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### A Novel Bio-Fermented Beverages from Dairy By-Products Based with Papaya Pulp and Stevia Leaves.



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#### ABSTRACT

Current trends and changing consumer needs indicate a great opportunity for innovations and developments in functional beverages. Scientific and clinical evidence is also mounting to corroborate the consumer perception of health from healthy beverages. So, the aim of this study was to develop probiotic cheese whey or milk permeate-based beverages fermented by 1.0% *Lactobacillus acidophilus* or *L. paracasei* and enriched with 10% papaya pulp (as prebiotic) and 1.0% stevia leaves extract (as natural sweetener). Four fermented probiotic whey and four milk permeate-based beverages were prepared and investigated for probiotic microbial counts, pH values, titratable acidity and sensorial attributes during storage period at  $5\pm 1^\circ\text{C}$  for 10 days. Results of pH values and titratable acidity of the beverages indicated no significant differences between the whey or permeate beverages. However, functional permeate beverages containing stevia extract had slightly higher probiotic viability than whey beverages during cooled storage. Fermented permeate-based beverages were appraised with relatively high overall score over the whey-based beverages. The final beverages were characterized with good sensory properties and a highly viable count of probiotic  $<10^8$  cfu/mL during 10 days of cooled storage. So, it is strongly advised to produce such a healthy probiotic whey or permeate beverages which fortified by papaya pulp and sweetened with stevia leaves extract.

**Keywords:** Probiotics, *L. paracasei*, whey, permeate, papaya pulp, stevia leaf, and fermented beverage

#### INTRODUCTION

Whey and permeate are a by-product of the cheese industry and represents a valuable source of biological and therapeutic ingredients. Several researchers have been proposed to utilize whey or permeate in the functional beverages industry due to its nutritional and biological values (Hattem *et al.*, 2011; Sakhale *et al.*, 2012; Singh and Singh, 2012 and Gomaa *et al.*, 2018). One or more flavoring agents or fruit juice are added to whey or permeate to forms functional beverages useful as a sports drink or as a therapeutic drink (Baljeet *et al.*, 2013; Tariq *et al.*, 2013; Rizk, 2016; Prashanth *et al.*, 2018 and Hailu *et al.*, 2019). To increase the biological value of whey and permeate, probiotic bacteria and prebiotic ingredients are one of the best choices of production functional fermented beverages based on whey and permeate (Atallah, 2015; Jambi, 2017; Aita *et al.*, 2019 and El-Shenawy *et al.*, 2019).

Papaya is cultivated for its fruits; it has a pulpy fresh yellow colored and it usually eaten as breakfast, and as ingredients in jellies, the juice makes a popular beverage (Panghal *et al.*, 2017). It is one best source of vitamins (A, B, C and E) and a rich source of antioxidant nutrients, potassium, magnesium and fiber (Gupta *et al.*, 2015 and Chen *et al.*, 2018). These nutrients are known to promote the health of the cardiovascular system and protect against colon cancer (Vij & Prashar, 2015 and Somanah *et al.*, 2018). Hence, for the present study, papaya was selected for preparation of functional beverage.

Stevia popularly known as sweet leaf has been used as a natural sweetening agent without caloric value (300 times sweeter than sucrose) in tea, beverages and food since a long time, allowing consumers to enjoy sweet taste without concerns about weight gain (Lisak *et al.*, 2011 and Kheynoor *et al.*, 2018). Stevia leaves contain a mixture of diterpene glycosides (steviosides) and is considered a good source dietary fiber, minerals and essential amino acids (Samuel *et al.*, 2018 and Lima *et al.*, 2019). Due to its chemical structure and health-promoting phytochemical components, stevia is suitable as a replacement for sucrose in beverages and for the production of functional food ingredients (Choi & Chung, 2018 and Kovačević *et al.*, 2018).

