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PRODUCTION OF PROBIOTIC-FERMENTED RICE MILK BEVERAGE FORTIFIED WITH CACTUS PEAR AND PHYSALIS PULP

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ABSTRACT: Fermented rice milk beverage fortified with cactus pear (*Opuntia* spp.) and physalis (*Physalis peruviana*) fruit pulps were prepared and stored at 5°C for 12 days. The fruit pulps were added at the rate of 10% and 20% (*W/W*) of probiotic rice milk. Physico-chemical of fermented rice milk beverages including total solids, protein, fat, fiber and ash were determined along the storage period. Acidity, pH, viscosity, microbiological and organoleptic properties were evaluated at zero time, 4, 8, and 12 days of storage at 5°C. There were significant differences between control and fermented rice milk beverages enriched with fruit pulps in terms of pH, total solids, protein, fiber, ash, content and titratable acidity during storage. The results revealed that acidity increased all over the storage time. The lowest value for viscosity was observed in fermented rice milk beverage containing 20% physalis pulps. A significant increase in both DPPH inhibition (%) and TPC as compared to control sample, 20%physalis showed significantly higher values for both DPPH inhibition (%) and TPC. The count of *S. thermophilus*, *L. acidophilus,* and *Bifidobacterium* BB-12 were above 7 log cfu mL^{-1} at the end of storage period in all treatments. Sensory evaluation revealed significant differences between control and fermented rice milk beverage samples. The fermented rice milk beverage containing 20% cactus pear pulp had the highest overall acceptability score when compared to control and other treatment samples. The results of the current investigation demonstrated that the addition of cactus pear and physalis pulps to fermented rice milk beverage significantly improved the quality of resultant fermented rice milk beverage.

Key words: *Oryza sativa* L., *Opuntia* spp., *Physalis peruviana*, dairy products, vegetable milk.

INTRODUCTION

Vegetable milks are of great interest due to the problems related to lactose intolerance **(Fiocchi *et al.,* 2010)**. The nutritional value of rice-based foods could be enhanced through fermentation by amyololytic lactic acid bacteria (LAB) such as some *lactobacilli* and *bifidobacteria* strains, which might improve the digestibility of starch in children and increase the availability of lysine **(Espirito-Santo *et al.,* 2014)**. The fermented rice extracts with 12 g/100g of waxy maize starch flavoured with strawberry syrup and strawberry aroma exhibited high antioxidant capacity, nutritional value, total phenolic compounds (TPC) content, and marketing potential, especially for consumers with special needs, such as those allergic to soybean protein or lactose, as alternative foods ready for consumption **(Costa *et al.,* 2017)**.

Among the dairy products, fermented products and yoghurt have high digestibility and include starter cultures protecting the microflora, which have inhibitory activities against harmful microorganisms, show anticarcinogenic, antitumor, and anticholesterol traits as well as they might be consumed safely by those with lactose intolerance **(Granato *et al.,* 2010)**. The consumption of such foodstuffs is continuously increasing as the customers' consider them as

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functional foods rather than traditional foods (**Uzuner *et al.,* 2016**). On the other hand **Ismail *et al.* (2018)** prepared functional rice rayeb milk using cow and rice milk mixture (50:50, *V/V*) with or without adding 4% honey. Functional foods are diverse food groups, including traditional foodstuffs, such as yoghurt. Yoghurt could be enriched with probiotics and fruit pulps **(Gonzalez *et al.,* 2011)**.

Rice (*Oryza sativa* L.) is a food of great nutritional value, highly energetic (around 80% of starch), proteins (7-8%), mineral salts (phosphorus, iron, and calcium) and B-complex vitamins, especially integral type. The high-quality protein, contains eight amino acids essential to human, is dispersed in the endosperm and bran of the grain. In addition, rice has a low amounts of lipids **(Bassinello and Castro, 2004)**. Rice is the second most important cereal grain consumed worldwide, and nutritionally provide carbohydrates, proteins, fats, fibers, minerals, and vitamins. It has high digestibility, biological value and protein efficiency ratio owing to the presence of high concentration of lysine among all the cereals **(Padma *et al.,* 2018)**.

Rice has the highest protein digestibility among a group of staples which included wheat, yam, maize, millet, sorghum, rye, oats, potato and cassava **(Juliano, 1993).** Rice milk could be consumed in several products such as rice-coconut yoghurt, probiotic yoghurt, fermented dairy products; like raybe milk and cheese **(Nakamura *et al.,* 2016; Uzuner *et al.,* 2016; Ismail *et al.,* 2018)**.

Physalis (*Physalis peruviana* L). belongs to the family Solanaceae and Physalis genus, commonly known as cape gooseberry or goldenberry in several countries. It is an exotic fruit that contains various bioactive compounds of pharmacological and nutritional interest **(Bravo and Osorio, 2016; Moura *et al.*, 2016)**, and plays a great role in nutrition as an excellent source for dietetic food products. Recent research showed that Physalis peruviana is rich source of many beneficial bioactive compounds **(Olievira *et al.,* 2015)**. The Physalis fruit is consumed fresh or dried like a raisin. It is also used in glazes for meat products, sauces, and seafood **(NRC, 1989)**. There are different products processed from Physalis fruits such as juice, jams, snacks sweetened with sugar, and chocolate-covered candies **(Ramadan and Moersel, 2009)**.

