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Production of fermented Guava and Coconut beverages supplemented with *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus*

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Abstract

Fermented functional beverages with (*Lactobacillus acidophilus* CH-2 and *Streptococcus thermophiles*) prepared from mixture (skim milk, 1% whey protein concentrate, 15% guava juice, 10% coconut extract, 5% fructose, 0.08% dariloid), homogenized, heated at 85 º C for 15 min, cold to 37º C and inoculated with starter for 2 hours. Samples were analyzed chemically, sensory evaluation and microbiologically at zero time and after 3, 7, 10 days of cold storage. Fermented functional beverages with 15% guava juice gained highest scores for flavour, taste and appearance than other treatments at zero time and during cold storage. In addition, fermented beverages with guava juice gained the highest content of total solids compared to other treatments and TS increased gradually during cold storage at 5º C±1. Fermented beverages with guava juice had a higher acidity than control. Fermented beverage with coconut extract and gradually increasing during cold storage. The pH values took an opposite trend of acidity in control and all treatments at zero time and during cold storage at 5º C±1. Fermented beverages with 15% guava juice had the highest viscosity, followed by fermented beverages with coconut extract and finally control. Minerals such as Fe, Na, K and Ca are the highest content in fermented beverages with guava juice than other treatments. Fermented beverages with guava juice had a highest PER1, PER2 (Protein efficiency ratio) and BV1 and BV2 (Biological value). Fermented beverages with guava juice had the highest content of arginine, threonine, alanine, proline, tyrosine and leucine than control and fermented beverages with coconut extract. These findings recommend that fortifying milk beverages with probiotics, guava juice, and coconut extract have the potential to produce functional fermented dairy products.

Keywords: Fermented beverages, viability, viscosity, nutritional value, sensory evaluation.

1. Introduction

Probiotics are defined as living microorganisms that, when given in sufficient proportions, confer health benefits on the host **[1, 2 and 3]**. Probiotics may play a beneficial role in several medical conditions such as reduce intestinal inflammation, lactose intolerance, cancer, allergies, hepatic diseases [4, 5]. During milk fermentation the metabolites contribute to the development of the flavor and sensory quality of dairy products [**6**]. Fermented milk beverages are an important part of the human diet as nutritious functional foods [**6**]. Also, probiotics decreased cholesterol and cure some illnesses. These products are a good delivery vehicle for viable probiotic strains [**7**]. During milk fermentation, the fermentation process produces different metabolites derived from protein, fat, and lactose, which benefit human health [8]. It is also worth noting that probiotic treatments for gastrointestinal inflammation are significantly less expensive than other forms [9, 10]. Meanwhile, there are several health benefits due to the consumption of dairy foods containing probiotic *Lactobacillus acidophilus*, and different studies have documented the health benefits of this probiotic strain. In addition, this strain has also been used for the treatment of intestinal disorders and dysfunctions and to improve the balance of gut microflora [11, 12, 13]. Whey protein is absorbed and digested rapidly. Whey protein contains a number of bioactive components, including β-lactoglobulin, α-lactoalbumin, serum albumin, immunoglobulin and lactoferrin. These components have positive effects on health, such as immune-improving and antioxidant characteristics that reduce hypertension, cancer, and inflammatory disease, and they are rich sources of branched-chain amino acids. On the other hand, coconut extract is a good source of lauric acid, which enhances the quantity of high-density lipoprotein cholesterol and contain a high level of arginine that is the major factor responsible for the hypolipidemic effects [14], improve the immune system functions and have antioxidant characteristics that cure inflammatory bowel diseases [15]. Guava juice had a high content of ascorbic acid (18.87 mg/1000). The aim of this work is the production of fermented functional beverages fortified with whey protein concentrate, skim milk, coconut extract and guava juice supplemented with probiotic bacteria in order to produce healthy beverages.