The development of a whey and permeate beverages containing papaya pulp as a prebiotic, stevia leaf extract as natural sweeteners and probiotic bacteria represents the possibility of producing a nutritive highly valuable whey or permeate beverages. Thus, the aim of this study was to develop probiotic whey and milk permeate beverages fermented by *L. acidophilus* and *L. paracasei* and to investigate the impact of the addition of stevia leaf extract (1.0%), papaya pulp (10%) and their potential interaction on the quality of beverages.

#### MATERIALS AND METHODS

##### Materials:

Fresh whey and permeate were obtained from a dairy pilot plant (Fac. Agric., Fayoum Univ., Egypt). Mature papaya fruits (*Carica papaya* L.) were purchased from the local market in Egypt. Dried stevia leaves (*Stevia*

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*rebaudiana*) were obtained from Crops Res. Institute, Agric. Res. Centre, Giza, Egypt. Two probiotic cultures of *L. acidophilus* and *L. paracasei* were obtained from Depart. Microbiol., Fac. Agric., Fayoum Univ., Egypt).

#### Methods:

##### Probiotic bacteria activation

*L. acidophilus* and *L. paracasei* were reactivated by inoculation in 10% sterilized reconstituted milk and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h before being used in the fermented beverages production.

##### Preparation of papaya pulp

The mature papaya fruit was washed and soaked in boiling water for 2 min to remove any impurities materials or bacteria and then peeled the fruit. The pulp was homogenized in an electric blender and added immediately to pasteurized fresh whey or permeate used in this study.

##### Preparation of stevia leaves extract

The stevia leaves were washed, dried in oven at  $100^\circ\text{C}$  for 24h and then grounded in a mixer grinder. The powder was dissolved in distilled water (1:2) at  $40^\circ\text{C}$  for 10 min with continuous stirring and filtered using a Whatman filter paper to obtain stevia extract. The obtained stevia extract was heated to  $100^\circ\text{C}/5$  sec.

##### Optimization of papaya pulp and stevia extract level in whey or permeate for beverage preparation

Preliminary experiments were performed to determine the optimal level of papaya pulp and stevia extract used in the preparation of functional whey or permeate beverage in terms of consumer acceptability. Fresh whey with different papaya pulp concentrations (0, 5, 10, 15, 20 and 30%) or with different levels of stevia extract (0, 0.5, 1.0, 1.5 and 2.0%) was evaluated for their sensorial attributes. The results revealed that, addition of 10% papaya pulp and 1.0% stevia extract in the preparation of whey or permeate beverage was found the most acceptable level among other treatments. Thus, the level of papaya pulp and stevia extract was fixed at 10% and 1.0%, respectively in this study.

##### Experimental procedure

Eight fermented probiotic beverages (four based on cheese whey and the other four based on milk permeate) were produced as follows:

**Experiment 1 (Whey-based beverages) and Experiment 2 (Permeate-based beverages):** pasteurized cheese whey or permeate (Four liters of each) was mixed at  $40^\circ\text{C}$  with 10% papaya pulp and then divided into four equal parts (liter of each part); the 1<sup>st</sup> and 2<sup>nd</sup> part were fermented individually by 1.0% *L. acidophilus* and *L. paracasei*, respectively; 1.0% stevia leaves extract was added to 3<sup>th</sup> and 4<sup>th</sup> part, then fermented individually by 1.0% *L. acidophilus* and *L. paracasei*, respectively. The resulting beverages were distributed in sterile plastic bottles in triplicates and fermented for 8 hrs at  $40^\circ\text{C}$  and then cooled stored for 10 days. Physicochemical, microbiological and sensorial evaluations were carried out on 1<sup>st</sup>, 3<sup>th</sup>, 6<sup>th</sup> and 10<sup>th</sup> days.

##### Physicochemical analyses

The pH values were determined using an Orion pH meter (Model 290A). Titratable acidity and lactose were determined in triplicate according to AOAC (2005). All the physicochemical analyses were analysed in triplicate.