Cactus pear (*Opuntia spp.*) is a tropical fruit, native to America, that grows in arid and semiarid areas**.** It contains highly important nutrients, such as amino compounds including taurine, betalains, vitamins, minerals as well as natural antioxidants, which could give benefit on a technological and nutritional functionality basis. Cactus pear is having excellent and wide scope for nutraceutical and food industry to prepared value-added products **Kamble *et al.* (2017)**. **Roghelia and Panchal (2016)** reported that cactus fruits contain high levels of natural colours, micronutrients, and could be utilized to develop value-added foodstuffs. Cactus pear pulp might be processed into several products such as sweeteners, marmalades, jellies, candies, alcoholic beverages, canned and frozen fruit, as well as flavoured yoghurt **(Abdel-Nabey, 2001; Moßhammer *et al.,* 2006; Matter *et al.,* 2016)**.

The present work was aimed to develop probiotic fermented dairy -like products using rice milk as milk substitute. Fortification of this product with fruits pulps with multi-functional properties and bioactive peptides, which could be used as a specific functional beverage, was also a goal of the study.

MATERIALS AND METHODS

Materials

Rice (*Oryza sativa* L.) Giza 171, Physalis (*Physalis peruviana* L.), and cactus pear (*Opuntia* spp.) were purchased from the local market (Zagazig city, Egypt). ABT-5 culture containing *Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium bifidum* were obtained from Christian Hansen Laboratory (Copenhagen, Denmark).

Methods

Preparation of rice milk and fruit pulps

Washed rice (one cup) and eight cups of water were placed on the heating mantle. The mixture was boiled for one hour to obtain soupy rice pudding. The content was later blended once until a smooth content was obtained. Ripe fresh fruits (physalis and cactus pear) were washed with tap water, peeled manually with a knife, and then subjected to pulp extraction. The resulted fruit homogenates were filled into jars, and heated at 95°C for 12 min.

Preparation of beverages

The resultant rice milk was mixed with 3% sucrose, and 4% skim milk powder (to increase Total Solids). Then it was heated (85 and 95°C for 30 min) in different containers, cooled to fermentation temperature (42-43°C), then inoculated with 5% of ABT-5 culture. Inoculated milk samples were incubated at 40°C until pH value reached 4.6. At this point, the beverages were put overnight in a refrigerator, then the samples of beverages were stirred and the fruit pulps were added to give six treatments as follows:

T_0:100% probiotic rice milk

T_1: 90% probiotic rice milk+10% physalis pulp

T_2: 80% probiotic rice milk+20% physalis pulp

T_3: 90% probiotic rice milk+10% cactus pear pulp

T_4: 80% probiotic rice milk+20% cactus pear pulp

T_5: 80% probiotic rice milk+10% cactus pear +10% physalis pulp

Beverages of all treatments were stored at 5°C for 12 days, while physicochemical, rheological, microbiological and sensory evaluation were carried out at 1, 4, 8 and 12 day intervals.

Chemical Analysis

The proximate chemical composition of the samples including total soluble solids (TSS), moisture, ash, lipids, crude protein, total acidity (as citric acid) and crud fiber contents, were determined according to **AOAC (2010)**. pH was determined using digital pH meter model (HANNA instrument) **(Ranganna, 1991)**. Total carbohydrates were calculated by difference according to **Guzman *et al.* (1999)**:

Total carbohydrates = Total Solids (TS) – (Fat – Protein + Ash)

Energy density was calculated according to **Insel *et al.* (2002)**:

E=4 [Protein (%)+Carbohydrates(%)+9 (Fat%)

Where

E: Energy density per 100 g of the product.

Rheological measurement

The viscosity of the beverage samples was analyzed using rotational viscometer (Type Lab. Model 5437) according to **Aryana (2003)**. Results were expressed as CP.

Determination of total phenolic content (TPC)

Ten ml of sample were stirred with 10 ml ethanol: water mixture (60:40, *V/V*) for 30 min at room temperature. The resulted mixture was centrifuged for 15 min at 5000 rpm (Hettig 1004 EBA 21 centrifuge, Germany). The collected supernatant was stored at 2°C and used to determine TPC and antioxidant activity of the samples.

TPC was determined using Folin-Ciocalteau method according to published procedure **(Roy *et al.,* 2014)**. Beverage (0.3 ml) was mixed with 0.2 N Folin-Ciocalteu reagents (1.5 ml). After 5 min, 1.2 ml of 0.7N Na_2CO_3 solution were added. The mixture was incubated at room temperature for 2 hr., then the absorbance was recorded at 765 nm, using UV-VIS spectrophotometer (Perkin Elmer Lambda 25 double beam) against a blank sample. Quantitative determinations were carried based on a five-concentrations (10, 25, 50, 75, 100 µg/ml) standard calibration curve of gallic acid dissolved in methanol (80%). The results were expressed as µg galic acid equivalents (GAE)/ ml beverage.

