Indian J Dairy Sci 77(4): 338-348
https://doi.org/10.33785/IJDS.2024.v77i04.006

RESEARCH ARTICLE

Preparation of synbiotic fermented milk and evaluation of short-chain fatty acids production during storage study

Mitali Makwana[1], JB Prajapati[2], Sreeja V[3] and Subrota Hati[3] (✉)

Received: 23 May 2023 / Accepted: 04 December 2023 / Published online: 23 August 2024

Abstract: The fermented milk product was formulated using *Lactobacillus cultures* i.e., V3 and M5 with addition of corn-starch. 8% sugar level was selected for the preparation of fermented milks based on sensory attributes and overall acceptability. Optimized fermented milk (T) along with control where corn starch was replaced with skim milk powder (C) were analyzed for their proximate composition, short chain fatty acids (SCFAs) production as well as changes in physico-chemical and sensory properties during storage at refrigerated temperature (5±2ºC) up to 28 days. The pH of fermented milks C and T had significant (P<0.05) differences during 28 days of refrigerated storage and decreased from 4.35 to 3.85 and 3.93, respectively. Acidity (% LA) of fermented milks C and T significantly (P<0.05) increased to 1.22% from 0.78 and 0.76%, respectively during storage. Average viable counts (log CFU/ml) of fermented milk T was 9.57 log CFU/ml, which decreased to 7.36 log CFU/ml after 28 days, which had no significant differences with C. All three short chain fatty acids (SCFA), acetic acid, propionic acid and butyric acid content (µg/mL) of fermented milks (C and T) significantly (P<0.05) increased during storage from 1.27 to 5.37 and from 4.17 to 11.00µg/mL, 2.00 to 2.40 and from 2.52 to 3.64 µg/mL, 2.25 and 4.72 from 2.11 and 3.18 µg/mL, respectively during storage. Treated fermented milks were acceptable up to 21 days under refrigeration temperature (5±2 ºC) and obtained higher sensory scores for all the attributes which decreased with elevated storage period.

[1]Dairy Microbiology Department, Anand Agricultural University, Anand, Gujarat, India

[2]VKCoE, IRMA, Anand-388110, Gujarat, India

[3]Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat

Subrota Hati (✉)
Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India
Email: subrota_dt@yahoo.com

Keywords: Fermented milk, Lactobacilli, Short-chain Fatty Acids, Synbiotics

Introduction

The term "functional food" was first used in 1984. A study on the relationship between Japan nutrition, sensory satisfaction, robustness and modulation of physiological systems to explain food products fortified with specific ingredients that have beneficial physiological effects. The purposes of functional foods are diverse: they improve the general conditions of the body such as prebiotics and probiotics, reduce the risk of certain diseases (for example, cholesterol-lowering products) and Can be used for Treatment of some diseases (Bigliardi and Galati 2013).The largest segment of the functional food market comprises of foods containing probiotics, prebiotics and synbiotics. According to FAO/WHO (2001), probiotics are defined as a "live microorganisms that when administered in adequate amounts confer a health benefit on the host". A number of potential benefits arising from the use of probiotics have been demonstrated, including increased resistance to infectious diseases (Kumar et al. 2012), alleviation of lactose intolerance, prevention of gut diseases, diarrhoea, vaginal and urogenital infections; reduced allergy and respiratory infections; reduced serum cholesterol concentration; increased resistance to toxins produced during cancer chemotherapy and decreased risk of colon cancer (Stavropoulou and Bezirtzoglou 2020). The diet and the intestinal milieu interact in a complex way with the bacterial population in the gut. Given the nutritive composition and natural buffering capacity, fermented milk is considered best career of probiotics. Positive health effects of fermented foods and especially of those with probiotic microorganisms are reported in many recent publications (Hasan et al. 2014).