##### Microbiological analyses

Under sterilized conditions, all functional beverages samples were analyzed when fresh and during storage period (3<sup>th</sup>, 6<sup>th</sup> and 10<sup>th</sup> days) at  $5 \pm 1^\circ\text{C}$ . The samples were mixed; appropriate dilutions were made with sterile tri-sodium citrate solution (0.1% w/v) and subsequently plated in duplicate onto selective media. Lactic acid bacteria ( $\log_{10}$  cfu/ml) was enumerated on MRS media (Merck, Darmstadt, Germany) at pH 5.4 after incubation at  $37^\circ\text{C}$  for 48 h as described in Oxoid (2006). Yeast and mold were enumerated on potato dextrose agar and incubated aerobically at  $25^\circ\text{C}$  for 3–6 days. Coliform counts were enumerated on MacConkey agar, the plates incubated at  $37^\circ\text{C}$  for 24h.

##### Sensory evaluation

Treatments were organoleptically evaluated when fresh and at 3<sup>th</sup>, 6<sup>th</sup> and 10<sup>th</sup> days by 15 panels of staff members of Dairy Sci. Depart. and Food Sci. and Technol. Depart., Fac. Agric., Fayoum Univ. Attributes evaluated were flavour (60 points), consistency (30 points), appearance & colour (10 points) and overall acceptability (100 points).

##### Statistical analysis

Experimental data were statistically analyzed using General Linear Models procedure of Statistical Package for Social Sciences (SPSS) Version 17.0.0 software. Multiple range tests were used to compare between the means (Duncan, 1955).

## RESULTS AND DISCUSSION

##### Physicochemical analysis

Changes occurred in the pH values, titratable acidity (TA %) and lactose content of the whey-based or permeate-based beverages during storage at  $5 \pm 1^\circ\text{C}$  for 10 days are presented in Table (1). Little variation was observed for either of the parameters studied during 10 days of cooled storage. Generally, probiotic beverages with 1.0% stevia extract showed higher acidity and lower pH than beverages prepared without stevia extract at both fresh and stored treatments. This could be attributed to the stevia extract addition accelerated the fermentation process of the beverages (Nagashima *et al.*, 2013).

The results in the same Table (1) show that all fresh samples had higher values of pH than that obtained during cooled storage. There is a slight difference in pH values between fresh treatments as they ranged from 4.2 to 4.63. The pH values decreased gradually during storage to reach 4.32 to 3.7 at the end of cooled storage (10 days).

The significant lowest ( $P < 0.05$ ) pH value was 3.7 for the treatment of probiotic milk permeate based beverage with *L. paracasei*, 1% stevia extract and 10% papaya at the 10<sup>th</sup> day of cooled storage. Results in Table (1) confirmed that permeate-based beverage fermented with *L. paracasei* and enriched with stevia extract when stored for 10 days, had the lowest value of pH, at the same time it recorded the significantly ( $P < 0.05$ ) highest TA of 1.35%. While, the fresh probiotic whey based beverage with *L. acidophilus* had the lowest TA (0.70%), may be that due to the presence of the entrapped probiotic bacteria. Post-acidification during storage time can be attributed to the progressive transformation of lactose into lactic acid, as a result of the continuation of activity and metabolism during refrigerated storage (Elewa, 1992 and Ramirez-Santiago *et al.*, 2010).

**Table 1. Some physicochemical analysis of the prepared functional beverages during storage periods at 5±1°C for 10 days.**

