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Developing of Grape-Flavored Whey Probiotic Beverage

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ABSTRACT

This study was conducted to develop Grape-Flavored Whey Probiotic Beverage and evaluate the probiotic potential of lactic acid bacteria (LAB) to be beneficial to dairy industry. Ten beverage formulations of different proportions (0,10,15 and 20)% of grape juice (GJ) were prepared and probiotic isolates were added as single (SI) and mixed (MI) isolates as following: T1:(0%GJ:100%Whey), T2:(10%GJ:90%Whey), T3:(15%GJ:85%Whey), T4:(20%GJ:80%Whey), T5:(10%GJ:90%Whey+SI), T6:(15%GJ:85%Whey+SI), T7:(20%GJ:80%Whey+SI), T8:(10%GJ:90%Whey+MI), T9:(15%GJ:85%Whey+MI) and T10:(20%GJ:80%Whey+MI) (vol/vol). The chemical and microbiological tests and sensory properties were conducted during storage periods. T7 was the best in sensory properties compared to the other treatments and its results at the end of study period were : pH (5.167), TA (1.221), fat content (0.417), SNF % (6.450), protein % (2.112) lactose % (3.689), probiotics counts 9.08 (log cfu/ml), riboflavin 3.249, lactic acid 17.095, acetic acid 7.452, propionic acid 6.524, and butyric acid 5.763. Results showed the significant components and desirable sensory properties of treatment 7 that may promise to a valuable whey pharmaceutical probiotic product.

KEYWORDS

Probiotic Beverage, Whey, Riboflavin, Short-Chain Fatty Acids.

Introduction

Dairy industry regards one of the most industries exposed to pollution because of the huge quantities of the produced whey. The residue of dairy industry (whey) regards a crucial environmental problem. Otherwise, the utilization of whey could be beneficial not just for the environment, but also for a sustainable and lasting economy. Whey is a profoundly nutritious product that is easy to digest and absorb (Teixeira *et al.*, 2019). Manufacturing of whey-based beverages shows up to be foremost conservative and least difficult arrangement for whey utilization in human nourishment. The require for improvement of whey-based beverages are closely related to dietary functional characteristics of whey proteins, in addition to satisfying the desires of present-day customers who request inventive products with improved characteristics (Chavan *et al*., 2015). Over the past twenty years, various whey-based beverages and comparative products containing separated whey ingredient (primarily whey proteins) have been set on the showcase as reviving, value-added and/ or useful nourishments (Chavan *et al*., 2015).

The main byproduct of dairy industry is whey which results from cheese or casein production.

Since almost a third of total milk production is utilized in cheese manufacturing, about 85-90% whey resulted from the milk volume that used in cheese manufacturing, a high quantity of whey is resulted which is about 19×10^6 ton per year (Pereira *et al.*, 2015). Whey is a yellow-greenish fluid composed of 50% milk solids including lactose, whey proteins, and mineral compounds, the fat content in whey is low, while the casein content which shapes the cheese curd, is insignificant. The whey content of total solid is about 6.7% (FAO, 2013). Whey proteins are wealthy in the essential amino acids, which give them a high biological value comparing with the other animal and vegetable proteins such as egg proteins, which regard as a referent for a long period, additionally, it contains lactose, minerals, and water-soluble vitamins (Bylund, 2015, Brandelli *et al.*, 2015; Hsieh *et al*., 2015). Furthermore, it has many health-enhancing effects (Petyaev *et al.*, 2012; Mann *et al*., 2019).

The utilize of whey in food manufacturing is one of the foremost attractive valuable strategies. Lately, many new whey products have been created. Whey-based beverage is a type of such items. Currently, much consideration has been paid to the advancement of probiotic whey beverages, since their benefits are increasingly recognized. Fermented beverages are well known by customers around the world for their healthy and therapeutic value. Taking into consideration the reality that the whey contains nearly 70% lactose from the milk, fermenting to yogurt-like drinks shows up to be a significant way of whey utilization, because fermentation process leads to decreasing the pH due to changing of the lactose into lactic acid, sweet whey results to be a good choice for production of fermented beverage (Barukčić *et al.*, 2019).

