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Formulation, Characterization and Storage Stability of Prickly Pear (*Opuntia ficus*) Fruit and Milk Based Fermented Beverage

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ABSTRACT

Background: Fermented milk based beverages are popular all over the world and cherished by people of all ages. Health conscious consumers *nowadays* desire for beverages with enhanced functionality. Prickly pear fruit crop having resilience to grow in arid and semi-arid lands, possess excellent antioxidant and natural food colorant potential. Present investigation was an attempt to combine prickly pear fruit with fermented milk.

Methods: Effect of milk fat, pulp level and sugar replacement with low calorie sweetener was investigated on the sensory acceptability. Optimized prickly pear fruit and milk based beverages was evaluated for nutritional, colour, antioxidant properties and consumer acceptance. Stability of pear fruit and milk based beverage under refrigeration storage was analyzed.

Result: Consumer acceptability test (n=100) of selected formulation indicated wide acceptability with overall liking score of 8.17±0.05 on a 9-point hedonic scale. Antioxidant potential of optimized prickly pear fruit and milk based beverage was significantly higher (648±6.11 TEAC/g) than the control samples (410.33±5.49 TEAC/g). Packed beverage in glass bottles remained acceptable up to 9th day.

Key words: Cactus pear, Fermented beverage, Prickly pear.

INTRODUCTION

Fermented milk-based beverages have been favourite among all ages worldwide since ancient times. Examples of some traditionally fermented milk based beverages are *kefir*, *koumiss*, *lassi*, *rabadi etc*. Yoghurt drinks, acidophilus milk and cultured buttermilk have grown in popularity while probiotic beverages are on the present trend list. To improve the palatability and provide a wide range of product options, fermented milk is occasionally combined with water, flavour, fruit pulp, sugar, salt, herbs and spices. *Lassi* is a typical example of thirst quenching fermented milk beverage and is popular in the South Asian region of the world. Consumers nowadays desire healthy foods containing ingredients of natural origin. On the other hand, food manufacturers are focusing on value added diverse product range to satisfy market demand. Drinks with fruit-yoghurt bases are now available in the market and beverage makers have continued to favour the judicious pairing of nutritionally dense fruits with fermented milk. In this way nutritional quality of milk, lactic acid bacteria and fruits complement each other for nutritional and functional benefits.

Opuntia ficus commonly known 'prickly pear' fruit has ability to grow in dry climates and contains wide range of health beneficial components including minerals, vitamins, amino compounds and antioxidants (Kumar *et al*., 2018). Additionally its betalain pigments have potential to be used as natural food colorants. Current research was an attempt to combine prickly pear fruit pulp with fermented milk to develop prickly pear fruit and milk based beverage and investigate the acceptability and storage stability.

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MATERIALS AND METHODS

The research work was carried out at College of Dairy Science, Amreli, Gujarat during year 2020-2022. Milk, prickly pear fruit (fully ripened, having red and purple colour), cane sugar, low calorie sweetener Sugar Free™ Green (stevia based) for the preparation of prickly pear fruit and milk based fermented beverage (PPFMB) was procured from the local market. Highly concentrated lyophilized lactic acid starter (DELVO YOG FVV-211) of DSM Food Specialties was obtained from Azelis India Pvt. Limited and used as per manufacture recommendations.

Preparation of prickly pear fruit and milk based fermented beverage

Prickly pear fruit pulp was extracted adopting the method of Davara *et al*. (2017) with slight modifications indicated in Fig 1. Milk was fermented (Robinson and Tamime, 1975)

and PPFMB was prepared according to manner described in Fig 1. Based on review of literature and preliminary trials levels of milk fat and prickly pear fruit pulp were decided and selected as factors for the development of fermented beverage and presented in Table 1.