Determination of antioxidant capacity

Radical scavenging activity (RSA) of the beverage samples were determined by bleaching of the purple solution of DPPH· **(Hanato *et al.,* 1988; Gulcin *et al.,* 2004)**. Sample (100 µl) was added to 2.9 ml of 0.1 mM DPPH· dissolved in ethanol and incubated of 60 min at room temperature. The absorbance was recorded against control at 517 nm. Percentage of antioxidant potential of DPPH· was calculated as follows:

$$\text{DPPH. scavenging activity(\%)}=\left[\frac{\text{A0-A1}}{\text{A0}}\right]\times 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract. Samples were analyzed in triplicate.

Colour determination

Colour characteristics of samples in terms of redness (a), lightness (L), and yellowness (b) were measured **(Modi *et al.*, 2009)** using Hunter Lab colour analyzer (Hunter Lab Color Flex EZ, USA). The system was calibrated using white and black ceramic reference standard. The mean values of three readings of each colour value for each sample were recorded. The overall colour changes were expressed by the delta-E relation according to the following equation:

$$\Delta E = \sqrt{(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2}$$

Microbiological examinations

The total viable bacteria were determined in beverage when fresh and then after 4, 8, and 12 days of storage period using plate count agar (PCA) medium. The enumeration of *Streptococcus thermophilus*, was performed at 37ºC for 48 hr., under anaerobic condition using M17agar (Oxide Ltd) **Rybka and Kailasaphaty (1996).** *Lactobacillus acidophilus* counts were determined using MRS-sorbitol agar (Oxide Ltd). The plates were incubated in anaerobic condition at 37ºC for 72 hr. **Dave and Shah (1996).** B. bifidum, was enumerated according to **Dinakar and Mistry (1994).** Using modified MRS agar (A mixture of antibiotics, including 2 g of neomycin sulphate, 4 g of paromomycin sulphate, 0.3 g of nalidixic acid, and 60 g of lithium chloride (NPNL, Sigma Chemical Co.), was prepared in 1 L of distilled water, filter-sterilized, and stored at 4°C until use. The mixture of antibiotics (5 ml) was added to 100 ml of MRS agar medium. Cysteine-HCl was added at the rate of 0.05% to decrease the redox potential of the medium). The plates were incubated in anaerobic condition at 37ºC for 72 hr.

Sensory evaluation of the prepared products

The prepared beverages were evaluated for organoleptic characteristics by five panelists of staff members, Food Sci. Dept., Fac. Of Agric, Zagazig Univ., Egypt in the following parameters, flavour 45 points, body and texture 35 points, colour and appearance 10, acidity taste 10 and overall acceptability 100 according to **Farag *et al.* (2007)**.

Statistical Analysis

The experiment was carried out in triplicate and the data were transferred to the **SPSS (2007)** version 16 program. Data were statistically analyzed by using one way ANOVA.

RESULTS AND DISCUSSION

Chemical Composition and Physicochemical Properties of Rice Milk, Cactus Pear and Physalis

Table 1 shows the composition and physicochemical properties of rice milk, cactus pear pulp, and physalis pulp. The results indicated that physalis pulp had higher moisture, fat, protein, fiber and ash compared to cactus pulp. Rice milk had low-fat content (0.32± 0.08%), whereas cactus and physalis contained 0.36 and 0.48%, respectively. Increasing of TS amount of rice milk might by explain on the basis of high carbohydrate levels. The results of chemical composition of rice milk in this study were near to those obtained by **Abou-Dobara *et al.* (2016)** who mentioned that TS, fat, and ash of rice milk were 12.3, 0.30, and 0.39%, respectively, However **Belewu, (2013)** reported very high level of rice milk components. Moisture, fat, protein, fiber, ash and carbohydrate contents of cactus pear pulp were 85.4, 0.36, 0.80, 0.53, 0.45, and 12.9 g/100g, respectively. These results are in line with the results obtained by **Matter *et al.* (2016)** and **Kamble *et al.* (2017)**.

Chemical composition of physalis agree to those obtained by **El-Sheikha *et al.* (2008).** In addition, Table 1 reveal that the TPC of rice milk, cactus pear, and physalis pulp extracts were 28.0, 38.1 and 42.6 mg GAE/100g, respectively. DPPH· inhibition (%) of ethanol rice milk, cactus pear, and physalis pulp extracts were 19.6, 59.6 and 71.4%, respectively. These results are in agreement with those previously reported for rice milk **(Walter *et al,* 2013)**, for cactus pear **(Matter *et al.,* 2016)**, and for physalis pulp **(Curi *et al.*, 2018)**.