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Often, a starter or probiotic culture which has the ability to metabolize lactose is used in whey fermentation (Chavan *et al.*, 2015; Sohrabi *et al*., 2016). Whey nutritional value could be enhanced by adding probiotic bacteria to make it a functional food. Dairy products with probiotic are a very important group which regards as a functional food that spreading recently in the global market (Granato *et al.*, 2010; Turkmen *et al*., 2019). Whey components enhance probiotic bacteria growth and survival (de Castro *et al.*, 2009; Bulatović *et al.*, 2014), in addition to improving lactic acid bacteria viability in gastrointestinal tract (Kar and Misra, 1999). Furthermore, Whey beverages with probiotic may reduce serum cholesterol level and blood pressure, stimulate immune system, and reduce cancer risk (Hernandez-Mendoza *et al.*, 2007; Shah, 2007). In addition to its health-promoting properties, the fermentation of whey may lead to more advantages, just like decreasing of the content of lactose, whey protein partial hydrolysis, shelf life increasing because of lactic acid, and aroma compounds production which enhances the sensory characteristics (Pescuma *et al.*, 2010; Chavan *et al.*, 2015).

The current study aims to develop a probiotic beverage of grape-flavored whey and study its physicochemical, microbial, and sensory characteristics, shelf life, lactic acid content and short-chain fatty acids, and riboflavin content.

Materials and Methods

Materials

- Raw Milk: Fresh, full-fat cow milk (the morning milking) obtained from the field of Animal Production Department - College of Agriculture - Basra University.
- Industrial grape juice: Purchased from Basrah local markets.
- Rennet: Christian Hansen- Copenhagen, Denmark.
- Single (*L. acidophilus*) and mixed (*L. plantarum, L. salivarius and L. rhamnosus*) isolates: Ready-made single and mixed isolates were used from (GREENMADE / Finland).
- MRS Agar media for the enumeration of probiotics bacteria.

Methods

- **Preparation of Whey**

Whey was obtained by adding rennet enzymatic coagulant to the raw milk (0.7 mL/L of milk). The mixtures of milk were stirred for 3 minutes and then allowed to sit for 30 min.

- **Preparation of Probiotic Beverages**

Four different concentrations (0,10,15 and 20) % of grape juice (GJ) were used in grape-flavored whey beverages and the remaining volume was completed with whey and distributed in sterilization bottles, sterilized then in an autoclave steam sterilizer at 121° C for 15 minutes, then left to cool to 37 °C. The probiotic isolates (10^8 cfu/ml) then were added as single (SI) and mixed (MI) isolates. Ten beverages treatments were prepared, containing T1:(0%**GJ**:100%**Whey**),T2:(10%**GJ**:90%**Whey**),T3:(15%**GJ**:85%**Whey**),T4:(20%**GJ**:80%**Whey**),T5:(10%**GJ**:90% **Whey**+**SI**),T6:(15%**GJ**:85%**Whey**+**SI**),T7:(20%**GJ**:80%**Whey**+**SI**),T8:(10%**GJ**:90%**Whey**+**MI**),T9:(15%**GJ**:85% **Whey**+**MI**)and T10:(20%**GJ**:80%**Whey** +**MI**) (vol/vol). Samples were incubated under anaerobic conditions in a shaker incubator at 37°C with a vibration motion of 55 rpm for 48 h. All prepared beverages were then stored at 4°C refrigerated temperature. The tests were conducted during storage periods of (0, 15 and 30) days**.**

- **Physicochemical and Microbial Analysis**

Different parameters such as titratable acidity, pH, solid non-fat (SNF), fat, protein, lactose content, probiotics counts were examined to study the impact of fermentation time and storage period on the development of probiotic beverages.

- **Lactic Acid and Short-chain Fatty Acid (SCFA) Analysis**

Lactic acid and short-chain fatty acids contents of grape-flavored whey beverages were analyzed at the labs of Ministry of Science and Technology / Baghdad, using the HPLC method according to Bujna *et al.* (2018).