Physico-chemical analysis, colour values and antioxidant potential

Fat and solids-not-fat (SNF) content of milk were estimated by methods as described in IS: SP: 18, part XI (1981). Prickly-pear fruit pulp was analyzed for total solids, fat (ether extract), protein using micro-Kjeldahl method (AOAC, 2016), pH, acidity as % citric (AOAC, 1995) and ash content. Fat content of PPFMB was determined by modified Gerber method as described by Arora and Kalra (1986). The acidity of curd was estimated using titration method as suggested in IS: SP: 18, part XI (1981), whereas acidity content of the PPFMB was measured using pH 8.3 as end point for titration. Selected formulation of PPFMB was analysed for pH (OAKTON, Model PC 2700), total solids (IS: SP: 18, part XI, 1981), protein content by micro-Kjeldahl method (AOAC, 2005), total carbohydrate (difference method) and ash content (IS: SP: 18, part XI, 1981). The colour of PPFMB on Hunter L, a and b scale was measured using the Colorflex EZ instrument (Hunter Associates Laboratory, Virginia, USA). Sample extract for ABTS radical scavenging activity was obtained by centrifugation at 10,000 × g for 10 min and filtration of supernatant through 0.45 ìm syringe driven filter. The antioxidant potential of the extract was measured by method of Awika *et al*. (2003). The extraction titration method of Deeth and Fitzgerald (1976) was used by taking 5 g of sample for the estimation of free fatty acids (FFA).

Microbial quality

Aerobic plate count, yeast and mold (Y and M) count and coliform count were enumerated as per methods described in IS: SP: 18, part XI (1981).

Sensory evaluation, consumer acceptance test and storage study

The samples of PPFMB maintained at 5±1°C were presented to the panel of sensory judges consisting of 7 trained

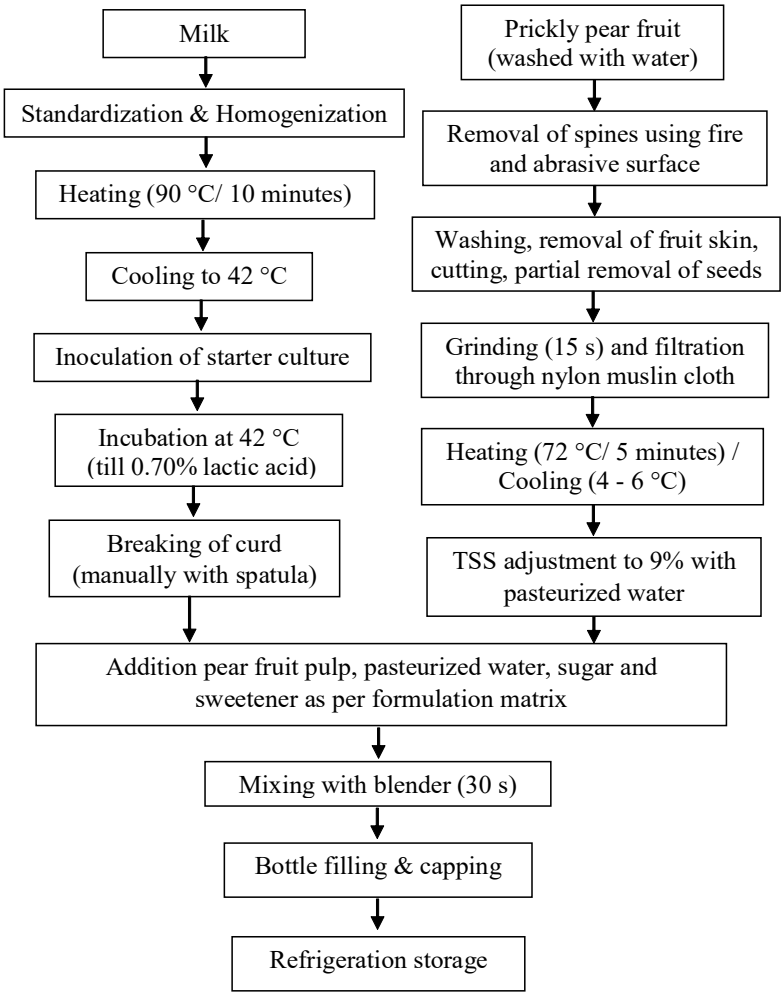


Fig 1: Flow chart for the preparation of prickly pear fruit and milk based fermented beverage.