Table 1. Composition and physicochemical properties of rice milk, cactus pear and physical

Ingredient	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)	*Carbohydrate (%)	Colour			pH	DPPH· (%)	Total phenolic (mg GAE / 100 g)
							L*	a*	b*			
Rice milk	83.12±0.38	0.32±0.08	1.48±0.25	0.23±0.00	0.51±0.03	14.85±1.31	82.22	-0.82	7.88	6.73±0.00	19.65±0.18	28.00±1.38
Cactus pear	85.41±0.50	0.36±0.01	0.80±0.13	0.53±0.02	0.45±0.08	12.90±1.00	35.77	4.72	16.36	5.73±0.01	59.67±0.38	38.12±2.38
Physalis	87.45±1.02	0.48±0.11	1.37±0.32	2.82±0.28	0.66±0.13	7.88±0.39	39.10	0.65	14.31	3.78±0.00	71.44±0.97	42.66±2.78

*Carbohydrate was calculated by difference.

L* (0, black; 100, white), a* (−a*: greenness, +a*: redness), b* (−b*, blueness; +b*, yellowness

Chemical Composition of Probiotic Rice Milk Beverage

The chemical profile of rice milk beverage are given in Table 2. The total solids content of different rice milk beverage varied from 13.8+4 ± 0.99 to 17.21±2.11%. TS content of T_0 was recorded as the highest (17.21±2.11%). The addition of fruit pulps caused a decrease in TS, protein, and carbohydrate contents of rice milk beverage treatments. This may be due to low TS content, protein and carbohydrate contents of fruit pulps. **Teshome *et al.* (2017)** reported that addition of mango and papaya juices at different proportions to yoghurt decreased TS, protein, and ash contents compared to control yoghurt. The fat content of rice milk beverage increased with increases in fruit percentage. This may due to higher fat content in fruit pulps compared to rice milk.

Table 2 shows that there was a slight increase in TS content of the beverage during storage. These results may due to the decrease in moisture content which caused an increase in dry matter. The differences between TS contents of the samples were not significant (p>0.05) during the storage. Fat content was not significantly decreased (p>0.05). The reduction in fat content could be a result of growth and activity of starter microorganisms. These results agree with **Uzuner *et al.* (2016)**.

The results indicated no significant decrease in protein content during the storage (p>0.05). Reduction in protein content during storage could be a result of proteolysis, which causes increase in peptides and amino acids.

pH, Titratable Acidity and Viscosity Values of Probiotic Rice Milk Beverage

Table 3 illustrates the impact of the addition of fruit pulps on pH, titratable acidity and viscosity. pH values of the beverage were decreased during storage. It means that in all treatments at zero time the highest pH values were recorded with limit (4.60- 5.53). The pH looks an opposite trend of acidity. These results agree with previous researchers **(Hassan *et al.,* 2012; Kumari *et al.,* 2015)**. The decrease of pH value for all treatments during storage could be due to accumulation of lactic and organic acids which produced by LAB and metabolic activity of probiotic bacteria (**Gamage *et al.,* 2016**).

Titratable acidity of all samples increased during storage period. A similar trend for fermented dairy products was recorded by several authors **(Hassan *et al.,* 2012; Ranadheera *et al.*, 2012; Kumari *et al.,* 2015)**. The increase in titratable acidity during storage time might be attributed to the activity of *Bifidobacterium* and *S. thermophiles* cultures. Acidity of beverages was increased with increasing of physalis pulp percentages. A similar observation was confirmed by **Roy *et al.* (2015)**. The result of present study indicates that T_2 (80% probiotic rice milk+20% physalis pulp) had the highest titratable acidity, but it had the lowest pH.

Fortification of rice milk beverages with fruits pulp significantly decreased viscosity compared with control. These results might be due to increasing TS content in rice milk. According to **Kumari *et al.* (2015)**, increasing TS content in yoghurt might result in high consistency and viscosity values. As given in

Table 2. Chemical composition of probiotic rice milk beverage during storage

Storage period (day)	Treatment	TS (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Energy density
0	T_0	17.21^{a}	0.32^{b}	4.30^{a}	0.23^{d}	0.50^{a}	12.09^{a}	68.44
	T_1	15.44^{b}	0.36^{a}	4.11^{a}	1.06^{a}	0.51^{a}	10.46^{b}	61.52
	T_2	13.84^{d}	0.37^{a}	3.84^{b}	1.28^{a}	0.55^{a}	9.08^{d}	55.01
	T_3	15.82^{b}	0.33^{b}	3.61^{b}	0.24^{c}	0.31^{c}	11.57^{a}	63.69
	T_4	14.52^{c}	0.34^{b}	3.50^{c}	0.27^{c}	0.26^{c}	10.43^{b}	58.69
	T_5	14.42^{c}	0.36^{a}	3.58^{c}	0.77^{b}	0.45^{b}	10.03^{c}	57.68
12	T_0	17.51^{a}	0.30^{c}	4.25^{a}	0.22^{d}	0.49^{a}	12.47^{a}	69.58
	T_1	15.74^{b}	0.34^{a}	3.89^{a}	1.03^{a}	0.50^{a}	11.01^{b}	62.66
	T_2	13.94^{d}	0.36^{a}	3.62^{b}	1.25^{a}	0.53^{a}	9.43^{d}	55.44
	T_3	16.12^{b}	0.32^{b}	3.58^{b}	0.24^{c}	0.26^{c}	11.96^{a}	65.04
	T_4	14.82^{c}	0.32^{b}	3.50^{c}	0.26^{c}	0.14^{d}	10.86^{b}	60.32
	T_5	14.72^{c}	0.33^{b}	3.47^{c}	0.77^{b}	0.41^{b}	10.51^{c}	58.89