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2. Materials and Methods:

2.1. Materials

2.1.1. Strains

Lactobacillus acidophilus CH-2 and *Streptococcus thermophilus* were obtained from the dairy microbiology Lab., National Research Centre, Cairo, Egypt. Strains were propagated in reconstituted sterile skim milk (12%TS) and subsequently incubated for 24 h at 37° C to prepare an addition culture.

Stabilizer: Dariloid 100 (Guar gum, Xanthan gum and locust bean gum) produced by the kelco division of Merck and co., Inc., U.S.A.

Whey protein concentrate: Ultrafiltered whey protein concentrate powder was obtained from Bio-pharm. Company A.R.E.

2.1.2. Preliminary experiments

Preliminary experiments were carried out to select the best ratio of Guava juice by adding 5, 10, 15, 20% guava juice. The organoleptic properties showed that 15% of guava juice is the best ratio and had the highest score for flavour, taste and appearance, and 10% of coconut extract is the best ratio [12]**.** The preparation of an aqueous extract of coconut with some modification, according to [12].

2.1.3. Preparation of fermented functional beverages with probiotic bacteria fortified with guava juice

Whey protein concentrate (1%) dissolved in skim milk and 15% guava juice, 5% fructose, 0.08 % Dariloid. All mixtures were homogenized and heated at 85° C for 15 minutes and cooled rapidly, and then inoculated with 2% of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1). The mixture was incubated at 37° C for 2 hours, and then transferred to the refrigerator at 5° C±1. Coconut and sugar were procured from the local market. Fresh cow milk was obtained from the faculty of Agric. Cairo, Univ. Fresh cow skim milk content 3.5% protein, 0.5% fat, 4.5% lactose, 0.9% ash, 12.82% total solid, 0.16% acidity and pH 6.6. Guava juice content 0.4% protein, 0.3% fat, 0.55% ash 5.5% fiber and pH 4.20. Whereas coconut extract content 0.52% protein, 0.15% fat, 0.45%ash, 4.415 carbohydrates, 0.15% acidity, and pH 5.96.

2.2. Methods

2.2.1. Chemical analysis

2.2.2. Acidity

Acidity was determined according to [13]. pH values of fermented beverage samples were measured using a digital pH meter equipped with a combined electrode (Hanna, Germany). Total solids (TS) and ash contents were determined according to [14].

2.2.3. Minerals

Mineral contents were determined by microwave digestion lab. Station, ICP technique optima 2000 DV. Lactose was determined according to [15].

2.2.4. Apparent viscosity

Apparent viscosity was measured using a Brookfield DV digital viscometer (Brookfield Engineering, Middleborough, MA, USA) with spindle no. 4 at 60 rpm. Apparent viscosity was expressed as Pascal - Pa.s.

2.2.5. Amino acids

The amino acids composition of functional fermented milk beverage samples was determined according to [16] using an amino acids analyzer (Biochrom30).

2.2.6. Protein efficiency ratios (PER)

The protein efficiency ratios of samples were based on their amino acids according to the recommendations of **Hassan *et al.*, (2023)** The following equations were used: PER1 = -0.684 + 0.455 = (leucine-0.047 (Proline) (for adults). PER2 = -0.468 + 0.454 (leucine) – 0.105 (tyrosine) (for juveniles).

2.2.7. Biological value (BV)

The biological value (BV) was assayed according to the following equation as recommended by [13] BV = 49.9 + 10.53 PER.

2.3. Microbiological analysis

2.3.1. *Streptococcus* and *Lactobacillus* counts

Fermented beverages samples were homogenized well then serially diluted up to 10^{-10} in sterile physiological saline (0.85% NaCl). After plating one milliliter of each dilution onto a sterile double petri dish, *Str. thermophilus* was counted in M17 agar (Oxoid) according to [17]. On the other hand, *L. acidophilus* was counted in MRS agar (Oxoid), according to [18]**.** Plates were incubated aerobically at 37° C for 24 hours and anaerobically at 37°C for 48 hours for *Str. thermophilus* and *L. acidophilus,* respectively. The viable cell numbers of all results were expressed as colony forming unit per ml (CFU/ml).