In 2008, prebiotics were defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Hill et al. 2014), a definition that is currently being further revised by ISAPP. Prebiotics are fermented by the gastrointestinal microbiota and contribute to healthy modulation of the gut (Di Bartolomeo et al. 2013). Synbiotics are a relatively new area that involve a combination of probiotic and prebiotic in one product; the prebiotic is intended to improve the survival/growth/performance of the probiotic or other beneficial bacteria in the colon, which in turn has beneficial health effects on the host (Pranckute et al. 2014). Functional foods are foods or dietary components that can provide a health benefit beyond basic nutrition. The use of the functional oligosaccharides in diet is a part of the management of dyslipidemia (Jaiswal and Sharma 2016). Optimal intake of the functional oligosaccharides reduces the risk of obesity, blood pressure and many other cardiovascular diseases. Fibers that lead to high amounts of SCFAs, lower the pH in the colon, which in turn affects the composition of the colonic microbiota and thereby the SCFA production.

SCFAs, especially acetic, propionic and butyric acid, are vital to maintain the normal function of intestine and human body. Acetate is absorbed and transported to the liver and peripheral tissues, less metabolized in the colon and acts as substrate for cholesterol synthesis and lipogenesis (Chakraborti 2015). Propionate is a primary precursor for gluconeogenesis and it reduces the synthesis of hepatic cholesterol (Cheng and Lai 2000). Butyrate is considered one of the most important colon metabolites, as it serves as the majorly preferred energy source for the colonocytes, has anti-inflammatory properties and regulates gene expression, differentiation, cellular proliferation and apoptosis in host cells, resulting in reduced risk of colon cancer (Hamer et al. 2008; Canani et al. 2011). Very few research works are carried out in combining the functional aspects of lactic acid bacterial fermentation of milk and generation of short chain fatty acids during its storage. Hence, the current study was planned mainly focusing on these three interesting SCFAs in fermented synbiotic product during storage study.

Materials and Methods

Preparation and Analysis of Fermented Milk

The study was planned with the use of best starter cultures *Lactobacillus helveticus* (V3) (MTCC 5463) (Prajapati et al. 2011) and *Lactobacillus fermentum* (M5) with the pre-chosen level of prebiotic (3% Corn-starch) (Makwana 2019) Standard procedure for probiotic lassi making with minor modification of the procedure adopted by Patidar and Prajapati (1998) was used. Complete flow diagram for preparation of fermented milk with added prebiotic is given in Fig. 1.

Optimization of sugar addition rate for preparation of fermented milk

The blend of MTCC 5463 (V3) *L. helveticus* and *L. fermentum* (M5) (1:1 by weight) was admixed with different rate of sugar (8, 9 and 10%) to suit to consumer taste. The samples of fermented milk admixed with different level of sugar were subjected to sensory evaluation by trained panel using affective testing method.

Sensory evaluation

Seven panellists were engaged for their liking and preference evaluation, which were performed on different occasions. Liking scales were defined using 9-point hedonic scale for sourness, flavour, appearance, consistency and overall acceptability as described by Stone and Sidel (2004). Coded samples of fermented blend were given to the panellists. The results of sensory evaluations were reported as mean value with standard error of mean.

Evaluation of stability of fermented milk during refrigerated storage

The influences of storage period (4°C for 28 days) on biochemical and sensory properties for fermented milk were analysed as follows. The samples of both blends were removed prior to incubation for analysis. Samples of both the types of fermented product were removed from the refrigerated storage (4 °C) at 0, 7, 14, 21 and 28 day. The samples were analysed for changes in pH and acidity, viability of Lactobacilli cultures, SCFAs production and sensory attributes (i.e. flavour, colour and appearance, body and texture and overall acceptability score).

Fresh toned milk (TM) having minimum 3.0 % fat and 8.5 % SNF was selected for preparing fermented milk.

