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Quality Attributes of Optimized Tiger Nut (*Cyperus esculentus*) milk Based Probiotic Drink

Malashree L.[1*], Ramachandra B.[2] and Prabha R.[3]
Department of Dairy Microbiology, Dairy Science College, Karnataka Veterinary Animal and Fisheries Sciences University, Bangalore-560024, (Karnataka), India.

(Corresponding author: Malashree L.)*
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ABSTRACT: **In this study objective was to prepare probiotic drink by blending of tiger nut aqueous extract (TNAE) and cow milk owing to dairy functional food. Tiger nut aqueous extract with dilution of 1:4 sterilized and inoculated with two probiotic strains *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 separately and had titratable acidity 0.66 % LA and direct microscopic count of log_{10} 7.54 per g. The blend of TNAE and cow milk was formulated to 75:25 had pH of 6.5; viscosity 0.98 cP at 20 °C, specific gravity of 1.001g/ml; titratable acidity of 0.15 % LA, lactose content of 0.40%, fat content of 4.02 %, protein of 2.80 %, total solid of 10.8 % with no lactose. The blended milk was heat treated at 85°C/30 min, 2% inoculum of *Bifidobacterium* BB-12, incubated at 37°C for 24 h yielded better titratable acidity of 0.86 % LA, DMC of 8.74 and best score for overall acceptability 8.25. Blend with 0.25 % stevia resulted in 0.85 % LA and 8.57 log_{10} cfu/ml and accepted with better score of 8.0 than that of 0.5 and 0.75 %. The prepared TNAE based probiotic drink had physico-chemical, microbiological characteristics such as pH of 4.9, viscosity (at 20°C) of 1.608 cP and specific gravity (at 20°C) of 1.069 g/ml. The titratable acidity of 0.85 % LA, lactose of 0.18 %, fat of 4.0 %, protein of 2.5% and total solids of 10.10% were noticed in the product. The viable count of *Bifidobacterium* BB-12 in the product had 8.68 log_{10} cfu/ml which was desired probiotic number in the product. This study contributed to open promising prospect for creating awareness about the cultivation of tiger nuts in India as it is economical crop with therapeutic benefits through functional drinks.**

Keywords: Tiger nut, Tiger nut aqueous extract, Probiotic drink, functional drink, Fermented beverage.

INTRODUCTION

Tiger nut is an edible perennial grass like plant of the sedge family with the scientific name *Cyperus esculentus lativum*. Tiger nuts are commonly called as earth almonds, rush nut, yellow nut sedge, chufa, cicoda in Hindi and *Bhadramusta* in Sanskrit. In spite of all these common names tiger nut tubers are familiar as they were originally cultivated 4000 years ago, during ancient Egyptian civilization (cradle of civilization) between the two rivers Tigris (Tiger) and Euphrates. Their cultivation was subsequently extended throughout other areas with temperate climate and fertile soil. Reports have shown that tiger nuts came to Spain from Africa (IHS, 2005). Tiger nuts are not actually nuts but tubers found on the root of a sedge plant. Characteristics of tiger nut tubers are oblong shaped, yellow, brown or black bumpy-skinned with an encircling leaf scar and ivory internal nut-like flesh, sweet taste and nutty flavour. In Egypt and the Mediterranean, nut tubers found their role in food, cosmetics and medicinal fields.

The "Horchata" is a vegetable milk extracted directly from tiger nut. It's a refreshing drink, superb as substitutive of traditional cow milk with a natural sweet taste. One of the under-utilized food ingredients in Africa and other developing economies is the Tiger nut milk (TNM. In 2019, Horchata became one of the most popular flavours in U.S, with 257 % hike in sales and considered national drink of Spain (www.thrillist.com, 2020).

Lactic acid fermentation of TNM is of particular interest because of the prospects to generate lactose-free, yogurt-like products of improved microbial stability and extended shelf life with acceptable sensory properties. Such fermented systems might be promising as a valuable alternative source of food nutrients, especially in many developing countries where the population shows high prevalence of lactose intolerance and has limited access to nutritious food Vesa *et al*., (2000).

Probiotics is a trendy concept for prevention of metabolic diseases in human beings. Probiotics can compensate for lactase insufficiency by the hydrolysis of lactose in the milk product in the intestine especially in colon region. The hydrolytic capacity of probiotic strains can be used to reduce the actual amount of lactose in the product, as occurs in yogurt. It can also be used to increase the overall hydrolytic capacity in the

small intestine. *Lactobacillus acidophilus* and species of *Bifidobacteria* are bile salt tolerant bacteria, inhabitants of gastro-intestinal tract having the ability to hydrolyse lactose and further used for acid production.