2.3.2. Total viable bacterial counts

Total viable bacterial counts of all samples were determined by using standard plate count agar medium (Oxoid) according to [19]. The plates were incubated at 35 ±1 ^{o}C for 48 h.

2.3.3. Mould and yeast counts

Mould and yeast counts of all samples were determined by using potato dextrose agar (Oxoid) according to [20]. The plates were incubated at 25 ^{o}C for 3 days and the result was expressed as colony forming units per ml (CFU/ml).

2.3.4. Sensory evaluation

Functional fermented milk beverages were sensory evaluated using scale of points 40 points for flavour, 40 points for taste and 20 points for appearance as described by [12].

2.3.5. Statistical analysis

All obtained data were subjected to the statistical analysis performed by the Vassar Stats (http: //www. faculty.vassar.edu/Lowry/anova2corr.html) computing site was used to analyze the obtained data statistically by A nova of two independent variables according to [21].

3. Results and discussion

Table 1: Sensory scores of fermented milk beverages shows that 15% Guava juice was the best ratios for beverages.

Storage period (weeks)	Properties	Control	Guava juice	Coconut extract	±SEM
Zero time	Flavour (40)	38^{a}	39^{a}	37^{a}	0.6085
3		37^{a}	37^{a}	34^{b}	0.6938
7		34^{b}	35^{a}	31^{c}	0.5773
10		32^{b}	33^{a}	28^{c}	0.8164
Zero time	Taste (40)	38^{a}	38^{a}	37^{a}	0.57735
3		36^{a}	36^{a}	34^{b}	0.6938
7		32^{b}	34^{a}	31^{c}	0.5773
10		30	32	28	0.8164
Zero time	Appearance (20)	19^{a}	19^{a}	18^{a}	0.5773
3		18	18	17	0.6085
7		16^{a}	16^{a}	15^{b}	0.5773
10		14	15	13	3.2716

Dissimilar superscripts at the same row for treatments are significantly differed ($P < 0.05$). Each value is a mean of 3 replicates

Table 2: Total solids, acidity and pH of fermented milk beverage during cold storage at 5ºC for 10 days.

	Storage period/ days			
	%Total solids			
	Fresh	3 days	7 days	10 days
Control	17.23^{c}	17.53^{c}	17.81^{c}	18.10^{c}
Guava juice	19.29^{a}	19.33^{a}	19.35^{a}	19.63^{a}
Coconut extract	18.18^{b}	18.19^{b}	18.42^{b}	18.53^{b}
±SEM	0.0157	0.0141	0.0083	0.0113
	%Acidity			
Control	0.16^{c}	0.19^{c}	0.21^{b}	0.23^{c}
Guava juice	0.27^{a}	0.28^{a}	0.29^{a}	0.31^{b}
Coconut extract	0.25^{b}	0.27^{b}	0.29^{a}	0.30^{a}
±SEM	0.19153	0.00333	0.00333	0.00333
	pH			
Control	6.33	6.28^{b}	6.09^{b}	6.01^{b}
Guava juice	5.94	5.93^{c}	5.85^{c}	5.69^{c}
Coconut extract	6.45^{a}	6.39^{a}	6.24^{a}	6.15^{a}
±SEM	0.01201	0.57264	0.02108	0.02009

Table (1) illustrates the effect of cold storage at 5 º C ±1on organoleptic properties of fermented functional milk beverages fortified with 15 % of guava juice and 10% coconut extract. It is clear that fresh fermented fortified with 15% of guava juice gained the highest score for flavour, taste and appearance, followed by fermented beverages fortified with 10% coconut and finally control. On the same table, we notice that total score points gradually decreased by increasing the cold storage 5º C ± 1 until 10 days. These results are in agreement with those obtained by [12, 22]. Statistical analysis shows that no significant at α 0.05 for cold storage until 10 days for control and treatments.