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- **Estimation of Riboflavin**

Riboflavin content was evaluated at labs of Ministry of Science and Technology / Baghdad, using the Shimadzu RF-535 HPLC chromatography system. Procedures for preparing standard solutions, standard curves, and quantification were performed to the samples according to the method described by Ashoor *et al*. (1983).

- **Sensory Evaluation**

The sensory properties of grape-flavored whey beverages were evaluated by an evaluation committee consisting of specialist members from Food Science Department-College of Agriculture-University of Basrah. A test form consisting of 4 sensory features: color and appearance, sedimentation, flavor, and overall acceptability. The nine-point pleasure scale was used to describe sensory characteristics numerically in which ranged from 1-9 points as: Like extremely (9) Like very much(8) Like moderately(7) Like slightly(6) Neither like nor Dislike(5) Dislike slightly(4) Dislike moderately(3) Dislike very much(2) Dislike extremely(1) (Kumari, 2010).

Statistical Analysis

Data were analyzed using Complete Random Design (CRD) and using the ready-made statistical analysis program SPSS, Version 24 (2016). The averages were compared using the lowest significant difference LSD under a probability level ($p<0.05$).

Results and Discussions

pH values of whey beverages were estimated during (0, 15, 30) days of storage periods on 4°C as illustrated in table 1. Non-probiotic beverage sample (T1-T4) kept its pH at the same value during storage periods with very slight decrease, although the slight differences among these samples which may be attributed to grape juice concentrations that was added to these samples which affected on the final pH value. pH values for whey beverages of single probiotic (T5, T6, T7) reduced from 6.107 to 5.167while the pH values of mixed probiotics whey beverages (T7, T8, T9) reduced from 6.104-5.122 and this reduction in pH values could be linked to converting of lactose into lactic acid by probiotic bacteria. Consequently, the pH of the prepared probiotic whey beverage of single and mixed probiotic bacteria was decreased, and these results were in agreement with Ndife (2019), who reported that the pH of Brown Rice Probiotic Beverages ranged from (6.21-5.32) at zero time and (5.96-4.78) in the fourth week.

Table 1. pH of whey beverages during storage periods at 4 °C

Treatments	**pH Through Storage periods**			**Treatment's mean**
	0 Time	**15 day**	**30 day**	
T1	6.185±0.061	6.182±0.110	6.180±0.064	**6.182** [a]
T2	6.084±0.054	6.083±0.844	6.081±0.050	**6.083** [b]
T3	6.053±0.061	6.051±0.615	6.048±0.050	**6.051** [b]
T4	6.031±0.064	6.029±0.178	6.028±0.100	**6.029** [b]
T5	6.107±0.155	5.407±0.018	5.244±0.057	**5.586** [c]
T6	6.051±0.063	5.394±0.113	5.212±0.011	**5.552** [c]
T7	6.029±0.141	5.281±0.101	5.167±0.061	**5.492** [c]
T8	6.104±0.120	5.383±0.041	5.229±0.060	**5.572** [c]
T9	6.048±0.055	5.284±0.061	5.204±0.055	**5.512** [c]
T10	6.033±0.063	5.261±0.582	5.122±0.612	**5.472** [c]
Period's mean	**6.073** [a]	**5.635** [b]	**5.552** [b]	

Titratable acidity (TA) of whey beverages increased for all samples along with storage periods as shown in table 2. The increase was very slight for the non-probiotic whey beverages samples (T1-T4) while there were differences among the samples because the addition percent of grape juice. Titratable acidity increased gradually from 0.570 to 1.221 in whey beverages of single probiotic (T5, T6, T7) while the increase was higher from 0.572 to 1.238 in whey beverages of mixed probiotics (T7, T8, T9). This increase in acidity could be resulting from the organic and amino acids production as a result for the activity of probiotic single and mixed isolates. Lactose and proteins are converted into lactic acid and amino acids which lead to increasing the acidity and reducing the pH in beverages (Kalra *et al.*, 1991). Study results were in one line with Al-Hindi and Abd El Ghani (2020) who reported that the acidity of fermented milk beverages inoculated with *Bifdobacterium longum* subsp *longum* and *Lactobacillus plantarum* and supplemented with peel extract of pomegranate increased remarkably (p<0.05) along with increasing the storage time regardless of its concentration.