panelists and analyzed for different sensory attributes like colour and appearance (C and A), consistency, sweetness, flavour and overall acceptability using a 9-point hedonic scale. For the consumer acceptance test, 100 potential consumers received packed samples of the PPFMB and evaluated on a 9-point hedonic scale. Stored PPFMB samples were analysed for changes in physico-chemical (acidity and FFA), colour values, sensory and microbial quality at an interval of 3 days up to 15 days.

RESULTS AND DISCUSSION

Physico-chemical analysis of prickly pear fruit pulp indicated total soluble solids (TSS), total solids (%), protein (%), fat (%), pH, acidity (% citric acid) and ash content (%) of 9.80±0.15°Bx, 10.13±0.21, 0.22±0.02, 0.91±0.03, 4.75±0.15, 0.13±0.01 and 0.71±0.04, respectively. Colour analysis of prickly pear fruit pulp indicated L value of 13.97±0.53, a value of 5.32±0.25 and b value of 0.69±0.07. Yield of pulp extract from fruit ranged 50.28 to 57.08% during experiments. A variation of±0.05% and±0.1% was tolerated in fat and SNF content of milk.

Since, 100% replacement of sugar with low calorie sweetener adversely affected the acceptability of PPFMB (prepared using toned milk and 10% pulp level) and scored 5.07±0.52 for overall acceptability, compared to 0% (7.43±0.35) and 50% (7.35±0.26) sugar replacement, hence 50% replacement was used in the study. Sensory attributes for the PPFMB as a result of different formulations are presented in Table 2. Main effect of pulp level and type of milk was analyzed individually using completely randomized design (CRD).

C and A and consistency scores among all sensory parameters were least affected by the pulp levels. Differences in C and A scores could be attributed to the changes in colour intensity of PPFMB due to pulp levels (Table 3). Our observations were supported by the findings of Desouky (2018) who reported increase in C and A scores initially when levels of cactus pear pulp was increased from 5% to 10%, which decreased by further increasing the pulp levels in goat milk based yoghurt drink. Decrease in sweetness scores were observed for PPFMB at higher pulp levels *i.e.* 15 and 20%, which could be attributed to decreased sugar ratio with increased pulp levels. Moreover,

Table 1: Experiment design matrix for prickly pear fruit and milk based fermented beverage.

Parameters	Levels
Milk fat and SNF (%)	<0.5 and 9.0 (SM: skim milk); 1.5 and 9.0 (DTM: double toned milk); 3.0 and 8.5 (TM: toned milk); 4.5 and 8.5 (STM: standardized milk)
Prickly pear fruit pulp level (%)	5; 10; 15 and 20 (on curd weight basis)
Partial sugar replacement with low calorie sweetener	0% replacement indicates 15 g cane sugar/100 g curd; 50% replacement indicates 7.5 g cane sugar and 0.75 g low calorie sweetener*/100 g curd)
Water	Equal to the quantity of calculated amount of sugar *i.e.* 15 g/100 g curd

*as per manufacturer description 100 g sugar free ™ green is equivalent to 1 kg sugar.

Table 2: Effect of prickly pear fruit pulp level and type of milk on the sensory attributes of fermented beverages.