Table (2) indicated the effect of cold storage at 5º C ± 1 on (total solids, acidity and pH) of functional fermented milk beverages fortified with guava juice and coconut extract. Total solids increased gradually during cold storage until 10 days. Functional fermented milk beverage fortified with guava juice had the highest content of TS than coconut and control. This is due to the high fiber content (5.5%) of guava juice. These results are in agreement with those obtained by [12, 23]**.** From the same table, we noticed that, the acidity increased by increasing the storage period at 5º C ± 1 until 10 days in the control and treatments fortified with guava juice and coconut extract, whereas the control had the lowest acidity than fermented milk beverages fortified with guava juice and coconut extract. In addition, fermented milk beverages fortified with guava juice had the highest acidity than control and treatment with coconut extract. This may be due to the high fiber content (5.5%) of guava juice. In contrast, the pH values showed an opposite trend of acidity. pH gradually decreased in control and treatments during cold storage at 5º C ± 1 until 10 days. Statistical analysis shows that is a significant at α 0.05 for total solids for cold storage until 10 days.

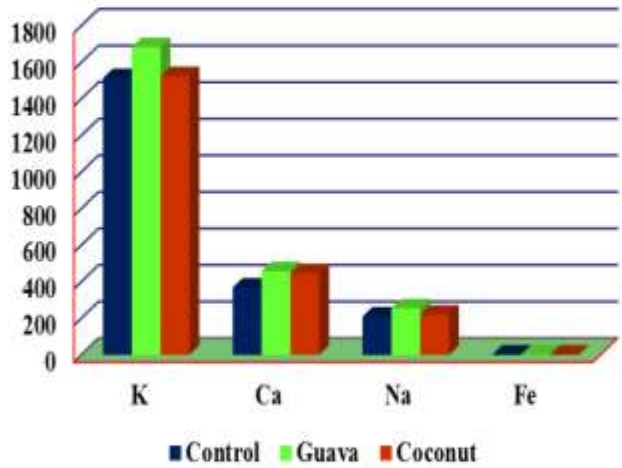

Fig 1: Minerals content (mg/100g) of functional fermented milk beverage fortified with guava juice and coconut extract

Figure (1) illustrate the minerals content (mg/100g) of functional fermented milk beverages fortified with guava juice and coconut extract. It is clear that (Fe, Na, K and Ca) had the highest content in fermented milk beverages fortified with guava juice followed by treatment with coconut extract and finally control. These minerals are very important for children and old people. These results are in agreement with those obtained by **[23,24].**

Table 3: Amino acids content of functional fermented milk beverages fortified with Guava juice and coconut extract

Amino Acids	Control	Guava juice	Coconut juice
	mg/ml of sample		
Aspartic acid	4.50	29.75	39.42
Glutamic acid	115.30	72.82	67.92
Serine	40.09	42.07	32.63
Glycine	31.63	36.64	23.26
Histidine	86.08	51.67	52.40
Arginine	49.68	70.66	56.97
Threonine	73.98	84.70	40.78
Alanine	11.45	14.96	6.78
Proline	62.51	80.82	48.10
Tyrosine	65.34	84.84	60.98
Valine	101.07	74.91	128.63
Methionine	34.50	39.52	60.11
Cysteine	11.61	18.01	19.92
Isoleucine	106.29	71.75	97.10
Leucine	57.01	85.48	61.05
Phenylalanine	43.61	48.60	91.47
Lysine	53.37	41.03	60.86