Table 2. Titratable acidity of whey beverages during storage periods at 4°C

Treatments	Titratable Acidity Through Storage Periods			Treatment's mean
	0 Time	15 day	30 day	
T1	0.553±0.047	0.555±0.022	0.556±0.033	**0.555 a**
T2	0.569±0.043	0.572±0.028	0.575±0.038	**0.572 a**
T3	0.578±0.060	0.579±0.012	0.581±0.062	**0.579 a**
T4	0.584±0.040	0.587±0.022	0.589±0.047	**0.587 a**
T5	0.570±0.040	1.153±0.040	1.209±0.053	**0.977 b**
T6	0.579±0.014	1.166±0.066	1.217±0.060	**0.987 b**
T7	0.586±0.049	1.174±0.180	1.221±0.050	**0.994 b**
T8	0.572±0.013	1.162±0.008	1.214±0.086	**0.983 b**
T9	0.576±0.045	1.171±0.011	1.231±0.073	**0.993 b**
T10	0.583±0.060	1.182±0.011	1.238±0.056	**1.001 b**
Period's mean	**0.575 a**	**0.930 b**	**0.963 b**	

Table 3 shows the changes in chemical composition for whey beverages during storage periods. There were significant differences (p<0.05) among treatments and among storage periods for fat and SNF which were almost close and stable during storage periods for (T1-T4) whey beverages samples, while there was a slight decrease for (T5-T7) samples and more clear decrease for (T8-T10) samples. Also, there were significant differences among the treatments for protein and lactose content. Protein and lactose content of whey beverages decreased more than the non-probiotic whey beverages, the reason may attribute to the activity of probiotic which affected on the utilizing both of protein and lactose. Study results were similar to the findings of Begum *et al*. (2019) in preparing a fermented (probiotic whey-based watermelon beverage) with the addition of the probiotic bacteria (*Lactobacillus acidophilus* NIAI L-54), they found that protein ratios ranged between (2.1-2.48)%, while the fat percentages ranged between (0.22-0.24)%.

Table 3. Chemical content of whey beverages during storage periods at 4°C

	Treatments	Chemical Content Through Storage periods			Treatment's mean
		0 Day	15 Day	30 Day	
Fat %	**T1**	0.470±0.036	0.473±0.036	0.472±0.036	**0.472 a**
	T2	0.465±0.023	0.467±0.023	0.468±0.023	**0.467 a**