Resistant Starch (RS) is a newly recognized healthy food for human and animals. RS is a prebiotic component present in tiger nut and improves the population of gut microbiota (Yang *et al.,* 2017). A few of the scientists have reported on the development of fermented tiger nut beverages along with the combination of soy milk, coconut milk and almond milk with the addition of probiotic strains which forms the recent concept of synbiotic products, a combination of probiotics and prebiotics.

Tiger nut has attracted very little scientific attention and it is an underutilized crop yet to be fully exploited. Development of new food products from tiger nut could enhance more interest in this crop and contribute to food security of many developing nation. The results of this study provide, a baseline data on tiger nut utilization. This will be a long way to diversify its use and in turn may lead to its increased production both at household as well as nation wise. Furthermore, it is expected that the thorough understanding of composition of tiger nut may further be ventured for its use in the prevention and treatment of some non-communicable diseases, for example lactose intolerance, gastro-intestinal disorders, diabetes and cardiovascular diseases.

MATERIALS AND METHODS

Probiotic strains. Probiotic strains *Bifidobacterium animalis* ssp. *lactis* BB-12 and *Lactobacillus acidophilus* were procured from CHR.HAN Company. Probiotic bacteria were maintained in 10 ml of sterile reconstituted skim milk as working culture and subcultured once in 7 days. Stock cultures were maintained in yeast glucose chalk litmus milk and subcultured once in 21 days. Both working and stock cultures were stored at refrigeration condition (5ºC).

Tiger nuts. A standard grade quality of raw, dried tiger nuts were purchased from the online amazon global store and preserved in air tight container at room temperature.

Cow milk. Aseptically, cow milk was collected from the Livestock farm complex of Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bengaluru -24 for the present study.

Preparation of Tiger nut aqueous extract (TNAE). To get aqueous extract from tiger nut, required quantity of dried tiger nuts were sorted to remove defective tubers and washed thoroughly in tap water for 3- 4 times. Tiger nuts were soaked in tap water at a ratio of 1:3 w/v at room temperature for minimum of 8 h to soften the fibrous tissues. Then the hydrated tiger nuts were washed, blended with potable water (1: final volumes w/v) using mixer at a maximum speed of 5 min. Thereafter, the aqueous extract from the homogenous slurry was extracted by sieving using a clean, dry muslin cloth. The chaff was removed (stored for further use) and the TNAE was packed in sterile plastic bottles and refrigerated till required for use (Nazir, 2017).

Standardization of dilution of TNAE. Standardization of dilution of TNAE was carried out by soaking of 25 g of tiger nuts for 6 h, ground and diluted to final volume by adding potable water at 1:31:4, 1:5 and 1:6 ratios. All the diluted TNAE samples and cow milk (control) were sterilized at 121ºC for 15 min and cooled to room temperature. Samples were inoculated with 1 % fresh probiotic strains of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 and incubated at 37ºC/18 h. Setting time, coagulum characteristics, titratable acidity and direct microscopic count were analyzed after incubation.

Formulation of blend. After the confirmation of growth of probiotic strains and curdling in fermented TNAE, formulation of blended or combined milk with TNAE and cow milk was carried at 0:100, 25:75, 50:50, 75:25 and 100:0 respectively. All these formulated combinations of samples, cow milk and TNAE as control throughout the study were sterilized at 121ºC for 15 min and cooled. Inoculated with 1% fresh *Lactobacillus acidophilus* and BB-12 separately, incubated at 37ºC/18 h. The setting time, direct microscopic count and titratable acidity were determined for the incubated samples.

As per Laboratory manual of FSSAI (2016) procedures, TNAE, fresh cow milk and blend were analyzed for the following physico-chemical properties such as pH (digital LAQUA twin pH-11 - HORIBA pH meter); Viscosity determined using Ostwald Viscometer at 20°C and expressed as centipoise (cP); specific gravity determined using pyknometer at 20°C; Lactose analysed by Lane - Eyon method; fat estimated by Gerber's method; total solids by gravimetric method and protein estimated by Micro-Kjeldhal method