Storage period (days)	Treatments							
	Probiotic whey-papaya pulp beverage fermented by				Probiotic permeate papaya-pulp beverage fermented by			
	<i>L.acidophilus</i>	<i>L.paracasei</i>	<i>L.acidophilus</i> + stevia extract	<i>L.paracasei</i> + stevia extract	<i>L.acidophilus</i>	<i>L.paracasei</i>	<i>L.acidophilus</i> + stevia extract	<i>L.paracasei</i> + stevia extract
pH								
Fresh	4.46 <sup>bc</sup>	4.40 <sup>c</sup>	4.30 <sup>ef</sup>	4.20 <sup>ghi</sup>	4.63 <sup>a</sup>	4.53 <sup>b</sup>	4.46 <sup>bc</sup>	4.41 <sup>c</sup>
3	4.44 <sup>c</sup>	4.38 <sup>cd</sup>	4.27 <sup>efg</sup>	4.18 <sup>hij</sup>	4.53 <sup>b</sup>	4.43 <sup>c</sup>	4.40 <sup>c</sup>	4.21 <sup>ghi</sup>
6	4.32 <sup>de</sup>	4.22 <sup>fghi</sup>	4.11 <sup>j</sup>	3.96 <sup>kl</sup>	4.41 <sup>c</sup>	4.19 <sup>ghi</sup>	4.23 <sup>fgh</sup>	4.01 <sup>k</sup>
10	4.15 <sup>hij</sup>	4.17 <sup>hij</sup>	3.93 <sup>kl</sup>	3.88 <sup>l</sup>	4.32 <sup>de</sup>	4.14 <sup>ij</sup>	4.01 <sup>k</sup>	3.70 <sup>m</sup>
Titratable acidity (%)								
Fresh	0.70 <sup>q</sup>	0.76 <sup>p</sup>	0.84 <sup>lmno</sup>	0.91 <sup>ij</sup>	0.80 <sup>op</sup>	0.82 <sup>no</sup>	0.83 <sup>mno</sup>	0.87 <sup>ijklm</sup>
3	0.86 <sup>klmn</sup>	0.90 <sup>ijk</sup>	0.93 <sup>i</sup>	1.01 <sup>h</sup>	0.84 <sup>lmno</sup>	0.90 <sup>ijk</sup>	0.86 <sup>klmn</sup>	0.88 <sup>ijkl</sup>
6	0.98 <sup>h</sup>	1.01 <sup>h</sup>	1.10 <sup>g</sup>	1.10 <sup>8f</sup>	1.00 <sup>h</sup>	1.09 <sup>g</sup>	1.10 <sup>g</sup>	1.21 <sup>ef</sup>
10	1.16 <sup>f</sup>	1.18 <sup>f</sup>	1.30 <sup>bc</sup>	1.34 <sup>ab</sup>	1.23 <sup>de</sup>	1.30 <sup>bc</sup>	1.26 <sup>cd</sup>	1.35 <sup>a</sup>
Lactose (%)								
Fresh	4.58 <sup>fghi</sup>	4.53 <sup>ij</sup>	4.56 <sup>hij</sup>	4.50 <sup>jk</sup>	4.89 <sup>a</sup>	4.79 <sup>b</sup>	4.78 <sup>b</sup>	4.76 <sup>bc</sup>
3	4.46 <sup>kl</sup>	4.42 <sup>lmn</sup>	4.45 <sup>klm</sup>	4.38 <sup>no</sup>	4.82 <sup>b</sup>	4.72 <sup>cd</sup>	4.65 <sup>ef</sup>	4.62 <sup>fgh</sup>
6	4.39 <sup>mn</sup>	4.32 <sup>opq</sup>	4.26 <sup>qrst</sup>	4.22 <sup>st</sup>	4.70 <sup>de</sup>	4.63 <sup>fg</sup>	4.59 <sup>fghi</sup>	4.55 <sup>ij</sup>
10	4.3 <sup>pqr</sup>	4.24 <sup>rst</sup>	4.21 <sup>t</sup>	4.13 <sup>u</sup>	4.57 <sup>ghi</sup>	4.29 <sup>qrs</sup>	4.36 <sup>mop</sup>	4.26 <sup>qrst</sup>

a, b,.... and u: Means having different superscripts within each column are significantly different (P<0.05).

Also, low pH values and high TA of permeate-based beverage fermented with *L. paracasei* and enriched with stevia extract can be attributed mainly to the high hydrolysis rate of lactose which is due to the prebiotic effect of grinded stevia extract. Similar results were obtained by Narayanan *et al.* (2014) reported that stevia was shown to enhance survival of *L. casei* in basal medium and fermented milk during fermentation.