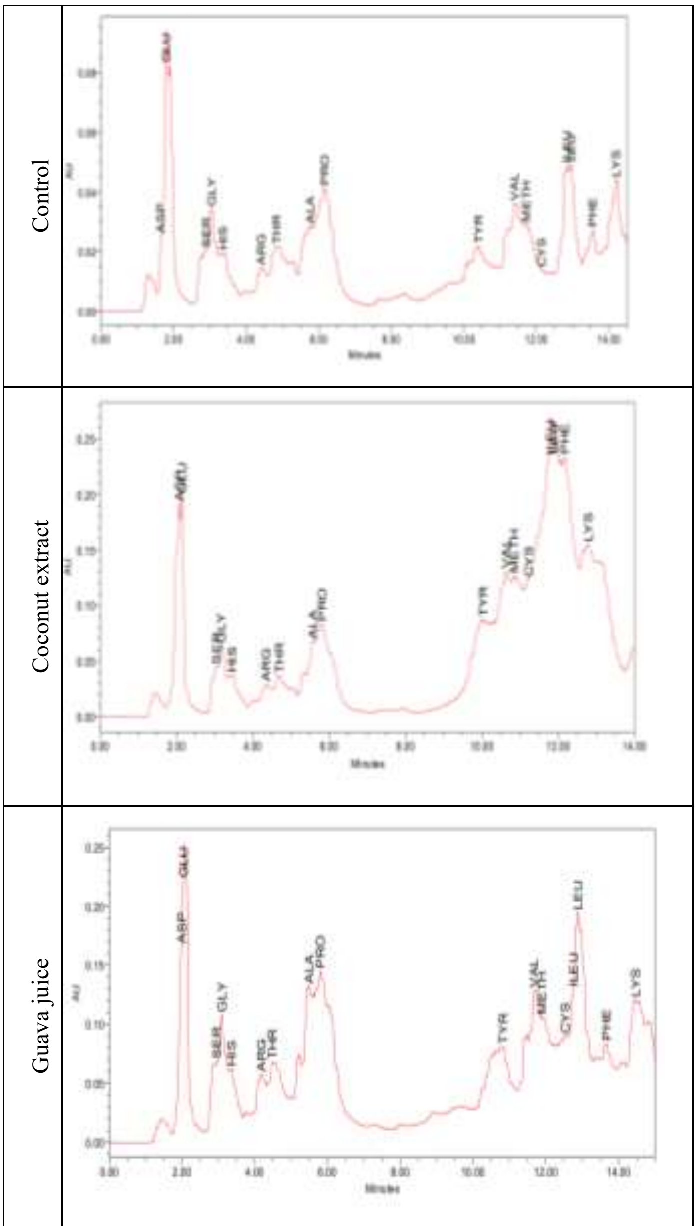

Fig.2 Chromatogram of amino acids determined by HPLC-Pico-Tag method [25].

Table (3) and Fig. (2) indicated the amino acids content of functional fermented milk beverages fortified with guava juice and coconut extract. It is clear that control and functional fermented milk beverage treatments fortified with guava juice and coconut extract had aspartic, glutamic, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleusine, leucine, phenylalanine and lysine. Functional fermented milk beverages fortified with guava juice had the highest content of arginine followed by fermented milk beverages fortified with coconut extract and finally control. L-arginine is useful for schoolchildren and old ages. Control had the highest content of glutamic acid compared to the treatments fortified with guava juice and coconut extract, whereas beverages with guava juice had the highest content of arginine, threonine, alanine, proline, tyrosine and leucine. On the other had beverages fortified with coconut had a highest content of valine, methionine, isoleucine, cysteine, phenylalanine and lysine.

Table 4**:** Protein efficiency ratios (PER) and biological value (BV) of functional fermented milk beverages fortified with guava juice and coconut extract

Treatments	PER1	PER2	BV1	BV2
Control	23.9	18.55	30.56	245.23
Guava juice	36.51	29.43	434.35	359.80
Coconut extract	26.0	21.78	323.68	279.24

C= Control G = Guava juice Co= Coconut extract
1= Adult 2= Juveniles

Table (4) show the protein efficiency ratios and biological value of functional fermented milk beverage fortified with guava juice and coconut extract. It is clear that treatment fortified with guava juice had the highest content of PER1, PER2. This may be due to the highest content of amino acids leucine, proline and tyrosine followed by treatment fortified with coconut extract and finally control. These results are in agreement with those obtained by [12, 26]. BV took the same trend. This may be due to the PER1, PER2 contents.