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	T3	0.457±0.035	0.460±0.035	0.459±0.035	**0.459 a**
	T4	0.448±0.041	0.449±0.041	0.451±0.041	**0.449 a**
	T5	0.463±0.026	0.441±0.026	0.432±0.026	**0.445 a**
	T6	0.455±0.030	0.436±0.030	0.425±0.030	**0.439 a**
	T7	0.445±0.020	0.426±0.036	0.417±0.068	**0.429 a**
	T8	0.460±0.015	0.439±0.015	0.428±0.015	**0.442 a**
	T9	0.452±0.032	0.431±0.032	0.419±0.032	**0.434 a**
	T10	0.442±0.047	0.421±0.047	0.413±0.047	**0.425 a**
	Period's mean	**0.456 a**	**0.444 a**	**0.439 a**	
SNF %	**T1**	6.496±0.015	6.498±0.035	6.499±0.041	**6.498 a**
	T2	6.484±0.015	6.485±0.043	6.487±0.036	**6.485 a**
	T3	6.477±0.015	6.479±0.055	6.480±0.028	**6.479 a**
	T4	6.469±0.030	6.471±0.020	6.473±0.010	**6.471 a**
	T5	6.486±0.030	6.474±0.030	6.465±0.011	**6.475 a**
	T6	6.478±0.041	6.465±0.075	6.657±0.047	**6.467 a**
	T7	6.471±0.040	6.458±0.040	6.450±0.020	**6.460 a**
	T8	6.485±0.020	6.472±0.020	6.463±0.025	**6.473 a**
	T9	6.477±0.030	6.464±0.030	6.456±0.122	**6.466 a**
	T10	6.470±0.030	6.457±0.030	6.449±0.020	**6.459 a**
	Period's mean	**6.479 a**	**6.472 a**	**6.468 a**	
Protein %	**T1**	2.733±0.025	2.735±0.025	2.738±0.025	**2.735 a**
	T2	2.700±0.036	2.701±0.036	2.703±0.036	**2.701 a**
	T3	2.689±0.020	2.691±0.020	2.694±0.020	**2.691 a**
	T4	2.676±0.030	2.678±0.030	2.681±0.030	**2.678 a**
	T5	2.703±0.049	2.273±0.049	2.129±0.049	**2.368 b**
	T6	2.691±0.047	2.258±0.047	2.116±0.047	**2.355 b**
	T7	2.678±0.043	2.251±0.043	2.112±0.043	**2.347 b**
	T8	2.702±0.049	2.269±0.049	2.118±0.049	**2.363 b**
	T9	2.688±0.061	2.261±0.061	2.121±0.061	**2.357 b**
	T10	2.676±0.070	2.247±0.070	2.103±0.070	**2.342 b**
	Period's mean	**2.694 a**	**2.436 ab**	**2.352 b**	
Lactose %	**T1**	4.273±0.055	4.275±0.055	4.277±0.055	**4.275 a**
	T2	4.262±0.020	4.263±0.020	4.265±0.020	**4.263 a**
	T3	4.256±0.015	4.259±0.015	4.261±0.015	**4.259 a**
	T4	4.249±0.020	4.251±0.020	4.252±0.020	**4.251 a**
	T5	4.264±0.026	3.757±0.026	3.695±0.026	**3.905 b**
	T6	4.255±0.030	3.748±0.030	3.690±0.030	**3.898 b**
	T7	4.247±0.026	3.750±0.026	3.689±0.026	**3.895 b**
	T8	4.261±0.015	3.749±0.015	3.687±0.015	**3.899 b**
	T9	4.254±0.030	3.741±0.030	3.684±0.030	**3.893 b**
	T10	4.248±0.025	3.734±0.025	3.677+0.025	**3.886 b**
	Period's mean	**4.257 a**	**3.953 b**	**3.918 b**	

Table 4 shows probiotic counts in probiotic whey beverages during storage periods at 4 °C. Grape juice addition rates affected slightly on probiotic counts at zero time with a simple superiority for the mixed probiotic on the single probiotic samples. There was an increase in probiotic count for (T5-T10) samples after fifteen days of storage, while probiotic counts decreased slightly after 30 days of cold storage, this may be attributed to the decrease in whey beverage pH during storage periods (Ndife, 2019). All probiotic whey beverages samples were within the acceptable limits of probiotic bacteria counts (more than 10^6) cfu/ml to be as therapeutic beverages during storage periods. The results were close to the results obtained by Shukla and Kushwaha (2017) as probiotic bacteria counts decreased from 8.25×10^8 to 5.2×10^7 in probiotic beverage fermented with *Lactobacillus acidophilus.*

Table 4. Probiotic bacteria counts in probiotic whey beverages during storage periods at 4°C

Treatments	Probiotics Bacteria Count During Storage Periods (log cfu/ml)			Treatment's mean
	0 Time	15 Day	30 Day	
T5	8.57±0.01	9.43±0.15	9.34±0.04	**9.11 a**
T6	8.56±0.15	9.31±0.07	9.21±0.07	**9.03 a**
T7	8.56±0.03	9.19±0.03	9.08±0.17	**8.94 a**
T8	8.57±0.04	9.45±0.11	9.37±0.08	**9.13 a**
T9	8.57±0.11	9.38±0.14	9.26±0.16	**9.07 a**
T10	8.56±0.13	9.24±0.06	9.13±0.12	**8.98 a**
Period's mean	**8.57 b**	**9.33 a**	**9.23 a**	