Estimation of SCFAs

Analysis of SCFAs in the curd was performed by slight modification of the method of Roopashri and Varadaraj (2014). Aliquot of 2 ml of homogenized fermented milk sample was taken in a 15 ml centrifuge tube, and 7 ml of 10 mM NaOH containing 0.1 mM crotonic acid was added. The mixture was kept in a shaker incubator for 6 h at 30 °C. One ml chloroform was then mixed with the sample to remove fat soluble substances. This mixture was centrifuged at 10,000 rpm for 15 min at 4 °C in a refrigerated centrifuge. The supernatant was isolated and filtered through 0.22 µm membrane filter. Using Hamilton syringe, 20 microlitre samples were injected in a Shimadzu HPLC model LC-20 (Shimadzu, Japan) to perform the HPLC analysis. An analytical column [C 18] was used for this purpose. 0.1 % (v/v) phosphoric acid isocratic mixture was used as the solvent for elution at 30 °C and at a flow rate of 0.6 ml/min, using an UV detector at 210 nm.

Determination of pH

The pH values of the blend during fermentation were monitored using a pH meter (Mettler Toledo, USA).

Determination of titratable acidity

The titratable acidity was estimated by the procedure described in (AOAC,1995).

Estimation of Protein Content

Protein content was determined by following macro-Kjeldahl method as described by AOAC (2006).

Estimation of Fat Content

The fat content was estimated by Mojonnier method (IS: SP-18, Part-X1,1981).

Estimation of Ash Content

Ash content of the product samples were determined by procedure describedin BIS handbook (BIS, 1981).

Microbiological Evaluation

***Lactobacilli* Enumeration**

MRS agar, 61.15 g was suspended in 1000 ml distilled water containing 1mL of tween 80 and boiled to dissolve the medium completely. It was then filled in flasks and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min. The pH ofthe medium was adjusted to 5.4 at the time of plating by using sterile glacial acetic acid. 1 mL of diluted sample (suitable dilution) was transferred in each of the Petridishes in triplicate, 10-15 mL of the melted agar (at 45ºC) was then poured and the contents were mixed well by