Viscosity: The data presented in figs (Fig3) shows the effect of cold storage at 5º C ± 1 on viscosity (Pa. s) of functional fermented milk beverage fortified with guava juice and coconut extract. . It is clear that treatment fortified with guava juice had the highest viscosity followed by treatment fortified with coconut extract and finally control. The viscosity (Pa. s) was increased by increasing the cold storage until 10 days. Fermented milk beverage fortified with guava juice had the highest viscosity than control and other treatment. This may be due to the highest acidity, total solids and fibers of guava juice. These results are in agreement with [10,27and 23].

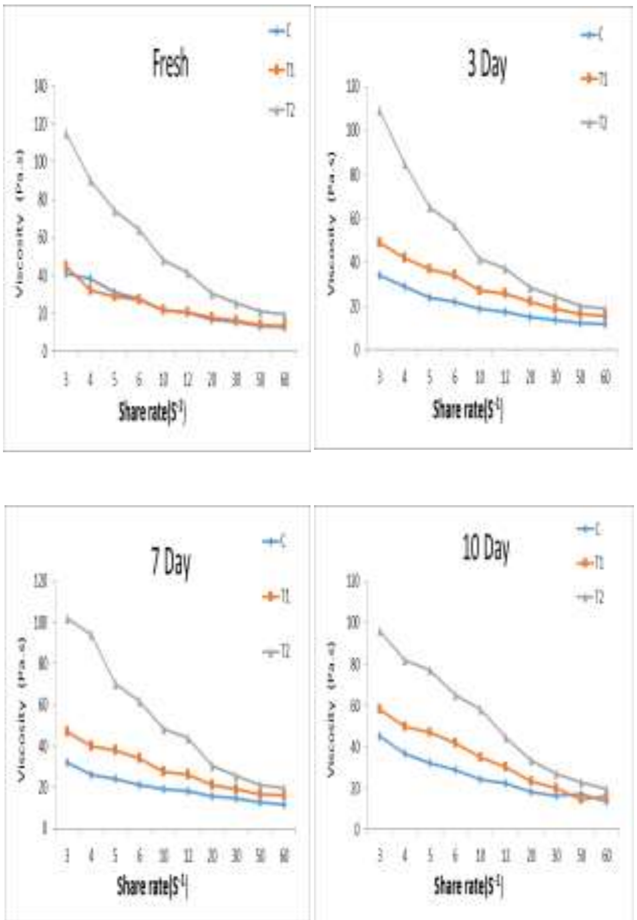

Figure 3: Viscosity of functional fermented milk beverage fortified with guava juice and coconut extract

Microbiological analysis

Viability of *Lactobacillus acidophilus* in fermented functional milk beverage

Table 5. Viability of *Lactobacillus acidophilus* (log CFU/ml) in fermented functional milk beverages during cold storage at 5 ±1° C

Treatments	Storage time (days)			
	Zero time	3	7	10
Control	ND	ND	ND	ND
T1	8.56[b]	9.12[b]	9.31[b]	9.59[a]
T2	8.81[a]	9.22[a]	9.32[a]	9.55[b]
±SEM	0.01943	0.00942	0.01805	0.01261

Dissimilar superscripts at the same row for treatments are significantly differed (P < 0.05). Each value is a mean of 3 replicates
Control = manufactured without cultures
T1: manufactured with adding 2 % of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1) + 10 % coconut extract + WPC+ Dariloid+ Fructose.
T2: manufactured with adding 2 % of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1) + 15 Guava juice +WPC+ Dariloid + Fructose.