Means followed by different letters in the same column are significantly different ($P < 0.05$)

Table 3. pH, acidity and viscosity values of probiotic rice milk beverage

Item	Storage day	T_0	T_1	T_2	T_3	T_4	T_5
pH	0	4.72^{Ca}	4.63^{Da}	4.60^{Da}	5.16^{ABa}	5.53^{Aa}	4.83^{Ba}
	4	4.60^{Ca}	4.46^{Da}	4.41^{Da}	4.98^{ABa}	5.40^{Aa}	4.77^{Ba}
	8	4.45^{Cb}	4.31^{Db}	4.29^{Db}	4.70^{Bb}	5.09^{Ab}	4.66^{Bb}
	12	4.31^{Cd}	4.19^{Dc}	4.00^{Ec}	4.61^{Bc}	4.93^{Ab}	4.44^{Cc}
Acidity (%)	0	0.60^{Cb}	0.67^{Bb}	0.71^{Ad}	0.57^{Dc}	0.53^{Dc}	0.60^{Cb}
	4	0.62^{Cb}	0.70^{Bb}	0.81^{Ac}	0.60^{Db}	0.58^{Db}	0.66^{Bb}
	8	0.770^{Ba}	0.81^{ABa}	0.91^{Ab}	0.68^{Ca}	0.66^{Ca}	0.72^{Ba}
	12	0.82^{Ba}	0.90^{ABa}	1.20^{Aa}	0.74^{Ca}	0.70^{Ca}	0.84^{Ba}
Viscosity CP	0	660^{Ad}	590^{Cc}	540^{Dc}	620^{Bc}	590^{Cc}	580^{BCc}
	4	670^{Ac}	600^{Cb}	550^{Db}	630^{Bb}	600^{Cb}	590^{BCb}
	8	690^{Ab}	625^{Ca}	570^{Ea}	640^{Ba}	610Da	600Da
	12	700^{Aa}	630^{Ca}	590^{Ea}	650^{Ba}	620^{Ca}	610Da

Means followed by (a-d) different letters in the same column are significantly different ($P < 0.05$)
Means followed by (A-E) different letters in the same rows are significantly different ($P < 0.05$)

Table 3, there was an increasing trend in viscosity of beverages during storage. **Akin and Konar (1999)** explained that the increase in the water holding capacity (WHC) of protein and tightening of gel structure during storage period.

DPPH Inhibition (%) and TPC of Rice Milk Beverage during Storage

Results presented in Table 4 indicate that fortification of rice milk beverage with cactus pear or physalis resulted in significant increase in both DPPH inhibition (%) and TPC as compared to the control sample. This increase was proportional with the level of their addition. Moreover, it could be noticed that fortification of probiotic rice milk beverage with 20% physalis showed significantly high values for both DPPH inhibition (%) and TPC than other treatments. This could be explained on the basis that physalis contained higher level of TPC than cactus pear. On the other hand, it could be observed that both inhibition (%) and TPC values decreased during storage period for all treatments

Colour Parameters

The changes in colour values (L*, b* and a*) of control and rice beverage enriched with cactus pear or physalis stored at 5°C during 12 days is presented in Figs. 1 and 2. In general, for all samples, colour values (L*, b* and a*) were influenced by type of fruits and percentage of addition. On the other side, storage time affected L*, b* and a* values. The L* value of rice beverages decreased at the end of storage period while a* and b* values of rice beverages fortified with cactus pear decreased, while rice beverage fortified with physalis and the mix was increased.

Microbiological Examination of Probiotic Beverage

The results in Table 5 indicate the effect of storage on *TBC, Streptococcus thermophiles*, *Lactobacillus acidophilus* and *Bifidobacterium. bifidum* counts in all treatments. Rice milk probiotic beverages had the highest count fro each of *Streptococcus thermophiles, Lactobacillus acidophilus*, *Bifidobacterium.bifidum* and TBC compared with sample fortified with fruit pulps but there were no significant differences in *Bifidobacterium bifidum* counts in rice milk probiotic beverage fortified with fruit compared to control rice milk beverage. Counts of *S. thermophilus*, *L. acidophilu,* and *Bifidobacterium* BB-12 remained above 8 log cfu ml^{-1}. The addition of fruit pulps improved the viability of *Streptococcus thermophiles, Lactobacillus acidophilus* but slightly decreased the viability *of Bifidobacterium bifidum*.The reduction in the count of *L. acidophilus* during the cold storage may be due to the production of antimicrobials compounds such as bacteriocins, H_2O_2or organic acids. Also, it could be observed that the beverage contains 20% physalis pulp had the lowest microbial load compared with the same percentage addition of cactus pear, it may be due to physalis pulp contains more phenolic compound (42.66 mg EGA /100g) than cactus pear pulp (38.12 mg GAE /100g)

These results are in harmony with **Hassan *et al.* (2012)** who reported a decrease in *Streptococcus thermophiles,* and *Lactobacillus acidophilus* during storage of cereal-based probiotic beverages. Similar results were reported by **Bakirci and Kavaz (2008)** who mentioned that viable counts of *S. thermophilus, L. acidophilus,*and*Bifidobacterium* spp. slightly decreased during the refrigerated storage, but remained at sufficient levels (>6 logcfu/g) for up to 14 days.