Type of milk+ Pulp %	Colour and appearance	Consistency	Flavour	Sweetness	Overall acceptability
SM+5%	8.12 ± 0.06^{A}	6.69 ± 0.19^{B}	7.33 ± 0.13^{B}	7.62 ± 0.11^{B}	$7.45\pm0.15^{a,B}$
SM+10%	8.10 ± 0.08^{A}	6.52 ± 0.16^{B}	7.26 ± 0.12^{B}	7.48±0.11	$7.31\pm0.14^{ab,B}$
SM+15%	7.98±0.09	6.57 ± 0.18^{B}	7.14 ± 0.09^{B}	7.48±0.13	$7.07\pm0.11^{bc,B}$
SM+20%	7.81±0.13	6.40 ± 0.16^{C}	6.95 ± 0.10^{AB}	7.29±0.13	6.90 ± 0.10^{c}
DTM+5%	7.79 ± 0.15^{AB}	8.00 ± 0.07^{A}	$8.10\pm0.17^{a,A}$	$7.98\pm0.11^{a,AB}$	$8.17\pm0.14^{a,A}$
DTM+10%	8.05 ± 0.08^{A}	7.93 ± 0.06^{A}	$7.86\pm0.11^{ab,A}$	7.69 ± 0.09^{ab}	$7.76\pm0.10^{b,A}$
DTM+15%	7.88±0.12	7.90 ± 0.12^{A}	$7.62\pm0.15^{bc,A}$	7.45 ± 0.15^{bc}	$7.67\pm0.12^{b,A}$
DTM+20%	7.98±0.17	7.93 ± 0.16^{AB}	$7.26\pm0.15^{c,A}$	7.19 ± 0.16^{c}	7.21 ± 0.13^{c}
TM+5%	7.67 ± 0.14^{B}	7.93 ± 0.12^{A}	$8.43\pm0.12^{a,A}$	$8.14\pm0.13^{a,A}$	$8.50\pm0.11^{a,A}$
TM+10%	7.74 ± 0.13^{B}	7.62 ± 0.11^{A}	$7.36\pm0.16^{b,A}$	7.52 ± 0.14^{b}	$7.71\pm0.09^{b,A}$
TM+15%	7.86±0.12	7.60 ± 0.10^{B}	$7.07^{\pm}0.07^{b,B}$	7.45 ± 0.10^{b}	$7.50\pm0.09^{b,A}$
TM+20%	7.69±0.15	7.69 ± 0.16^{B}	$6.71\pm0.14^{c,B}$	7.17 ± 0.13^{b}	7.02 ± 0.07^{c}
STM+5%	7.62 ± 0.18^{B}	8.12 ± 0.09^{A}	$8.10^{\pm}0.18^{a,A}$	$7.88\pm0.15^{a,AB}$	$8.14\pm0.15^{a,A}$
STM+10%	7.88 ± 0.11^{AB}	7.90 ± 0.12^{A}	$7.88\pm0.13^{ab,A}$	7.83 ± 0.13^{a}	$8.00\pm0.10^{a,A}$
STM+15%	7.93±0.12	7.86 ± 0.15^{A}	$7.57\pm0.17^{b,A}$	7.48 ± 0.15^{ab}	$7.81\pm0.13^{a,A}$
STM+20%	7.95±0.19	8.24 ± 0.16^{A}	$6.81\pm0.19^{c,B}$	7.17 ± 0.18^{b}	7.19 ± 0.18^{b}

Values in each cell represent mean±SE, n=7; Means with different superscripts in small letters within a column vary significantly ($p<0.05$) and indicates the main effect of pulp level at same milk fat content. Means with different superscripts in capital letters at same pulp level in a column vary significantly ($p<0.05$) and represent the main effect of fat content.

a decreasing trend in the C&A score and increasing trend in consistency scores was observed with increasing milk fat percentage, which could be attribute to scattering effect of milk fat globules (Katsumata *et al*., 2020) and improved mouthfeel of beverage due to high fat content, respectively.

Increase in the prickly pear fruit pulp level has led to decrease in the flavour score of beverage (Table 2). Highest, flavour score of 8.43±0.12 and overall acceptability score (OAS) of 8.50±0.11 was observed for the PPFMB prepared using toned milk (TM) with 5% pulp level. OAS of PPFMB decreased significantly ($p<0.05$) with increasing pulp level, which could be due to perceivable astringency of prickly pear fruit at higher pulp levels due to characteristic shrinking effect on the tongue surface (Kgatla *et al*., 2010). Desouky (2018) reported improvement in flavour of goat milk and cactus pear pulp based yoghurt drinks with increasing pulp level from 5% to 15%, which was attributed to masking of goaty milk flavour with added pulp. Effect of milk fat content on flavour and OAS was significant ($p<0.05$) and indicated positive influence up to certain level on the acceptability of PPFMB due to better mouthfeel. Preference for milk fat content depends on consumer segment like 'low' or 'high' fat milk drinkers (McCarthy *et al*., 2017).