Results in Table (1) show the effect of interaction between treatments and storage period on the lactose content is significant (p <0.05). The lactose content of the prepared probiotic beverages decreased significantly during storage at 5±1°C up to 10 days and there were significant differences (P<0.05) between all treatments during storage period. The lactose content of freshly produced probiotic beverages varied from 4.5 to 4.89%. These values decreased slightly to 4.13 to 4.57% at the end of storage period. The decrease in lactose content during the storage periods may be due to the microbial activity. The results are also confirmed with those reported by Vahedi *et al.* (2008).

**Viability of probiotic bacteria**

Results presented in Table (2) shows the probiotic counts in functional whey-based and permeate-based beverages. Probiotic bacteria in permeate based beverage with *L. paracasei*, stevia extract 1% and papaya 10% recorded the highest viability of 11.38 log cfu/ ml after 10 days of storage. Whereas, the probiotic whey based beverage with *L. acidophilus* and papaya 10% when fresh had the lowest number of *L. acidophilus* 10.38 log cfu/ml. Cell counts of all treatments remained above 10<sup>8</sup> log cfu/ml, complying with the recommended minimum numbers of 10<sup>6</sup> log cfu/ml of live probiotic cells at the time of consumption, to be considered as probiotic food product (Ferdousi *et al.*, 2013).

As well, *L. acidophilus* and *L.paracasei* in both of whey and permeate with stevia and papaya registered higher number of viable probiotic count than the other treatments. This can be partially explained by Adesh *et al.*

(2012) reported that stevia is rich in beta carotene, ascorbic acid, calcium, iron, magnesium and phosphorous. On the other hand, Papaya is a rich source of nutrients such as pro-vitamin A, carotenoids, vitamin C, vitamin B, lycopene, minerals and dietary fiber.

Changes occurred in the total viable counts (TVC) of the prepared functional beverages during storage periods at 5± °C for 10 days are showed in Table (2). The TVC in all treatments decreased as storage progressed, the treatments without stevia extract had high TVC as compared to treatments with stevia extract either with whey or permeate beverage. This decrease in the TVC might be due to the presence of *L. acidophilus* or *L. paracasei* which have antagonistic effect on a lot of microorganisms. The *L. acidophilus* whey-based beverage with 1% stevia extract and 10% papaya had the highest TVC 6.76 log cfu/ ml when fresh, whereas, permeate with 10% papaya and *L. acidophilus* or *L. paracasei* registered significantly lower TVC (4 and 4.01 log cfu/ ml) than the other treatments at the end of cooled storage period. All probiotic beverages treatments were free from fungi when fresh and till the end of refrigerated storage period (10 days), that might be due to the antifungal effect of used probiotic starter (*L. acidophilus* and *L. paracasei*). All treatments were free from coliform either when fresh or during storage periods, that might be as a result to the good hygienic practice or because of the antibacterial activity of used probiotic starter. Beside of the positive effect on probiotic, stevia has medicinal properties as anti-bacterial, anti-fungal and anti-viral as reported by Panpatil and Polasa (2008).

In addition to enumeration of microbial counts, all fermented beverages were subjected to counts of yeasts and molds and coliforms to evaluate the hygienic or sanitary conditions of the process. Based on the results, yeast & mold and coliform were not detected in all treatments either when fresh or during storage. Also, this may be due to the antifungal effect of used probiotic starter (*L.acidophilus* and *L. paracasei*).