Sensorial Properties of Probiotic Beverage

Results in Table 6 reveal that there were significant differences between control and different types of rice milk beverages for overall acceptability. Control rice milk beverage had the lowest values, while rice milk beverage fortified with cactus pear pulp had the highest values. The highest value was related to sample enriched with 20% cactus pear. The storage time had slightly impact on all attributes. According to these results, cactus pear enriched rice milk beverage had the most acceptability from consumers. These results are in agreement with those reported by **Matter *et al.* (2016)** who found that addition of cactus pear pulp to yoghurt enhanced the sensory evaluations of cactus pear-enriched yoghurt.

Table 4. DPPH· inhibition (%) and TPC of probiotic rice milk during storage

Item	Storage period (day)	T_0	T_1	T_2	T_3	T_4	T_5
DPPH· inhibition (%)	**0**	15.50^{Da}	20.22^{Ca}	23.56^{Aa}	19.12^{Ca}	21.41^{Aba}	22.12^{Ba}
	4	15.00^{Ea}	19.29^{Ca}	23.41^{Aa}	18.52^{Da}	21.00^{Aba}	21.55^{Ba}
	8	13.28^{Eb}	18.64^{Cb}	23.22^{Aa}	17.61^{Db}	20.54^{Bb}	20.61^{Bb}
	12	11.61^{Ec}	17.33^{Cb}	22.59^{Ab}	16.52^{Dc}	19.10^{Bb}	19.28^{Bb}
TPC (mgGAE/100 g)	**0**	22.5^{Da}	25.05^{Ca}	27.30^{Aa}	24.08^{Ca}	26.21^{Aba}	26.35^{Ba}
	4	20.21^{Db}	24.70^{Ca}	27.29^{Aa}	23.66^{Ca}	25.75^{Aba}	25.84^{Ba}
	8	19.19^{Ec}	23.90^{Cb}	26.55^{Aa}	22.51^{Db}	23.76^{Bb}	24.41^{Bb}
	12	17.12^{Ed}	21.81^{Cb}	25.00^{Ab}	21.23^{Dc}	23.24^{Bb}	23.74^{Bc}

Means followed by (a-d) different letters in the same column are significantly different (P < 0.05)

Means followed by (A-E) different letters in the same rows are significantly different (P < 0.05)

Table 5. Microbiological examination of probiotic rice milk beverage during storage

Micro organism	Storage period (day)	T_0	T_1	T_2	T_3	T_4	T_5
TBC	**0**	5.12×10^6	1.81×10^5	4.2×10^4	2.5×10^6	1.62×10^6	2.1×10^5
	4	6.24×10^6	2.11×10^5	4.33×10^4	2.61×10^6	1.71×10^6	2.30×10^5
	8	7.20×10^6	2.33×10^5	4.41×10^4	2.71×10^6	1.77×10^6	2.37×10^5
	12	7.81×10^6	2.52×10^5	4.55×10^4	3.00×10^6	1.81×10^6	2.87×10^5
S. thermophiles **log cfu ml^{-1}**	**0**	8.75	8.72	8.62	8.74	8.68	8.35
	4	8.20	8.36	8.14	8.40	8.22	7.38
	8	8.00	7.85	7.75	7.92	7.81	7.33
	12	7.60	7.42	7.24	7.60	7.70	7.20
L. acidophilus **log cfu ml^{-1}**	**0**	9.75	9.70	9.50	9.73	9.66	9.22
	4	9.25	9.00	8.81	9.18	8.90	8.22
	8	8.50	8.46	8.20	8.60	8.54	8.00
	12	8.28	8.24	8.16	8.40	8.32	7.86
Bifidobacterium BB-12 **log cfu ml^{-1}**	**0**	9.87	9.74	9.66	9.70	9.61	9.50
	4	9.38	9.30	9.14	9.20	9.02	8.70
	8	8.66	8.63	8.48	8.54	8.42	8.30
	12	8.43	8.32	8.31	8.38	8.20	7.96