Total solids content of PPFMB gradually decreased when levels of prickly pear fruit pulp were increased (Table 3), however, significant ($p<0.05$) differences were observed in samples prepared using TM. Rise in the acidity of PPFMB was significant ($p<0.05$) with increasing pulp levels. A significant difference in fat percentage was recorded when prepared using TM and standardized milk (STM) due to increasing prickly pear pulp levels, which could be attributed to low fat content in the pulp. Hallim *et al*. (2019) reported increase in the TS and decrease in the fat content of stirred yoghurt prepared using cow milk fortified with cactus fruit juice, which could be attributed to the higher TS and low fat content of fruit extract. In our study TSS content of prickly pear fruit pulp was adjusted to 9°Brix and small amount water was added during preparation of beverage.

Colour values (Table 3) varied significantly ($p<0.05$) with prickly pear fruit and milk fat percentage. Hallim *et al*. (2019) reported decrease in L* values, increase in a* values and decrease in b* values of stirred yoghurt fortified with cactus pear fruit juices, which supported observation of our studies.

Based on our findings for flavour scores and OAS, toned milk with 5% prickly pear fruit pulp level was selected for consumer survey and storage studies. Physico-chemical properties, colour values, antioxidant potential and sensory attributes of selected formulation of PPFMB *i.e*. TM, 5% prickly pear fruit pulp level and 50% sugar replacement with low calorie sweetener in comparison with control samples are recorded in Table 4. However, only 12.3% reduction in calorie could be achieved with 50% sugar replacement with low calorie sweetener. Surprisingly, reduced calorie PPFMB samples were awarded with significantly higher acceptability scores than the control samples. Responses from 100 randomly chosen potential consumers for PPFMB indicated, 93 of them have rated the beverage ≥ 8 for 'overall liking' on a 9-point hedonic scale. Average score of 100 responses for overall liking was 8.17±0.05, respectively.

Trolox equivalent antioxidant capacity (TEAC) of PPFMB samples was significantly ($p<0.05$) higher than the control samples. Hallim *et al*. (2019) also reported increase in radical scavenging activity and total phenolic compounds when fortified the stirred yoghurt with 5% cactus pear pulp.

Table 3: Effect of prickly pear fruit pulp level and type of milk on the physico-chemical quality and colour of fermented beverages.