**Table 2. Changes in viability of probiotic bacteria and total viable counts (log cfu/ ml) of the prepared functional beverages during storage periods at 5±1°C for 10 days.**

Storage Period (days)	Treatments							
	Probiotic whey-papaya pulp beverage fermented by				Probiotic permeate papaya-pulp beverage fermented by			
	<i>L.acidophilus</i>	<i>L.paracasei</i>	<i>L.acidophilus</i> + <i>L.paracasei</i> + stevia extract	<i>L.paracasei</i> + stevia extract	<i>L.acidophilus</i>	<i>L.paracasei</i>	<i>L.acidophilus</i> + <i>L.paracasei</i> + stevia extract	<i>L.paracasei</i> + stevia extract
Probiotic bacteria counts (log <sub>10</sub> cfu/ ml)								
Fresh	10.38 <sup>r</sup>	10.37 <sup>r</sup>	10.88 <sup>jk</sup>	10.80 <sup>jk</sup>	10.56 <sup>p</sup>	10.47 <sup>q</sup>	10.86 <sup>k</sup>	10.93 <sup>i</sup>
3	10.61 <sup>o</sup>	10.56 <sup>p</sup>	10.91 <sup>ij</sup>	10.86 <sup>k</sup>	10.48 <sup>q</sup>	10.58 <sup>op</sup>	10.89 <sup>jk</sup>	10.97 <sup>h</sup>
6	10.94 <sup>hi</sup>	10.66 <sup>n</sup>	11.24 <sup>d</sup>	11.06 <sup>f</sup>	10.71 <sup>m</sup>	10.49 <sup>q</sup>	11.19 <sup>e</sup>	11.20 <sup>e</sup>
10	10.99 <sup>m</sup>	10.65 <sup>n</sup>	11.29 <sup>c</sup>	11.07 <sup>f</sup>	11.01 <sup>g</sup>	10.86 <sup>k</sup>	11.34 <sup>b</sup>	11.38 <sup>a</sup>
Total viable count (log <sub>10</sub> cfu/ ml)								
Fresh	6.41 <sup>c</sup>	6.40 <sup>c</sup>	6.76 <sup>a</sup>	6.51 <sup>bc</sup>	6.01 <sup>e</sup>	6.47 <sup>bc</sup>	6.57 <sup>b</sup>	6.51 <sup>bc</sup>
3	5.33 <sup>g</sup>	6.01 <sup>e</sup>	6.07 <sup>de</sup>	6.09 <sup>de</sup>	4.72 <sup>i</sup>	6.20 <sup>d</sup>	6.02 <sup>e</sup>	6.09 <sup>de</sup>
6	5.06 <sup>h</sup>	4.30 <sup>k</sup>	5.61 <sup>f</sup>	5.36 <sup>g</sup>	4.15 <sup>lm</sup>	4.53 <sup>j</sup>	4.61 <sup>ij</sup>	5.36 <sup>g</sup>
10	4.61 <sup>ij</sup>	4.04 <sup>mn</sup>	4.96 <sup>h</sup>	4.30 <sup>k</sup>	4.00 <sup>o</sup>	4.01 <sup>o</sup>	4.25 <sup>kl</sup>	4.09 <sup>mn</sup>

a, b,.... and s: Means having different superscripts within each column are significantly different (P<0.05).

**Sensory evaluation**

Table (3) shows the results of the sensory evaluation of the cheese whey-based beverages and milk permeate-based beverages. For appearance & colour, and consistency, there was significant difference among whey-based and permeate-based beverages. The mean for flavour score was higher for the whey-based beverage than for permeate-based. The permeate-based beverage obtained the highest scores for colour, consistency and total quality scores. This could have been due to the denaturation of whey protein during pasteurization, which effect on appearance and consistency, in whey-based beverages, in contrast to permeate ones. However, whey-based beverages recorded significantly (P<0.05) higher flavor score than permeate-based beverages. The probiotic whey-based beverage with *L.paracasei*, stevia extract 1% and papaya 10% had the highest flavor score of 58.6 when fresh, whereas probiotic

permeate-based beverage with *L. acidophilus* and papaya 10% had significantly lower flavor score 49.4 at the 10<sup>th</sup> day of refrigerated storage than other treatments. The *L. paracasei* permeate-based beverage with 1% stevia extract and 10% papaya, when fresh had the highest total scores of 94.6, whereas the lowest one was recorded by *L. acidophilus* whey-based beverage with papaya it recorded 84.8 at the 10<sup>th</sup> day of cooled storage. This illustrated that, both of whey or permeate beverages with stevia is more acceptable in flavor than that without stevia.