Table 6. Sensory evaluation of probiotic rice milk beverage during storage

Item	Storage period (day)	T_0	T_1	T_2	T_3	T_4	T_5
Flavour (45)	0	38.40^{Ca}	38.20^{Ca}	37.40^{Da}	40.60^{Ba}	42.40^{Aa}	40.00^{Ba}
	4	37.56^{Cb}	37.02^{Ca}	36.11^{Da}	38.69^{Bb}	41.25^{Aa}	39.10^{Bb}
	8	34.33^{Cc}	34.00^{Cb}	32.45^{Db}	36.21^{Bc}	38.29^{Ab}	36.65^{Bc}
	12	29.12^{Dd}	31.24^{Cd}	30.22^{Ed}	33.2^{Bd}	35.91^{Ac}	34.65^{Ad}
Body and texture (35)	0	32.00^{Aa}	31.40^{Ba}	30.00^{Ca}	30.00^{Ca}	31.50^{Ba}	30.00^{Ca}
	4	31.12^{Aa}	30.01^{Bb}	29.58^{Ca}	29.75^{Ca}	30.66^{Ba}	29.32^{Ca}
	8	29.63^{Ab}	28.55^{Cc}	28.07^{Cb}	27.52^{Db}	29.31^{Bb}	28.02^{Cb}
	12	25.34^{Ee}	27.55^{Cd}	26.33^{Dc}	26.66^{Dc}	29.31^{Ab}	28.00^{Bb}
Colour and appearance (10)	0	7.40^{Ca}	8.00^{Aa}	7.80^{Ba}	8.00^{Aa}	7.50^{Ca}	7.90^{Aa}
	4	7.30^{Db}	7.81^{Ab}	7.66^{Bb}	7.81^{Ab}	7.39^{Cb}	7.46^{Bb}
	8	7.00^{Cc}	7.33^{Bc}	7.02^{Cc}	7.50^{Ac}	7.12^{Cc}	7.33^{Bc}
	12	6.78^{Ce}	7.12^{Bd}	7.00^{Dc}	7.50^{Ac}	7.01^{Dd}	7.12^{Bd}
Acidity taste (10)	0	8.60^{Aa}	6.60^{Da}	6.50^{Da}	8.20^{Ba}	\`8.30^{Ba}	8.00^{Ca}
	4	8.41^{Aa}	6.43^{Da}	6.36^{Db}	8.00^{Cb}	8.20^{Ba}	7.90^{Ca}
	8	8.04^{Aa}	6.33^{Db}	6.24^{Dc}	7.88^{Bc}	8.00^{Ab}	7.87^{Cb}
	12	7.14^{Cc}	6.32^{Eb}	6.24^{Ec}	7.60^{Bd}	8.00^{Ab}	7.80^{Db}
Overall acceptability (100)	0	79.39^{Ea}	81.27^{Ca}	80.71^{Da}	84.16^{Ba}	85.09^{Aa}	83.78^{Ba}
	4	76.22^{Db}	80.21^{Ca}	79.78^{Cb}	82.9^{Bb}	83.43^{Ab}	81.66^{Cb}
	8	76.00^{Db}	79.22^{Cb}	79.78^{Cb}	80.32^{Bc}	81.33^{Ac}	79.87^{Bc}
	12	70.22^{Ec}	78.69^{Cd}	75.00^{Dc}	78.77^{Bd}	80.00^{Ac}	78.59^{Bc}

Means followed by (a-d) different letters in the same column are significantly different ($P < 0.05$)
Means followed by (A-E) different letters in the same rows are significantly different ($P < 0.05$)

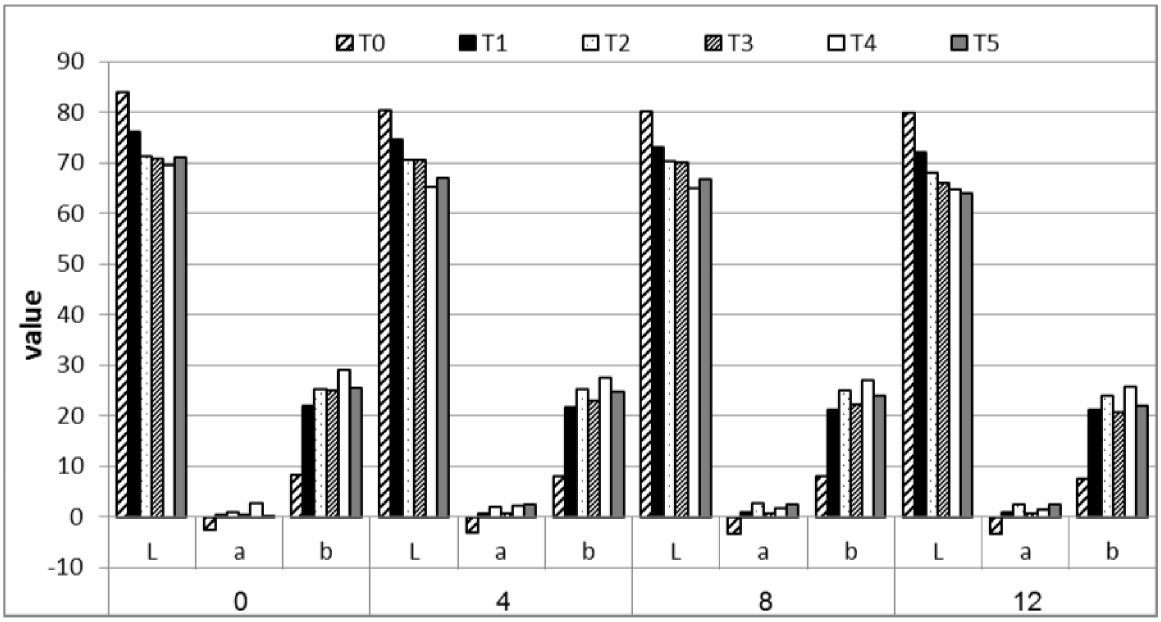

Fig. 1. Changes in the colour of probiotic rice milk beverage during storage period