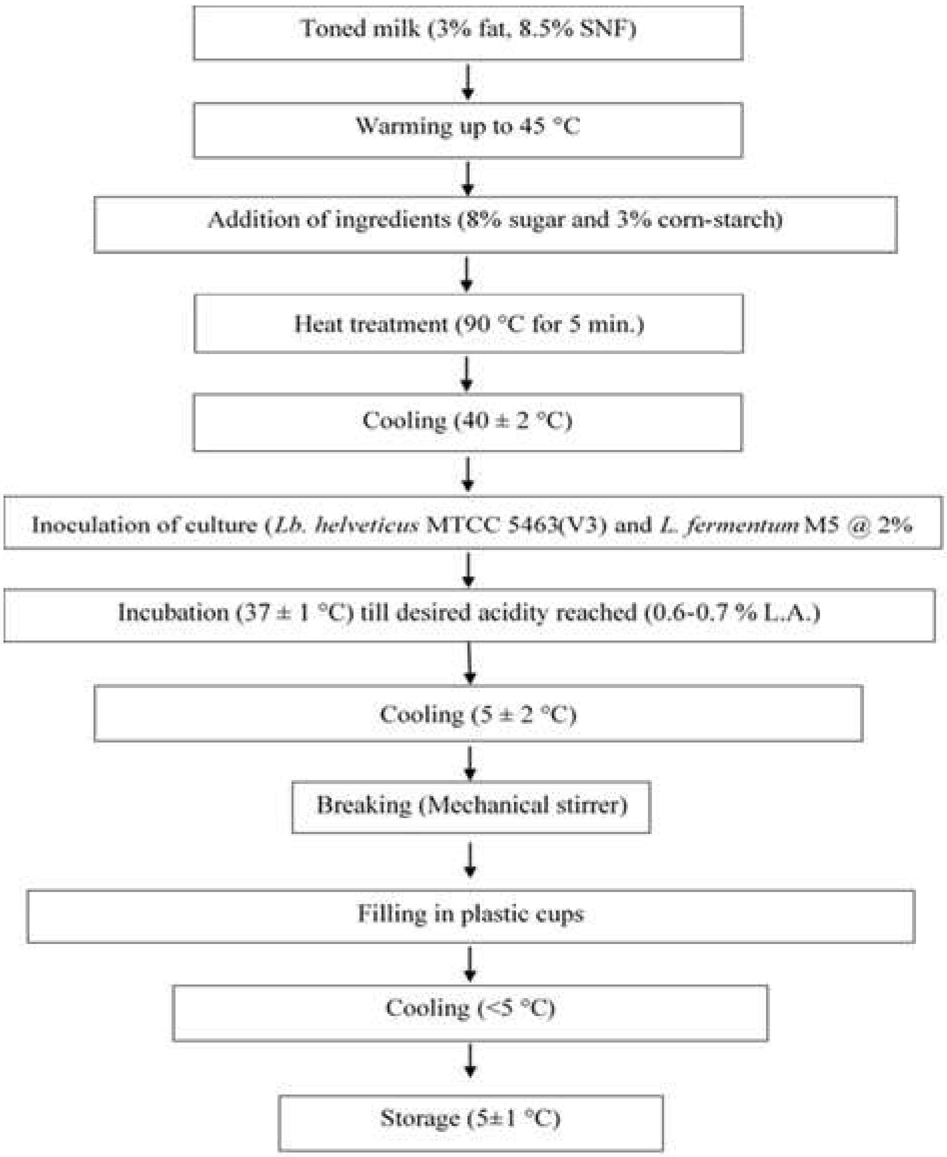

Fig.1 Flow chart for preparation of fermented milk

rotating in a horizontal position. The contents were allowed to solidify and a second of same agar (4-5 mL) was overlaid in each plate. The plates were then inverted and incubated at 37°C for 24-48 h and colonies formed on the medium were counted after incubation period.

Results and Discussion

Standardization of the sugar addition level for preparation of fermented milks

The selected LAB strains (V3 and M5 @ 2%) with 3% level of addition of corn-starch in toned milk medium were considered for optimization of rate of sugar addition. Varying amount of sugar (8%, 9% and 10%) were added. Sugar addition rate was optimized based on sensory evaluation of the blends. Different sensory properties viz. flavour, body, colour and appearance, sourness and overall acceptability were evaluated using affective testing method based on 9-point hedonic scale by seven expert panel members. The mean scores for sensory attributes of fermented milks as affected by addition of different level of sugar addition to fermented blend are presented in Table 1 and 2.

Mean scores for flavour, consistency, sourness, colour and appearance as well as overall acceptability as perceived by panellists were not significantly affected by the level of sugar. Also, there was no significant difference observed within the different level of sugars as the highest mean score observed for flavour was for 8% sugar (8.68) followed by 9% (8.59) and 10% (8.51). It was significantly ($P<0.05$) higher as compared to control (6.94).

Assessment of biochemical characteristic and sensory attributes of fermented milk during refrigerated storage

The fermented milk (T), prepared with 8% sugar addition was studied for storage stability and other fermentation properties at refrigeration temperature along with the control product (C) for comparison replacing corn-starch (CS) with supplementation of SMP and fermented using the same method as given in Fig. 1. The both prepared fermented milks are shown in Fig. 2. They were analysed for changes in pH, viability of probiotic organisms and sensory attributes during refrigerated storage (5±1°C) for 28 days. Different sensory properties viz. flavour, body, appearance, sourness and overall acceptability were evaluated using affective testing method based on 9-pointhedonic scale by seven expert panel members.