Effect of heat treatments on growth of probiotic culture and sensory quality in fermented blend. In view of studying the effect of heat treatments on the growth of probiotic strains and sensory attributes, each 100 ml of TNAE, cow milk and optimized blend samples were subjected to various heat treatments such as pasteurization at 63°C/30 min, steaming at 85°C/30 min and sterilization at 121°C/15 min (Ukuwuru and Ogbodo 2011), cooled to room temperature and inoculated with 1% fresh *Lactobacillus acidophilus* and BB-12 and incubated at 37°C/18 h. The setting time, direct microscopic count and titratable acidity were analyzed for the incubated samples along with sensory characteristics of test sample and control. Sensory attributes of fermented optimized blend was compared with the fermented TNAE and cow milk. The various parameters like colour and appearance, flavour, taste, consistency and overall acceptability were judged by a panel by using a nine point hedonic scale (Nazir, 2017). The score given by the judges were then statistically analyzed.

Optimization of per cent of probiotic inoculum and stevia addition in blend. The optimized blend was further optimized for different levels of inoculum of *Lactobacillus acidophilus* and BB-12 at 1 %, 2 % and 3

% individually and also in combination of these two strains. Further stevia was added at 0.25 %, 0.5 % and 0.75 %. The inoculated test samples were incubated at 37 ºC till the milk sets. Then the setting time, titratable acidity and direct microscopic count and sensory attributes were determined as mentioned in the above section.

Physico-chemical and microbiological characteristics of developed probiotic drink. For the obtained optimized TNAE based probiotic drink, various physico-chemical properties like pH, TA, viscosity, lactose content, protein content, fat and total solids content were studied (FSSAI, 2016). Viability of *Bifidobacterium* BB-12, coliform, yeast and mold counts in the probiotic drink were analyzed through pour plating method **(**Harrigan, 1998).

Statistical analysis. The results obtained for physico-chemical, organoleptic and microbiological analyses of the research work with respect to all the parameters were average of three trials. The data was analyzed using one way/two way ANOVA using R software [R. version 3.1.3 (2015-03-09)].

RESULTS AND DISCUSSION

For both the probiotic cultures, dilution at the rate of 1:4 ratio was found best with firm coagulum formation, mild syneresis and mild acidic flavour while dilution ratio 1:5 and 1:6 were rejected as the dilutions did not result in desired coagulation characteristics along with less probiotic growth. There was significant difference (P=.05) among dilutions of TNAE with respect to titratable acidity and probiotic counts in both the types of fermented milks when compared with fermented cow milks (Table 1). The resistant starch which is a natural prebiotic in tiger nut might have encouraged the better growth of BB-12 than *L. acidophilus.* Okudu and Ogubuike, (2016) made tiger nut milk by wet milling of 300 g soaked tiger nut seeds with 1 L of potable water accounting for 1:3 dilution and the same was followed by El-Shenawy *et al.*, (2019) and they further used the tiger nut milk heating at 70°C for 20 min for probiotic beverage preparation using three probiotic bacteria mixture including *L. plantarum, L. acidophilus* culture and *B. breve* culture.

Table 1: Standardization of dilution of Tiger nut aqueous extract (TNAE).

Samples	Dilution Ratio	Parameters analyzed				
		Setting time (h)	*Lactobacillus acdophilus*		BB-12	
			% LA	DMC (log_{10}/ ml)	% LA	DMC (log_{10}/ml)
Cow milk : Water (Control)	100:0	18	Firm body, no syneresis sweet & acidic		Firm body, no syneresis sweet & acidic	
			0.75^{a}	8.41^{a}	0.84^{a}	8.75^{a}
Tiger nut : Water	1:3		Very firm body, no syneresis, sweet & acidic		Very firm body, no syneresis, sweet & acidic	
			0.65^{a}	7.71^{b}	0.70^{b}	7.92^{b}
	1:4		Firm body, mild syneresis bland & mild acidic		Firm body, mildsyneresis, bland & mild acidic	
			0.60^{a}	7.28^{c}	0.66^{c}	7.54^{c}
	1:5		Loose body syneresis bland & less acidic		Loose body syneresis bland & less acidic	
			0.42^{ba}	6.24^{d}	0.40^{db}	6.15^{db}
	1:6		No firm body syneresis bland & non acidic		No firm body syneresis bland & non acidic	
			0.30^{c}	5.24^{e}	0.28^{eb}	5.15^{eb}
CD *(*P=.05)			0.28	0.23	0.12	0.27

Note: Results were average of three trials (n = 3),
Similar superscripts in the column indicate non-significance while different superscripts indicate significant difference (P=.05).