Table (5) shows riboflavin content of whey beverages during storage periods at 4 °C. There was a significant increase ($p < 0.05$) in riboflavin content, the treatment (T5) had the highest content of riboflavin, reaching (3.362 mg/liter) compared to the rest of the samples, and there were no significant differences between the treatment of beverages (T5, T6, T7, T8, T9 and T10). No significant differences were observed between samples (T1, T2, T3, and T4). The table also shows that there was a significant increase ($p < 0.05$) in riboflavin content after 15 days of storage. These results were consistent with Guru and Viswanathan (2013) regarding the ability of *Lactobacillus acidophilus* to produce riboflavin, and in a higher percentage, compared with other Lactobacillus isolates when this isolates were grown on the whey as fermentation medium.

Table 1. Riboflavin content of whey beverages during storage periods at 4°C

Treatments	Riboflavin Content Through Storage Periods (mg/L)			Treatment's mean
	(0 Time)	(15 day)	(30 day)	
T1	1.080±0.014	1.070±0.013	1.061±0.010	**1.071 b**
T2	0.973±0.022	0.969±0.020	0.964±0.021	**0.969 b**
T3	0.917±0.021	0.914±0.017	0.911±0.019	**0.914 b**
T4	0.859±0.018	0.856±0.015	0.848±0.015	**0.854 b**
T5	0.968±0.009	2.721±0.043	3.362±0.102	**2.350 a**
T6	0.921±0.018	2.657±0.097	3.296±0.060	**2.291 a**
T7	0.861±0.009	2.613±0.017	3.249±0.073	**2.241 a**
T8	0.975±0.004	2.693±0.101	3.316±0.075	**2.328 a**
T9	0.914±0.024	2.628±0.062	3.269±0.081	**2.270 a**
T10	0.857±0.017	2.547±0.073	3.182±0.095	**2.195 a**
Period's mean	**0.933 b**	**1.967 a**	**2.346 a**	

Table 6 illustrates whey beverages content of short chain fatty acids and lactic acid during storage periods at 4 °C. There was a significant increase ($p<0.05$) in lactic acid content, (T5) was the higher in lactic acid content (13.087 g/l) comparing with the rest samples, and there were no significant differences among whey beverages treatments. There was a decrease in lactic acid content at the end of storage period and this may be attributed to converting part of lactic acid into short chain fatty acids by probiotic bacteria (Hernandez-Hernandez *et al*., 2011). T8 was higher significantly in its content of acetic acid, propionic acid, and butyric acid which were (5.352, 4.703, 4.035) g/l respectively comparing with the rest samples, noting that there were no significant differences among probiotics whey beverages samples. The table also shows that there was a significant increase ($p<0.05$) in the level of these short chain fatty acids with the progress of storage periods. These results were consistent with the findings of Oliveira *et al*. (2012); LeBlanc *et al.*, (2017); Mieszkin *et al.*, (2017); Hati *et al.*, (2019) on the ability of probiotic bacteria to produce lactic acid and short-chain fatty acid with different percentage when grown it on the whey as fermentation medium.

Table 2. Lactic acid and short-chain fatty acids content of whey beverages during storage periods at 4°C

	Treatments	Lactic acid & Short-chain Fatty Acid content Through Storage periods (g/l)			Treatment's mean
		0 Day	15 Day	30 Day	
Lacti c	T1	2.600±0.010	2.594±0.010	2.589±0.015	**2.594 b**
	T2	2.347±0.005	2.342±0.015	2.339±0.045	**2.343 b**