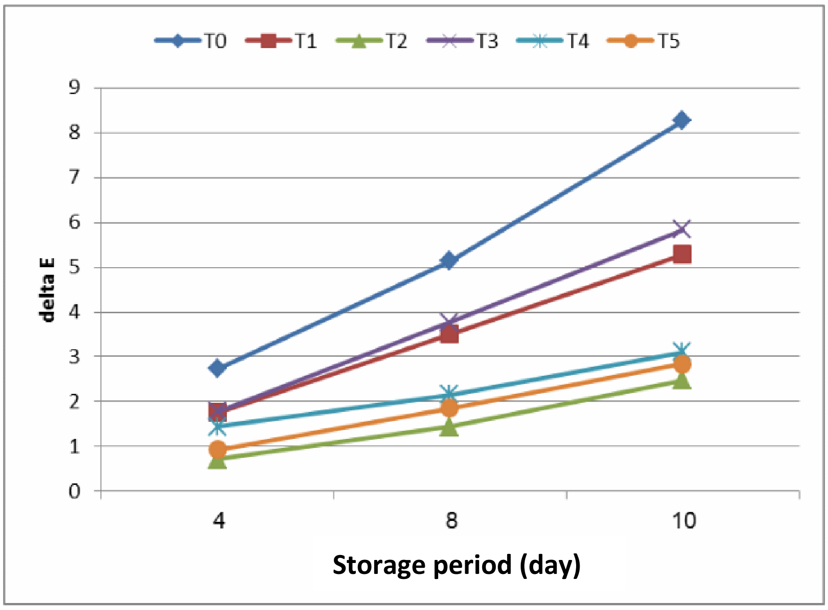

Fig. 2. Colour changes L, a and b (expressed as delta E) for probiotic rice milk beverage during storage

Conclusion

The study showed a new possibility to produce acceptable fermented probiotic rice milk beverages enriched with fruits pulp during storage for 12 days for probiotic strains. The prepared beverages combined interesting nutritional qualities and probiotic characteristics. The results could facilitate the development of new fermented, non-dairy, nutritionally well-balanced functional food products with unique physical and chemical properties.

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إنتاج مشروب لبن الأرز المتخمر الحيوى المدعم بلب التين الشوكى والحرنكش

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تم تحضير مشروب لبن الأرز المتخمر المدعم بلب فاكهة التين الشوكى والحرنكش بمعدل ١٠% و٢٠% (وزن/ وزن) من لبن الأرز الحيوى وتم تخزين العينات لمدة ١٢ يوماً على درجة حرارة ٥ْم، وتم تقدير الخواص الفيزوكيميائية للمشروب بما فى ذلك المواد الصلبة الكلية والبروتين والدهون والألياف والرماد على طول فترة التخزين كما تم تقدير الحموضة ودرجة ال pH واللزوجة والخصائص الميكروبيولوجية والحسية بعد التصنيع مباشرة وبعد مرور ٤، ٨ و١٢ يوما من التخزين، وقد أوضحت النتائج أن هناك فروقاً ذات دلالة احصائية بعينة المقارنة مقارنة بمشروب لبن الأرز المدعم بلب الفاكهة من حيث pH والحموضة حيث زادت الحموضة بمرور وقت التخزين، وقد لوحظ زيادة الحموضة فى المعاملات بمرور وقت التخزين، كما لوحظ أقل قيمة للزوجة لمشروب لبن الأرز المتخمر فى العينة المدعمة ب٢٠% من لب الحرنكش، كما تبين زيادة معنوية فى كلا محتوى الفينولات الكلية ومضادات الأكسدة وكانت أعلى قيم لكلايهما فى العينات المدعمة ٢٠% حرنكش، كانت أعداد S. *thermophilus* و *L. acidophilus,* و*Bifidobacterium* أعلى من $7 \log cfu\ ml^{-1}$ فى نهاية فترة التخزين فى جميع المعاملات، وأظهر التقييم الحسى أن هناك إختلافات معنوية بين عينات مشروب لبن الأرز المتخمر، وأن المشروب المحتوى على ٢٠% من لب التين الشوكى أعطى أعلى درجة من القبول العام بالمقارنة مع الكنترول وباقى المعاملات الأخرى، وأظهرت نتائج الدراسة الحالية أن إضافة لب التين الشوكى والحرنكش إلي مشروب لبن الأرز المتخمر قد حسنت بشكل كبير من جودة المشروب.

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