Type of milk+ Pulp %	T.S. (%)	pH	Acidity (% LA)	Fat (%)	L	a	b
SM+5%	16.13 ± 0.76^{C}	4.64 ± 0.01	0.58 ± 0.01^{c}	0.05 ± 0.00^{D}	60.57 ± 1.74^{a}	26.44 ± 2.19^{c}	-7.05 ± 0.62^{a}
SM+10%	15.83 ± 0.71^{C}	4.61 ± 0.02	0.61 ± 0.00^{ab}	0.05 ± 0.00^{D}	$51.85\pm0.20^{b,B}$	33.00 ± 1.24^{b}	-9.34 ± 0.12^{b}
SM+15%	15.89 ± 0.78^{B}	4.60 ± 0.03	0.61 ± 0.00^{b}	0.05 ± 0.00^{D}	$46.09\pm0.48^{c,B}$	36.74 ± 1.43^{ab}	-10.14 ± 0.16^{b}
SM+20%	15.38 ± 0.95^{B}	4.56 ± 0.01^{AB}	$0.63\pm0.01^{a,B}$	0.05 ± 0.00^{D}	$42.00\pm0.08^{d,B}$	41.03 ± 0.44^{a}	-10.21 ± 0.21^{b}
DTM+5%	16.80 ± 0.25^{BC}	4.62 ± 0.01	0.60 ± 0.01^{b}	1.27 ± 0.03^{C}	62.64 ± 2.16^{a}	24.28 ± 2.70^{b}	-7.11 ± 0.56^{a}
DTM+10%	16.57 ± 0.26^{BC}	4.60 ± 0.01	0.62 ± 0.01^{ab}	1.20 ± 0.06^{C}	$55.87\pm2.38^{ab,AB}$	29.50 ± 3.17^{ab}	-9.32 ± 0.24^{b}
DTM+15%	16.14 ± 0.33^{B}	4.60 ± 0.01	0.62 ± 0.01^{ab}	1.15 ± 0.03^{C}	$51.20\pm2.47^{bc,AB}$	34.68 ± 2.52^{a}	-9.84 ± 0.23^{b}
DTM+20%	15.82 ± 0.15^{B}	4.59 ± 0.01^{A}	$0.64\pm0.00^{a,AB}$	1.13 ± 0.03^{C}	$47.55\pm2.31^{c,AB}$	36.89 ± 2.59^{a}	-10.22 ± 0.20^{b}
TM+5%	$18.14\pm0.26^{a,AB}$	4.63 ± 0.01^{a}	0.59 ± 0.01^{c}	$2.47\pm0.03^{a,B}$	63.05 ± 1.57^{a}	25.39 ± 2.24^{b}	-7.44 ± 0.48^{a}
TM+10%	$17.45\pm0.23^{ab,B}$	4.61 ± 0.00^{a}	0.61 ± 0.01^{b}	$2.37\pm0.03^{ab,B}$	$56.37\pm2.37^{ab,AB}$	31.56 ± 2.45^{ab}	-9.62 ± 0.52^{b}
TM+15%	$17.24\pm0.03^{b,AB}$	4.59 ± 0.01^{b}	0.62 ± 0.00^{b}	$2.27\pm0.03^{bc,B}$	$51.18\pm2.14^{bc,AB}$	35.97 ± 1.99^{a}	-10.47 ± 0.54^{b}
TM+20%	$17.13\pm0.27^{b,AB}$	$4.55\pm0.01^{c,B}$	$0.64\pm0.01^{a,AB}$	$2.17\pm0.03^{c,B}$	$47.75\pm2.29^{c,AB}$	38.56 ± 1.87^{a}	-10.41 ± 0.43^{b}
STM+5%	19.19 ± 0.37^{A}	4.65 ± 0.02^{a}	0.58 ± 0.00^{c}	$3.67\pm0.03^{a,A}$	65.88 ± 0.91^{a}	24.41 ± 1.33^{c}	-6.76 ± 0.48^{a}
STM+10%	18.88 ± 0.34^{A}	4.60 ± 0.01^{ab}	0.61 ± 0.01^{b}	$3.57\pm0.03^{a,A}$	$59.13\pm1.50^{b,A}$	30.08 ± 1.69^{b}	-8.94 ± 0.59^{b}
STM+15%	18.52 ± 0.40^{A}	4.58 ± 0.02^{bc}	0.62 ± 0.01^{b}	$3.43\pm0.03^{b,A}$	$54.72\pm1.53^{bc,A}$	33.53 ± 1.65^{ab}	-9.72 ± 0.47^{b}
STM+20%	18.10 ± 0.39^{A}	$4.53\pm0.02^{c,B}$	$0.65\pm0.00^{a,A}$	$3.27\pm0.03^{b,A}$	$51.10\pm1.64^{c,A}$	36.31 ± 1.64^{a}	-10.21 ± 0.35^{a}

Values in each cell represent mean±SE, n=3; Means with different superscripts in small letters within a column vary significantly ($p<0.05$) and indicates the main effect of pulp level at same milk fat content. Means with different superscripts in capital letters at same pulp level in a column vary significantly ($p<0.05$) and represent the main effect of fat content.

Table 4: Physico-chemical, colour, antioxidant properties, energy value, sensory quality and microbiological quality of the optimized product.