With the confirmation of growth of probiotic strains and curdling characteristics in TNAE, the formulated ratio of 75:25 was selected with titratable acidity of 0.72 % LA and probiotic count of 8.30 log_{10}/ml for *L. acidophilus* culture while 0.82 % LA and 8.52 log_{10} / ml for BB-12 (Table 2). This was the only formulation available where, TNAE was more and cow milk ratio less and lactic acid production and probiotic counts were nearly similar to plain fermented cow milk. This was denoted by non-significant difference (P=.05) among formulations with respect to titratable acidity and probiotic counts both in *L. acidophilus* and BB-12 compared with cow milk as control sample. Some of the investigators formulated the tiger nut milk with soy milk, coconut milk and almond milk at different concentrations. The growth of probiotic bacteria in tiger nut was substantiated by Ndikom and Elutade, (2016) in their study as they isolated LAB from the surface of tiger nut tubers.

The physico-chemical properties analyzed are depicted in Table 3. Statistically significant difference (P=.05) among viscosity, lactose, fat, protein and total solids occurred whereas pH, specific gravity and titratable acidity did not show significant difference among cow milk, TNAE and blend, respectively.

Table 2: Formulation of blend (TNAE: Cow milk).

Ratio (TNAE : Cow milk)	Parameters analyzed				
	Setting time (h)	*Lactobacillus acidophilus*		BB-12	
		% LA	DMC (log_{10}/ ml)	% LA	DMC (log_{10}/ ml)
0:100 (Cow milk as control)	18	0.76[a]	8.45[a]	0.82[a]	8.71[a]
100:0 (TNAE as control)		0.62[a]	7.30[a]	0.68[a]	7.60[a]
25:75		0.80[a]	8.40[a]	0.85[a]	8.60[a]
50:50		0.70[a]	8.32[a]	0.79[a]	8.43[a]
75:25		0.72[a]	8.30[a]	0.82[a]	8.52[a]
CD (P=.05)		1.52	1.46	1.49	1.51

Note: • Results were average of three trials (n = 3)
• Similar superscripts in the column indicate non-significance while different superscripts into significant difference (P=.05).
• Dilution optimized (1:4) TNAE + cow milk is heat treated to 85°C/ 30 min, inoculated with *Lactobacillus acidophilus* and BB-12 at 1 % with incubation at 37 °C.

Cow milk, TNAE and blend analyzed for physico-chemical parameters revealed variation in viscosity, lactose, fat, protein and total solids. TNAE and blend showed less viscosity by 0.74 cP and 0.82 cP respectively in comparison with cow milk i.e. 1.80 cP. The pH values and titratable acidity for both control and sample were almost similar. The acidity of TNAE was nearly comparable to cow milk because of acidic amino acids such as aspartic acid and glutamic acid being prominent in TNAE. There was noticeable variation with respect to lactose content among the three types sample. TNAE was completely devoid of lactose and the blend contained a small portion of lactose accounting for 0.40 % which was due to blending of 25 % cow milk with the 75 % TNAE. Blend and TNAE showed fat of 4.02 % and 8.0 % respectively. On par with present study, Chima *et al.*, (2013) reported on pH of 6.64, acidity of 0.15 % LA, fat of 25.5 %, protein of 8.19 % and total solids of 12.28 % in tiger nut milk (1:3). Udeozor, (2012) determined the proximate composition of tiger nut – soy milk drink (50:50) both extracts of plant origin with pH value of 6.10; moisture of 57.3 %; carbohydrate of 4.8 %; protein of 7.95 %; fat of 27.2 % and crude fiber of 0.24 %. Till date, literature pertaining to present study on physico-chemical characteristics of blend of TNAE and cow milk at 1:4 ratio are meagre.

As per Table 4, blend inoculated with 1% *L. acidophilus* with the steaming at 85 °C/30 min resulted in titratable acidity of 0.75 % LA, probiotic count of 8.34 log_{10} and over all acceptability of 7.0 score which were lesser than that of BB-12 strain. Among the three heat treatments, BB-12 strain inoculated in steamed sample at 85 °C/30 min scored better than pasteurization and autoclave temperatures. There was significant difference (P=.05) in acidity, DMC and overall acceptability (OA) among the BB-12 fermented steamed cow milk, TNAE and blend. Both *L. acidophilus* and BB-12 fermented milks prepared using steaming at 85°C/30 min for cow milk, TNAE and blend found better with acidity and DMC compared with pasteurized and sterilized milk prepared probiotic milks and thus applied in further studies. Treatment of steaming of blend at 85°C/30 min had not been tried by any of the researchers for the preparation of tiger nut based probiotic drink.