There were statistically significant differences in flavour between the samples without and with stevia extract addition as well as between the whey-based and permeate-based beverages. The maximum value (the most pleasant treatment) was permeate-based beverages and the lowest score was whey-based beverages without stevia extract.

**Table 3. Sensory evaluation of the prepared functional beverages during storage periods at 5±1°C for 10 days.**

Storage period (days)	Treatments							
	Probiotic whey-papaya pulp beverage fermented by				Probiotic permeate papaya-pulp beverage fermented by			
	<i>L.acidophilus</i>	<i>L.paracasei</i>	<i>L.acidophilus</i> + <i>L.paracasei</i> + stevia extract	<i>L.paracasei</i> + stevia extract	<i>L.acidophilus</i>	<i>L. paracasei</i>	<i>L.acidophilus</i> + <i>L.paracasei</i> + stevia extract	<i>L.paracasei</i> + stevia extract
Flavour (60)								
Fresh	54.00 <sup>gh</sup>	55.19 <sup>ef</sup>	57.80 <sup>ab</sup>	58.60 <sup>a</sup>	51.80 <sup>klmno</sup>	52.40 <sup>ijkl</sup>	53.59 <sup>ghi</sup>	54.59 <sup>fg</sup>
3	54.00 <sup>gh</sup>	54.40 <sup>gh</sup>	57.40 <sup>bc</sup>	57.80 <sup>ab</sup>	50.80 <sup>pp</sup>	51.80 <sup>klmno</sup>	52.59 <sup>ijkl</sup>	54.00 <sup>gh</sup>
6	52.80 <sup>ijk</sup>	53.40 <sup>hij</sup>	56.80 <sup>bed</sup>	57.20 <sup>bc</sup>	50.40 <sup>pq</sup>	51.00 <sup>nop</sup>	51.59 <sup>lmno</sup>	52.80 <sup>ijk</sup>
10	52.19 <sup>klm</sup>	52.59 <sup>ijkl</sup>	56.00 <sup>ed</sup>	56.60 <sup>de</sup>	49.40 <sup>r</sup>	49.80 <sup>qr</sup>	51.19 <sup>mnp</sup>	52.00 <sup>klmn</sup>
Consistency (30)								
Fresh	24.99 <sup>c</sup>	25.59 <sup>b</sup>	24.99 <sup>c</sup>	25.59 <sup>b</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>
3	25.59 <sup>b</sup>	25.19 <sup>bc</sup>	25.19 <sup>bc</sup>	25.59 <sup>b</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>
6	25.59 <sup>b</sup>	25.39 <sup>bc</sup>	25.39 <sup>bc</sup>	25.39 <sup>bc</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>
10	25.39 <sup>bc</sup>	25.19 <sup>bc</sup>	25.39 <sup>bc</sup>	25.19 <sup>b</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>	30.00 <sup>a</sup>
Appearance and colour (10)								
Fresh	7.60 <sup>b</sup>	7.60 <sup>b</sup>	7.40 <sup>b</sup>	7.60 <sup>b</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>
3	7.60 <sup>b</sup>	7.60 <sup>b</sup>	7.40 <sup>b</sup>	7.60 <sup>b</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>
6	7.40 <sup>b</sup>	7.60 <sup>b</sup>	7.40 <sup>b</sup>	7.40 <sup>b</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>
10	7.20 <sup>b</sup>	7.40 <sup>b</sup>	7.20 <sup>b</sup>	7.40 <sup>b</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>	10.00 <sup>a</sup>
Total quality Score (100)								
Fresh	86.60 <sup>no</sup>	88.41 <sup>m</sup>	90.40 <sup>ghij</sup>	91.80 <sup>def</sup>	91.80 <sup>def</sup>	92.40 <sup>cde</sup>	93.60 <sup>abc</sup>	94.60 <sup>a</sup>
3	87.20 <sup>mn</sup>	87.20 <sup>mn</sup>	89.80 <sup>hijk</sup>	90.80 <sup>fghi</sup>	90.80 <sup>fghi</sup>	91.80 <sup>def</sup>	92.60 <sup>cd</sup>	94.00 <sup>ab</sup>
6	85.80 <sup>op</sup>	86.20 <sup>no</sup>	89.60 <sup>ijkl</sup>	89.80 <sup>hijk</sup>	90.40 <sup>ghij</sup>	91.00 <sup>fgh</sup>	91.60 <sup>defg</sup>	92.80 <sup>bcd</sup>
10	84.80 <sup>p</sup>	85.40 <sup>op</sup>	88.60 <sup>kl</sup>	89.60 <sup>ijkl</sup>	89.40 <sup>ijk</sup>	89.80 <sup>hijk</sup>	91.20 <sup>efg</sup>	92.00 <sup>def</sup>