Parameters	Optimized PPFMB	Optimized PPFMB (without sugar replacement)	Control* (without pulp)
Total solids (%)	18.57±0.26^{c}	21.62±0.16^{a}	19.27±0.12^{b}
pH	4.60±0.06^{ab}	4.62±0.15^{a}	4.56±0.02^{b}
Acidity (% lactic acid)	0.61±0.01^{b}	0.63±0.01^{b}	0.72±0.01^{a}
Fat (%)	2.47±0.03^{a}	2.43±0.03^{a}	2.27±0.03^{b}
Protein (%)	2.52±0.13	2.47±0.11	2.44 ±0.08
Ash (%)	0.57±0.04	0.56±0.06	0.56±0.06
Total carbohydrate (%)	13.02±0.08^{c}	16.15±0.09^{a}	14.00±0.13^{b}
L value	56.36±0.57^{c}	60.59±0.91^{b}	83.90±0.88^{a}
a value	33.88±0.28^{a}	25.57±0.89^{b}	-0.75±0.07^{c}
b value	-10.3±0.295^{c}	-5.47±0.24^{b}	13.53±0.56^{a}
ABTS radical scavenging activity (µM Trolox equivalent/g)	648±6.11^{a}	615.67±6.98^{b}	410.33±5.49^{c}
Energy (Cal/100 ml)$^{£}$	82.83±1.11^{b}	94.45±0.71^{a}	84.52±0.50^{b}
Sensory attributes			
Colour and appearance	8.29±0.07^{a}	8.05±0.03^{b}	8.36±0.05^{a}
Consistency	8.24±0.09^{a}	7.90±0.06^{b}	7.67±0.07^{c}
Flavour	8.21±0.14	7.93±0.06	8.21±0.14
Sweetness	8.07±0.11	7.90±0.06	8.02±0.06
Overall acceptability	8.14±0.06^{a}	7.76±0.06^{b}	8.07±0.04^{a}

Values in each cell represent mean±SE, n=3, except n=7 for sensory attributes; means with different superscript in a row vary significantly ($p<0.05$).

*5% water in place of pulp, 15 g cane sugar/100 g curd, rose flavor 0.01%.

$^{£}$Calculated using factors suggested by Merrill and Watt (1973).

Observations were also supported by findings of Desouky (2018) for antioxidant capacity of cactus pear pulp fortified goat milk based yoghurt bio-drink.

Storage stability of PPFMB

Initial acidity of 0.61±0.00 (% lactic acid) increased to 0.79±0.00 on 9th day of storage, which led to decrease in the overall acceptability score (OAS) from 8.33±0.05 to 7.14±0.05. Continuing the storage on 12th day, acidity of PPFMB increased to 0.83±0.01 which rendered the beverage less acceptable with OAS of 6.64±0.05, which further decreased to 5.71±0.06 on 15th day of storage. FFA of PPFMB samples increase from 10.82±0.14 to 16.31±0.62 (µeq/g) at the end of storage study. Increase in the acidity and FFA content of PPFMB could be attributed the microbial activity. Slight changes in L value: 58.73±0.25 to 59.41±0.15; a value: 31.26±0.01 to 28.54±0.24 and b value -6.59±0.11 to -5.33±0.10 was recorded during storage causing significant reduction in the C and A score of PPFMB. Changes in the colour values can be attributed to the oxidation of phytochemicals and degradation of pigments (Stintzing *et al*., 2005) of cactus pear fruit during storage. Aerobic plate count (Log_{10}CFU/g) of PPFMB significantly ($p<0.05$) increased from 4.86±0.06 to 6.69±0.07 on 9th day of storage, then after a decreasing trend was observed. Coliform and Y and M count in the PPFMB were not detected throughout storage study.

CONCLUSION

Prickly pear fruit pulp when combined with fermented milk to develop fermented beverage indicated excellent acceptability among consumers. Prickly pear fruit and milk based fermented beverage exhibited better antioxidant potential than control samples.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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