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	T3	2.213±0.005	2.209±0.011	2.205±0.011	**2.209 b**
	T4	2.089±0.010	2.083±0.045	2.077±0.049	**2.083 b**
	T5	2.351±0.005	19.586±0.910	17.324±0.856	**13.087 a**
	T6	2.211±0.005	19.443±0.755	17.206±0.777	**12.953 a**
	T7	2.092±0.011	19.332±1.090	17.095±0.883	**12.840 a**
	T8	2.349±0.005	19.579±1.010	17.318±0.955	**13.082 a**
	T9	2.208±0.005	19.437±0.837	17.195±0.920	**12.947 a**
	T10	2.091±0.005	19.326±0.998	17.089±0.900	**12.835 a**
	Period's mean	**2.255 b**	**12.593 a**	**11.244 a**	
Acetic acid	**T1**	1.306±0.015	1.302±0.015	1.298±0.015	**1.302 b**
	T2	1.184±0.015	1.181±0.020	1.180±0.020	**1.182 b**
	T3	1.112±0.015	1.109±0.020	1.107±0.020	**1.109 b**
	T4	1.053±0.020	1.051±0.020	1.048±0.011	**1.051 b**
	T5	1.187±0.015	7.174±0.020	7.516±0.025	**5.292 a**
	T6	1.115±0.015	7.099±0.036	7.539±0.046	**5.251 a**
	T7	1.058±0.020	7.037±0.030	7.452±0.030	**5.182 a**
	T8	1.186±0.015	7.213±0.037	7.657±0.036	**5.352 a**
	T9	1.114±0.015	7.145±0.020	7.581±0.020	**5.280 a**
	T10	1.056±0.015	7.089±0.020	7.534±0.037	**5.226 a**
	Period's mean	**1.137 b**	**4.740 a**	**4.991 a**	
Propionic acid	**T1**	1.180±0.010	1.176±0.010	1.173±0.005	**1.176 b**
	T2	1.063±0.015	1.059±0.005	1.055±0.011	**1.059 b**
	T3	0.997±0.015	0.992±0.005	0.987±0.015	**0.992 b**
	T4	0.939±0.020	0.935±0.020	0.931±0.015	**0.935 b**
	T5	1.065±0.015	6.286±0.072	6.658±0.040	**4.670 a**
	T6	0.999±0.015	6.213±0.030	6.586±0.055	**4.599 a**
	T7	0.941±0.020	6.166±0.041	6.524±0.049	**4.544 a**
	T8	1.066±0.015	6.334±0.015	6.709±0.030	**4.703 a**
	T9	0.998±0.015	6.262±0.043	6.627±0.015	**4.629 a**
	T10	0.938±0.038	6.209±0.040	6.581±0.020	**4.576 a**
	Period's mean	**1.019 b**	**4.163 a**	**4.383 a**	
Butyric acid	**T1**	0.713±0.062	0.709±0.062	0.704±0.035	**0.709 b**
	T2	0.643±0.043	0.641±0.041	0.638±0.032	**0.641 b**
	T3	0.607±0.072	0.604±0.072	0.598±0.037	**0.603 b**
	T4	0.572±0.087	0.569±0.070	0.565±0.052	**0.569 b**
	T5	0.645±0.055	5.522±0.066	5.819±0.066	**3.995 a**
	T6	0.605±0.047	5.495±0.127	5.796±0.070	**3.965 a**
	T7	0.574±0.080	5.464±0.050	5.763±0.050	**3.934 a**
	T8	0.646±0.055	5.576±0.066	5.883±0.514	**4.035 a**
	T9	0.608±0.073	5.542±0.543	5.839±0.577	**3.996 a**
	T10	0.577±0.055	5.514±0.511	5.821±0.055	**3.971 a**
	Period's mean	**0.619 b**	**3.564 a**	**3.743 a**	

Table (7) shows the sensory properties (color and appearance, sedimentation, flavor, and overall acceptability) of whey beverages. T7 was significantly ($P<0.05$) the best in all sensory properties except for sedimentation after 15 days of storage period comparing with the rest samples and this may be attributed to whey fermentation which may lead to production of lactic acid, and aroma compounds which enhanced the sensory characteristics (Chavan *et al*.,2015). The results were consistent with Punnagaiarasi *et al.* (2017) who reported that adding watermelon juice increased consumer acceptance, and among all the drinks, the whey drink with (15%) watermelon juice showed the highest sensory scores compared to the other drinks.