a, b,.... and s: Means having different superscripts within each column are significantly different (P<0.05).

**CONCLUSION**

This study demonstrates the possibility of developing highly nutritive beverages prepared from cheese whey or milk permeate. The addition of stevia

leaves extract was the most important factor influencing probiotic viability. The beverages prepared in this study are new products with a high organoleptic quality and a high number of probiotic bacteria.

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### مشروبات متخمرة حيويًا جديدة من مخلفات الألبان مع أوراق الاستيفيا ولب الباباظ ولاء محمد سعد بهنس\* ، خالد عبد الحكم عبد السلام ، و داد عزب متری و نعمت على حسن قسم الألبان، كلية الزراعة ، جامعة الفيوم ، مصر

تم تصنيع أربع مشروبات مختلفة من الشرش وأربع مشروبات أخرى من البرمبييت متخمرة بإضافة بكتريا حيوية بنسبة 1% من *L. acidophilus* أو *paracasei* كعينات مقارنة مع التذعيم بـ 10% لب الباباظ لجميع المشروبات وتحلية بنسبة 1% من مستخلص الاستيفيا لمعاملتين فقط من الشرش المتخمرة وأخرى من البرمبييت المتخمرة بواسطة السلالتين محل الدراسة. ثم تخزين المشروبات الناتجة على درجة حرارة  $5 \pm 1^\circ\text{C}$  لمدة 10 أيام وأجريت خلالها التحليلات الكيميائية والميكروبيولوجية والحسية أثناء التخزين على فترات (طازجة ، 3 و 6 و 10 أيام). وقد دلت النتائج بعد تحليلها الإحصائي أن جميع المشروبات إنخفضت قيم الأس الهيدروجيني لها، وزاد معدل الحموضة فيها. وكان لاختلاف المعاملات تأثير معنوي على حيوية البكتريا المدعمة حيويًا، العدد الكلي للميكروبات، حيث كان هناك تزايد تدريجي في قيم البكتريا المدعمة حيويًا كلما زادت فترة التخزين بينما كان هناك انخفاض تدريجي للعد الكلي للميكروبات. كما لوحظ تزايد في أعداد البكتريا المدعمة حيويًا في المعاملات المحتوية على مستخلص الاستيفيا مقارنة بالخالية منها. كما سجلت المشروبات المصنعة بواسطة *L. paracasei* قيم أعلى في حيوية بكتريا البادئ من المشروبات المصنعة بواسطة *L. acidophilus*. ولم يلاحظ وجود كل من الفطريات والخمائر وبكتريا القولون في كل المعاملات خلال فترة التخزين. وقد حصل مشروب البرمبييت الطازج المتخمرة بواسطة *L. paracasei* مع إضافة مستخلص أوراق الاستيفيا 1% على أعلى درجات التقييم الحسي، بينما حصل مشروب الشرش مع *L. acidophilus* على مجموع درجات أقل بكثير في اليوم العاشر من المعاملات الأخرى خلال فترة التخزين.