In the present study, sensory profile evaluation of optimized blend showed statistically significant difference in colour, taste and consistency followed by flavour and overall acceptability. The significant (P<0.05) difference in appearance and overall acceptance of milk was attributed to the much brown colour of TNAE (4.5 and 6.0 score). The taste of TNAE was sweet while cow milk tasted less. TNAE possessed nutty flavour as compared to cow milk flavour (bland). The blend scored better (7.50) than the control on 9 - point hedonic scale.

Table 3: Evaluation of Physico-chemical properties of optimized blend.

Samples	Physical properties			Chemical properties (%)				
	pH	cPat 20°C	Sp. gr (g/ ml)	%LA	Lactose	Fat	Protein	Total Solids
Cow milk(Control)	6.6[a]	1.80[a]	1.019[a]	0.16[a]	4.50[a]	3.80[a]	3.20[a]	11.5[a]
TNAE(Control)	6.7[a]	1.06[b]	0.990[a]	0.14[a]	0.00[b]	8.00[b]	5.10[b]	22.0[b]
Blend**	6.5[a]	0.98[c]	1.001[a]	0.15[a]	0.40[c]	4.02[ac]	2.80[ac]	10.8[ac]
CD (P=.05)	0.75	0.43	1.01	1.01	0.57	1.00	0.99	1.00

Note: All the values are average of three trials (n=3).
• Similar superscripts indicate non-significance while different superscripts indicate significant difference.
• Blend** - Combination of TNAE(1:4 diluted) and Cow milk at 75:25 ratio.
• cP – Viscosity expressed as Centipoise at 20°C.
• Sp.gr- Specific gravity (g/ml) measured at 20°C

Table 4: Effect of heat treatments on TNAE, cow milk and Blend.

Type of samples	Heat treatments								
	Pasteurization (63 °C/30 min)			Steaming (85 °C/30 min)			Autoclave (121 °C/15 min)		
	Lactobacillus acidophilus								
	% LA	log_{10}/ml	OA*	% LA	log_{10}/ml	OA*	% LA	log_{10}/ ml	OA*
Cow milk	0.68[a]	8.00[a]	7.50[a]	0.76[a]	8.42[a]	8.00[a]	0.70[a]	8.04[a]	8.00[a]
TNAE	0.52[a]	6.32[b]	6.00[b]	0.62[a]	7.30[b]	6.50[b]	0.40[a]	6.57[b]	6.20[b]
Blend**	0.66[a]	7.20[a]	7.20[c]	0.75[a]	8.34[ac]	7.80[c]	0.62[a]	7.10[a]	7.20[c]
CD (P=.05)	1.01	0.90	0.82	1.00	1.00	0.85	0.98	1.00	0.83
	***Bifidobacterium animalis* ssp. *lactis* BB-12**								
Cow milk	0.74[a]	8.21[a]	8.00[a]	0.84[a]	8.72[a]	8.20[a]	0.70[a]	8.14[a]	8.00[a]
TNAE	0.64[a]	7.51[a]	6.20[b]	0.62[a]	7.58[b]	6.80[a]	0.42[a]	7.17[b]	6.30[b]
Blend**	0.71[a]	7.82[a]	7.50[ac]	0.83[a]	8.54[a]	8.00[a]	0.68[a]	7.65[a]	7.30[ac]
CD (*P=.05*)	**0.82**	**0.85**	**0.85**	**1.01**	**0.99**	**0.86**	**0.97**	**0.88**	**0.83**

Note: • All the values are average of three trials (n=3).
- Similar superscripts indicate non-significance while different superscripts indicate significant difference.
- Blend** - Combination of TNAE (1:4 diluted) and Cow milk at 75:25 ratio.
- OA* – Overall acceptability with 9 point hedonic scale.

There was no significant difference with respect to titratable acidity and overall acceptability however significant difference (P=.05) was noticed for DMC for all the three levels of *L. acidophilus* as inoculum (Table 5). There was no significant difference in titratable acidity values, however there was significant difference (P=.05) for DMC and overall acceptance for all the three levels of BB-12 inoculum (Table 6). There was no significant difference in titratable acidity, however there was significant difference (P=0.05) in DMC and overall acceptance of all the three levels of combined inoculum (Table 7).