Table (7) shows the viable counts of *L. acidophilus* (log CFU/ml) in fermented functional milk beverages. It is clear that the viable counts of the added strain were nearly the same and recorded 8.56, 8.74, 8.81 and 8.95 in T1, T2 and control, respectively. After 10 days of cold storage, the viability of the examined strain was higher in all fermented treatments and the counts in the samples increased slightly (1 log cycle) during refrigerated storage and throughout the storage period. Finally, control had the highest count of 9.78 log CFU/ml and the counts were still above the recommended therapeutic levels. Results are in harmony with those mentioned by [28, 29 and30**]** who mentioned that dairy products are the major vehicle for delivering and enhancing the viability of probiotics because the milk medium contains a number of active compounds and nutrients that promote microbial growth and viability. Statistical analysis shows that there is no significant at α 0.05 for cold storage until 10 days.

Table 6. Viability of *Streptococcus thermophilus* (log CFU/ml) in fermented functional milk beverage during cold storage at 5 ±1° C

Treatments	Storage time (days)			
	Zero time	3	7	10
Control	ND	ND	ND	ND
T1	8.67[a]	8.70[a]	8.81[b]	8.82[b]
T2	8.52[b]	8.65[b]	8.84[a]	8.90[a]
±SEM	0.01347	0.01666	0.00881	0.00666

Dissimilar superscripts at the same row for treatments are significantly differed (P < 0.05). Each value is a mean of 3 replicates
Control = manufactured without cultures
T1: manufactured with adding 2 % of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1) + 10 % coconut extract + WPC+ Dariloid+ Fructose.
T2: manufactured with adding 2 % of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1) + 15 Guava juice +WPC+ Dariloid+ Fructose.

Table (6) indicated the viability of *Streptococcus thermophilus* (log CFU/ml) in fermented functional milk beverage during cold storage at 5 ±1° C. The counts of *Streptococcus thermophilus* was slowly increased during the storage period. On the other hand, the counts of *Streptococcus thermophilus* were recorded 8.82, 8.87 and 8.90 log CFU/ml in treatments T1 and T2, respectively, at the end of cold storage. The counts are still above the recommended therapeutic levels, which agreement with those reported by [31, 32 and 33]. They reported that fermented milk and dairy products are the best carriers of probiotics through gastrointestinal conditions, contributing to a variety of health benefits. Statistical analysis shows that there is a significant at α 0.05 for cold storage until 10 days.

Table 7. Total viable counts (log CFU/ml) in fermented functional milk beverage during cold storage at 5 ±1° C

Treatments	**Storage time (days)**			
	Zero time	3	7	10
Control	3.23[c]	4.25[c]	4.60[c]	4.86[a]
T1	4.96[a]	5.65[a]	5.96[a]	6.48[b]
T2	4.82[b]	5.41[b]	5.80[b]	6.35[c]
±SEM	0.95369	0.02268	0.08388	0.02687

Dissimilar superscripts at the same row for treatments are significantly differed (P < 0.05). Each value is a mean of 3 replicates
Control = manufactured without cultures
T1: manufactured with adding 2 % of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1) + 10 % coconut extract + WPC+ Dariliod+ Fructose.
T2: manufactured with adding 2 % of *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* (1:1) + 15 Guava juice +WPC+ Dariliod + Fructose.

Total viable counts (log CFU/ml) in fermented functional milk beverages are illustrated in Table 7. The counts were varied in all treatments. From the obtained results, we observed that total viable counts gradually increased with extending storage period in all beverage samples. The total viable counts ranged from 3.23 to 4.86 log CFU/ml in fresh. The treatment T2 had the highest count at the end of cold storage, whereas the fresh treatment recorded the lowest count at the end of the storage period. Statistical analysis shows that there is no significant at α 0.05 for cold storage until 10 days for .

Mould and yeast count: samples of fermented milk beverages are free from mould and yeast in fresh samples and during cold storage at 5 ±1° C for 10 days also, samples are free from coliform.

4. Conclusion:

Functional fermented milk beverages supplemented by *Lactobacillus acidophilus* CH-2 and *Streptococcus thermophilus* and fortified with 15 % guava juice are useful for schoolchildren and old people followed by fermented milk beverages fortified with 10% coconut extract. These may be due to the highest content of L-arginine. We can preparation of healthy fermented milk as functional milk beverage for adult and juveniles.

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