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Table 7. Sensory properties of whey beverages during storage periods at 4°C

Parameters	Treatment	Storage Periods (Day)		Treatment's Mean
		15	30	
Color and appearance	T1	5.2±0.96	5.1 ±1.10	5.1 c
	T2	5.3±1.04	5.2 ±1.13	5.2 c
	T3	5.3±1.07	5.0 ±1.15	5.1 c
	T4	5.4±0.76	5.3 ±0.94	5.3 c
	T5	8.2±0.63	8.1 ±0.56	8.1 a
	T6	8.4±0.54	8.2 ±0.42	8.3 a
	T7	8.5±0.75	8.4 ±0.69	8.4 a
	T8	6.6±0.46	6.4 ±0.51	6.5 b
	T9	6.8±0.69	6.3 ±0.51	6.5 b
	T10	6.9±0.71	6.4 ±0.84	6.6 b
	Period's mean	6.6 a	6.4 a	
Sedimentation	T1	6.2±1.08	6.0 ± 0.99	6.1 c
	T2	6.3±1.02	6.1 ± 0.91	6.2 c
	T3	6.5±0.99	6.1 ± 0.91	6.3 c
	T4	6.6±0.59	6.1 ± 0.63	6.3 c
	T5	7.7±0.72	7. 5 ± 0.52	7.6 a
	T6	8.3±0.95	7.8 ± 1.03	7.9 a
	T7	8.1±0.98	7.7 ± 0.94	8.0 a
	T8	6.7±0.87	6.4 ± 0.78	6.5 b
	T9	6.9±1.01	6.5 ± 1.17	6.7 b
	T10	6.4±0.75	6.2 ± 0.63	6.3 b
	Period's mean	6.9 a	6.6 a	
Flavor	T1	5.3±0.92	5.1±1.12	5.2 c
	T2	5.4±0.32	5.2±1.34	5.3 c
	T3	5.4±0.59	5.1±1.11	5.2 c
	T4	5.5±1.05	5.3±1.01	5.4 c
	T5	7.9±0.67	7.8±1.31	7.8 a
	T6	8.2±0.72	7.7±0.63	7.9 a
	T7	8.4±0.81	7.9±0.87	7.2 a
	T8	6.6±0.53	6.3±0.48	6.4 b
	T9	6.9±0.88	6.4±1.07	6.6 b
	T10	7.3±0.76	6.8±0.91	7.0 b
	Period's mean	6.6 a	6.3a	
Overall acceptability	T1	5.3±1.43	5.2±1.11	5.2 c
	T2	5.5±1.03	5.3±1.21	5.4 c
	T3	5.6±1.14	5.4±1.26	5.5 c
	T4	5.5±1.07	5.3±1.29	5.4 c
	T5	7.9±0.88	7.7±0.67	7.8 a
	T6	8.1±0.76	7.7±0.67	7.9 a
	T7	8.7±0.52	8.6±0.63	8.6 a
	T8	6.8±0.67	6.5±0.52	6.6 b
	T9	6.4±0.73	6.2±0.63	6.3 b
	T10	7.1±0.97	6.7±0.82	6.4 b
	Period's mean	6.6 a	6.4 a	

Conclusions

The incubation of the probiotic's cultures of single and mixed cultures in the grape-flavored whey probiotic beverages maintained a viable probiotic population ($\geq 10^6$ cfu/mL) during fermentation and storage under refrigeration for 30 days. riboflavin and organic acids, such as lactic, acetic, propionic, and butyric acids, were produced and maintained the low pH (around 5) of the beverage, which is important for the food safety, taste, and aroma of the beverages. Furthermore, the fermentation of whey by the probiotics enhanced the sensory characteristics of these beverages which may promise to a valuable whey pharmaceutical probiotic product.

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