Table 5: Optimization of levels of inoculum of *Lactobacillus acidophilus* in blend

Samples	Inoculum level (%)								
	1			2			3		
	% LA	DMC (log_{10}/ml)	OA*	% LA	DMC (log_{10}/ml)	OA*	% LA	DMC (log_{10}/ ml)	OA*
Cow milk(Control)	0.76[a]	8.42[a]	8.00[a]	0.82[a]	8.62[a]	8.12[a]	0.85[a]	8.74[a]	8.22[a]
TNAE(Control)	0.62[a]	7.30[b]	7.00[b]	0.66[a]	7.50[b]	7.20[a]	0.67[a]	7.52[b]	7.30[a]
Blend**	0.75[a]	8.34[ac]	7.80[a]	0.80[a]	8.40[a]	8.00[a]	0.83[a]	8.50[ac]	8.10[a]
CD (P=.05)	1.00	1.00	0.92	1.00	0.99	1.00	0.99	0.97	1.00

Note: • All the values are average of three trials (n=3).
- Similar superscripts indicate non-significance while different superscripts indicate significant difference.
- Blend** - Combination of TNAE (1:4 diluted) and Cow milk at 75:25 ratio and steamed.
- OA* – Overall acceptability with 9 point hedonic scale.

Table 6: Optimization of levels of inoculum of *Bifidobacterium animalis* ssp. *lactis* BB –12 in blend

Samples	Inoculum level (%)								
	1			2			3		
	% LA	DMC (log_{10}/ml)	OA*	% LA	DMC (log_{10}/ml)	OA*	% LA	DMC (log_{10}/ml)	OA*
Cow milk	0.84[a]	8.72[a]	8.20[a]	0.87[a]	8.80[a]	8.75[a]	0.89[a]	8.82[a]	8.75[a]
TNAE	0.62[a]	7.58[b]	6.00[b]	0.65[a]	7.80[b]	7.00[b]	0.66[a]	7.82[b]	7.25[b]
Blend**	0.83[a]	8.54[a]	8.00[ac]	0.86[a]	8.74[a]	8.25[ac]	0.88[a]	8.80[a]	8.30[ac]
CD (P=.05)	1.00	1.00	1.00	1.00	1.01	0.97	1.01	1.01	0.94

Note: • All the values are average of three trials (n=3).
- Similar superscripts indicate non-significance while different superscripts indicate significant difference.
- **Blend - Combination of TNAE (1:4 diluted) and Cow milk at 75:25 ratio and steamed.
- OA* – Overall acceptability with 9 point hedonic scale.

Table 7: Optimization levels of inoculum of combined probiotic cultures in blend.

Samples	Inoculum of *Lactobacillus acidophilus* + *Bifidobacterium animalis* ssp. *lactis* BB-12 level (%)								
	1			2			3		
	% LA	DMC (log_{10}/ ml)	OA*	% LA	DMC (log_{10}/ ml)	OA*	% LA	DMC (log_{10}/ ml)	OA*
Cow milk (Control)	0.74[a]	8.32[a]	8.00[a]	0.80[a]	8.58[a]	8.00[a]	0.76[a]	8.50[a]	8.12[a]
TNAE (Control)	0.60[a]	7.10[b]	7.00[b]	0.63[a]	7.20[b]	7.15[b]	0.64[a]	7.42[b]	7.25[b]
Blend**	0.72[a]	8.14[ab]	7.60[a]	0.76[a]	8.10[a]	7.80[a]	0.80[a]	8.30[a]	8.00[a]
CD (P=.05)	1.00	1.00	0.88	0.96	0.96	0.94	0.98	0.99	1.00

Note: • All the values are average of three trials (n=3).
- Similar superscripts indicate non-significance while different superscripts indicate significant difference.
- **Blend - Combination of TNAE (1:4 diluted) and Cow milk at 75:25 ratio and steamed.
- OA* – Overall acceptability with 9 point hedonic scale.

An inoculum of 2 % *Bifidobacterium animalis* ssp. *lactis* BB-12 was selected for further studies as the titratable acidity, probiotic count and overall acceptability were better than 1 and 3 % inoculum and also that of probiotic bacteria *L. acidophilus* as well in combination of probiotic cultures. The inoculum level of 3 % probiotic cultures did not show much difference with respect to titratable acidity, probiotic counts and overall acceptability score.

