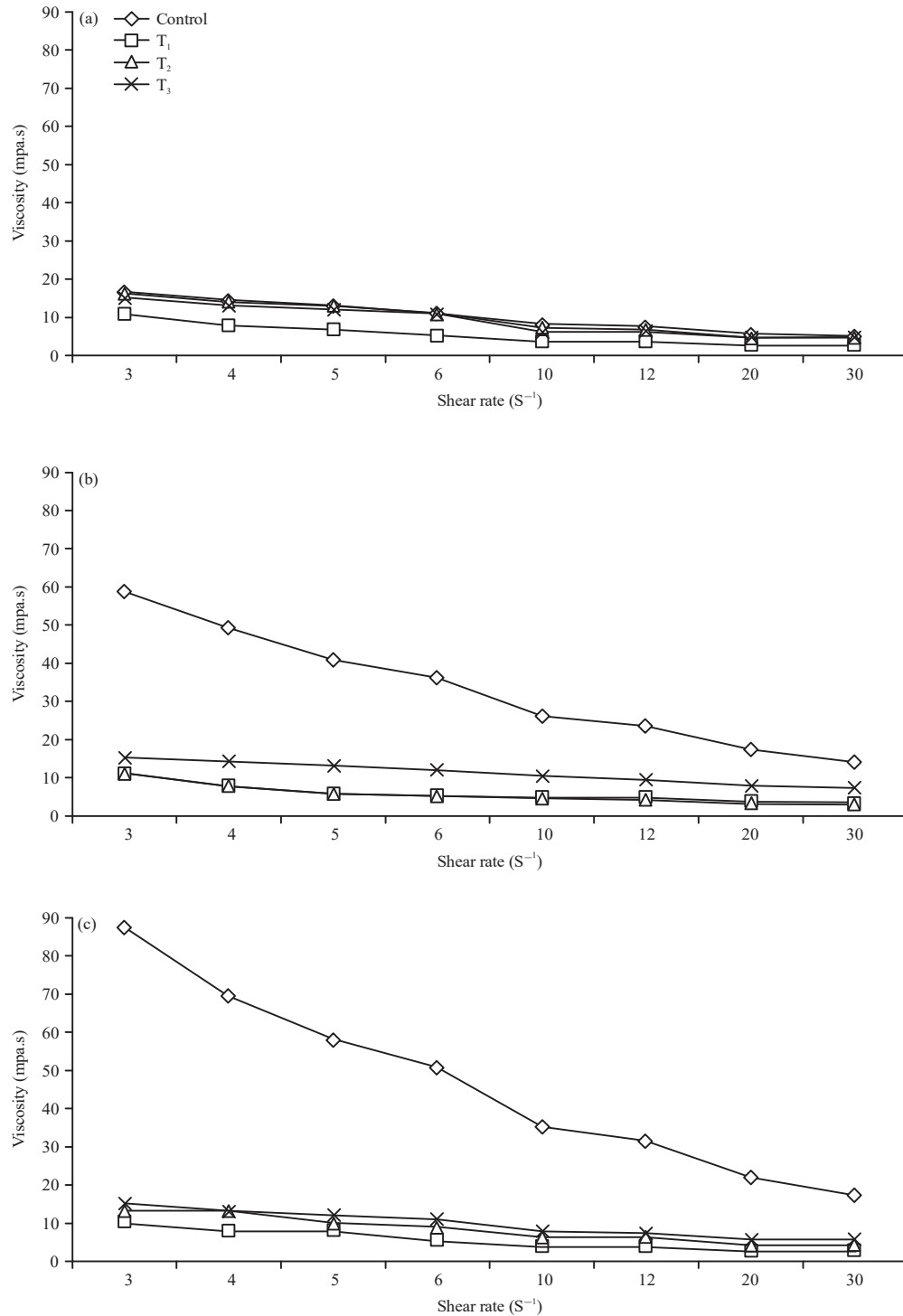


Fig. 1: Structure viscosity of permeate-tiger nut beverage during storage period at 4±2°C for 10 days

Control: UF-milk permeate with 30% TNE and 5% sugar, T₁: Control beverage with 1% *L. plantarum* and 1% *L. acidophilus*, T₂: Control beverage with 1% *Bifidobacterium breve* and 1% *L. plantarum*, T₃: Control beverage with 1% *L. plantarum*, *Bifidobacterium breve* and 1% *L. acidophilus*

(p>0.05) of probiotics containing beverages compare with control beverage at different storage periods. Also, the differences were not significant in flavor and taste attributes

among control beverage, T₁ and T₂. At the same time, the flavor and taste attributes of T₃ was the lowest value at day 5 and 10, which was not clean and more acidic.

Table 5: Sensory properties of probiotic permeate-tiger nut beverage during storage period at $4 \pm 2^\circ\text{C}$ for 10 days

Beverage treatments	Storage period (days)	Consistency	Flavor	Taste	Color
Control	1	8.40 ^a	7.90 ^a	7.75 ^a	7.70 ^a
	5	9.17 ^a	7.83 ^a	7.92 ^a	8.50 ^a
	10	9.21 ^a	7.54 ^a	7.54 ^a	7.70 ^a
T ₁	1	8.40 ^a	8.10 ^a	8.00 ^a	8.00 ^a
	5	9.17 ^a	7.90 ^a	8.17 ^a	9.00 ^a
	10	9.12 ^a	7.45 ^a	7.83 ^a	8.54 ^a
T ₂	1	8.50 ^a	7.30 ^a	7.10 ^a	7.80 ^a
	5	9.17 ^a	7.50 ^a	7.17 ^a	9.00 ^a
	10	8.90 ^a	7.65 ^a	7.33 ^a	8.10 ^a
T ₃	1	8.40 ^a	7.50 ^a	7.45 ^a	8.00 ^a
	5	9.33 ^a	5.50 ^b	5.00 ^b	9.00 ^a
	10	8.45 ^a	5.45 ^b	5.00 ^b	8.45 ^a

Control: UF-milk permeate with 30% TNE and 5% sugar, T₁: Control beverage with 1% *L. plantarum* and 1% *L. acidophilus*, T₂: Control beverage with 1% *Bifidobacterium breve* and 1% *L. plantarum*, T₃: Control beverage with 1% *L. plantarum*, *Bifidobacterium breve* and 1% *L. acidophilus*

CONCLUSION

From the previous results, the use of 65% UF-milk permeate, 30% tiger-nut extract and 5% sugar fortified with probiotic strains of *Lactobacillus acidophilus* with *Lactobacillus plantarum* (1:1) or *Lactobacillus acidophilus* with *Bifidobacterium breve* (1:1) can produce acceptable and stable healthy food drink for storage up to 10 days at $4 \pm 2^\circ\text{C}$.

SIGNIFICANCE STATEMENT

This study was a trail to combined the healthy benefits of the tiger-nut extract with the milk permeate, drain-waste in dairy industries and the probiotic bacteria, as a mixture, to produce a healthy/nutritive beverage that can be beneficial for abdominal/gastroenteritis discords and even for healthy consumers. It will help the researcher to uncover the possible different uses of tiger-nut extract as well as the milk permeate that not complete yet.

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