In order to fix the inoculum of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* BB-12 at 1%, 2% and 3% were used in the preparation of probiotic milk from optimized blend, cow milk and TNAE. Inoculum of 2 % in case of both probiotic cultures worked well compared to 1 and 3 per cents. Best overall acceptability scores (8.25) were obtained for 2% inoculum of BB-12 compared to *Lactobacillus acidophilus* (8.00), hence for latter studies 2% was optimized for the preparation of probiotic drink. Combined cultures at 2 % inoculum level, the quality of the curd was not enhanced compared to the fermented milk using the culture of Bifidobacteria which may be due to non-symbiotic effect among the cultures. In substantiation to the present study, regarding the inoculation of probiotic strain, (Agbaje *et al.,* 2015) demonstrated the ability of *Lactobacillus plantarum* to grow and acidify the tiger nut based beverage. El-Shenawy *et al.,* (2019) investigated and reported on development of probiotic beverage from a mixture of UF-milk permeate (65 %), tiger-nut aqueous extract (30 %) and 5 % sugar, fortified with 1 % mixture of probiotic cultures such as *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) produced a healthy stable beverage. The beverage with 1:1:1 upon storage at 4°C for 10 days showed counts of *L. acidophilus* 7.93, *B. breve* of 8.13 and *L. plantarum* of 7.75 log viable counts.

Table 8: Optimization of addition of Stevia in TNAE based probiotic drink.

Sample	Stevia addition (%)								
	0.25			0.5			0.75		
	% LA	DMC (log_{10}/ ml)	OA*	% LA	DMC (log_{10}/ ml)	OA*	% LA	DMC (log_{10}/ ml)	OA*
Cow milk(Control)	0.85^{a}	8.79^{a}	8.50^{a}	0.88^{a}	8.80^{a}	7.50^{a}	0.90^{a}	8.83^{a}	7.00^{a}
TNAE(Control)	0.64^{a}	7.60^{b}	6.50^{b}	0.67^{a}	7.81^{a}	6.50^{b}	0.65^{a}	7.84^{a}	6.00^{b}
Blend**	0.85^{a}	8.57^{a}	8.00^{a}	0.85^{a}	8.75^{a}	7.80^{a}	0.89^{a}	8.82^{a}	7.50^{ab}
CD (P=.05)	**1.01**	**0.99**	**0.98**	**1.00**	**1.01**	**0.98**	**0.93**	**1.01**	**0.97**

Note: • All the values are average of three trials (n=3).
- Similar superscripts indicate non-significance while different superscripts indicate significant difference.
- Blend** - Combination of TNAE (1:4 diluted) and Cow milk at 75:25 ratio, steamed with 2 % BB-12.
- OA* – Overall acceptability with 9 point hedonic scale.

In order to add taste to the probiotic drink, addition of stevia was standardized. Stevia is a prebiotic, but addition is very less and might not have affected the number of probiotic culture in the product. Blend with 0.25 % stevia was accepted with highest score than 0.5 % and 0.75 %. The blend with 0.5 and 0.75 % were not accepted by the judges due to more sweetness in the product. There was significant difference (P=.05) between DMC and overall acceptability of stevia incorporated fermented cow milk, TNAE and Blend. Literature regarding addition of stevia, natural sweetener to tiger nut based probiotic drink does not exist.

The decrease in pH could be as a result of fast growth rate of *Bifidobacterium* BB-12 which broke down carbohydrate that resulted in the increase in quantity of lactic acid released into the TNAE based drink as fermentation progressed. The decrease in pH observed during fermentation of probiotic drink by LAB is in agreement with a related study carried out by Wakil *et al.,* (2014) who studied the pH of starter developed fermented tiger nut milk with 1:2.5 dilution. The least pH (4.6) was observed in fermented tiger nut milk at 24 h of fermentation.

The viscosity of probiotic drink decreased (1.608 cP) as compared to cow milk (1.679 cP**)** along with the increase of the shear rate, which reflected on shear-thinning behaviour. The growth of Bifidobacteria led to production of amylase enzyme, decreased the molecular association between starch chains and hydrolyze the starch chains, thus decrease the viscosity was observed. However, TNAE exhibited the lower viscosity (1.541 cP) compared with cow milk and probiotic drink (1.679 cP and 1.608 cP).

The specific gravity of TNAE and blend (1.065 and 1.069) increased as compared to cow milk (1.062 g/mL). The specific gravity was mainly due to the presence of water content and concentrations of fat, protein, vitamin and mineral in the tiger nut milk. The highest specific gravity of probiotic drink would mean that there was more water than milk solids. The amount of water would depend on the quantity of tiger nut used for extraction.

Significant difference *(*P=.05) among the fat, protein, total solids and viable counts among the control samples and TNAE based probiotic drink were noticed (Table 9).

The reduction of 2 % in lactose due to hydrolysis was observed for cow milk after fermentation using probiotic bacteria. The analysis of TNAE for lactose resulted in absence of lactose content since TNAE was lactose free. Blend resulted in 0.18 % reduction in lactose after fermentation with *Bifidobacterium* BB-12 strain.

The decrease in the fat content of cow milk, TNAE and blend might be as a result of *Bifidobacteria* species utilizing lipids for the synthesis of cell membrane in order to increase their population indicated the synthesis of extracelluar lipase by probiotics (Dellali *et al*., 2020).

Table 9: Evaluation of Physico-chemical and microbiological characteristics of optimized TNAE probiotic drink.

Samples	Characteristics								
	Physical			Chemical					Microbiological
	pH	Viscocity	Sp. gr	Titratble acidity	Lactose	Fat	Protein	Total solids	BB-12
		cP		% LA	%				(log_{10} cfu/ ml)
Cow milk	4.8[a]	1.679[a]	1.062[a]	0.88[a]	2.19[a]	3.12[a]	3.00[a]	10.90[a]	8.80[a]
TNAE	5.0[a]	1.541[a]	1.065[a]	0.64[a]	0.00[a]	7.80[b]	4.85[b]	21.20[b]	7.80[b]
Probiotic drink	4.9[a]	1.608[a]	1.069[a]	0.85[a]	0.18[a]	4.00[ac]	2.58[ac]	10.1[ac]	8.68[a]
CD (P=.05)	**0.94**	**1.00**	**0.92**	**0.99**	**0.57**	**0.99**	**0.99**	**1.00**	**1.00**

Note: • All the values are average of three trials (n=3).
- Similar superscripts indicate non-significance while different superscripts indicate significant difference.
- Probiotic drink - Combination of TNAE (1:4 diluted) and Cow milk at 75:25 ratio, steamed + 2 % BB-12 + 0.25% stevia optimized.
- Coliform as well as yeast and mold counts were nil in all the three types of products.
- cP – Viscosity at 20°C, Sp.gr - Specific gravity at 20°C (g/ ml)

The protein content in cow milk, TNAE and blend showed slight reduction due to protein hydrolysis which might be due to exoproteinase of probiotic bacteria. Highest total solids were recorded in TNAE (21.20%) followed by cow milk (10.90 %) then blend (10.1 %). High total solid created richness in the probiotic drink prepared from blended tiger nut based milk which might be accountable of resistant starch of tiger nut (Maduka *et al.*, 2017).

The viable log *Bifidobacterium* BB-12 count in probiotic drink prepared from cow milk, TNAE and probiotic drink were 8.80, 7.80 and 8.68 respectively. As per FSSAI (2017) the viable probiotic number in any probiotic drink should be greater than or equal to 10^8cfu/g of the product.

On par with the present study, Bristone *et al.,* (2015) analyzed the physico-chemical properties of yoghurt prepared by blend of cow milk and tiger nut milk at the ratio of 50: 50 and 20:80. Per cent moisture content was 88.61, 90.52, carbohydrate of 6.01, 5.20, fat 2.17, 1.56, protein of 2.60, 2.16, total solids of 11.39, 9.48, pH 3.97, 3.97 and titratable acidity of 1.09 and 1.13 % LA respectively. Microbiological analysis of yogurt prepared had total bacterial count of 6.0×10^5, mold count of 5.8×10^5 for 50: 50 ratios and 7.1×10^5, 6.3×10^5 for 20:80 ratio respectively. This study matched with present study but differed with respect to ratio ie. 25:75 (cow milk : TNM) and use of Bifidobacteria as probiotic culture instead of yogurt cultures. The proximate composition of optimized tiger nut milk based probiotic drink obtained in this study was in accordance with the study of beverage prepared by Gambo and Da'u, (2014).

CONCLUSION

This study was a trail to combine the health benefits of the tiger nut aqueous extract with the cow milk and the probiotic bacteria to produce a probiotic drink that can be effectively consumed by consumers. It would help the researchers to uncover various possible uses of tiger nut or tiger nut milk with respect to therapeutic benefits which are not fully explored by the Indian population.

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Conflict of Interest. None.

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