Changes in overall acceptability of fermented milks

The mean score of overall acceptability for fermented milks is presented in Table 3. On average, the score for the control product (6.35) was significantly lower ($P<0.05$) than the developed product (7.88). However, the acceptability score significantly declined

Table 1: Sensory evaluation of fermented milks added with different levels of sugar

Treatments with sugar	Sensory Attributes				
	Flavor	Body	Sourness	Color & Appearance	Overall Acceptability
Control (0%)	6.94±0.5	8.55±0.1	8.65±0.1	8.73±0.08	7.41±0.4
8%	8.68±0.04	8.71±0.1	8.73±0.04	8.78±0.1	8.77±0.1
9%	8.59±0.04	8.68±0.09	8.7±0.1	8.75±0.1	8.64±0.1
10%	8.51±0.04	8.72±0.07	8.68±0.1	8.73±0.08	8.68±0.02
Sem	0.13	0.06	0.05	0.04	0.11
$CD_{(0.05)}$	0.40	NS	NS	NS	0.36
CV%	3.16	1.42	1.13	1.10	2.80

Values represent mean (7 panellists × 3 replication) ± SD

Table 2: Optimization of sugar addition rate for preparation of fermented milk

Treatments	Physicochemical Attributes		
	pH	Acidity (%L.A.)	*Lactobacillus* Count (log CFU/ml)
Control (0%)	4.15±0.01	1.09±0.01	9.48±0.08
8%	4.16±0.03	1.09±0.03	9.50±0.1
9%	4.06±0.05	1.06±0.01	9.49±0.09
10%	4.15±0.06	1.09±0.02	9.49±0.1
Sem	0.05	0.01	0.05
$CD_{(0.05)}$	NS	NS	NS
CV%	1.38	2.50	1.13

Values represent mean (7 panellists × 3 replication) ± SD during the storage period ($P<0.05$). Mean score 8.73 at 0 day declined significantly ($P<0.05$) to 7.01 at 14 d for product T and subsequently declined during the storage. Similarly, for the product C, mean score 7.88 at 0 day declined significantly to 6.95 at day 14 and continually declined further during the storage study.

Changes in pH of fermented milks

The changes in pH of the fermented milk during the storage are presented in Table 4. During storage at 5° C, both types of fermented milks showed decrease ($P<0.05$) in pH on 7th day followed by decrease ($P<0.05$) up to 28 days of storage (Table 4). The average decline in pH was significantly higher on 7th day of storage. Subsequently further decline up to 21 days of storage was observed for both the milks. However, in the last week of storage study, mean pH value further declined significantly ($P<0.05$) to 3.89.

Changes in viability of LAB

The changes in the total viable counts of *Lb. helveticus* MTCC 5463 + *Lb. fermentum* M5 are presented in Table 5. For both the fermented milks, the total viable counts of the products C and T significantly declined ($P<0.05$) on7th day, then after remained unaffected up to 21 days. However, significant loss in viability of starter bacteria ($P<0.05$) were also observed on 28thday for both the milks.

Fig. 2 Prepared fermented milk (T) and Control (C)

Changes in titratable acidity of fermented milks

The changes of the acidity in both the types of fermented milks is presented in Table 6. In both the products, the acidity significantly increased ($P<0.05$) on7thday and 14th day, then after remained unaffected up to 28 days. However, the effect of treatments and the interaction effects of starter cultures during storage resulted in insignificant changes ($P>0.05$) in the acidity of two different milks.

Overall, mean score value of all sensory properties remained above 6 (liking slightly) throughout the storage period up to21 days indicating the product is acceptable. However, consumer to consumer variation, temperature of storage, packaging material and conditions, post production handling and other microbial

Table 3: Changes in overall acceptability of fermented milks during storage

Treatments	Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	7.88±0.28	7.59±0.37	6.95±0.08	5.87±0.27	5.51±0.06	6.35
T	8.73±0.21	8.2±0.24	7.01±0.2	6.58±0.15	5.98±0.11	7.88
Mean (P)	7.84	7.30	7.04	6.97	6.43	
Source		SEm	$CD_{(0.05)}$		CV%	
Period (P)		0.09	0.26			
Treatments (T)		0.05	0.17		3.14	
P*T		0.12	NS			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

Table 4: Changes in pH of fermented milks during storage

Treatments	Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	4.35±0.1	4.08±0.0 3	4.07±0.05	4.10±0.008	3.85±0.03	4.11
T	4.35±0.05	4.14±0.06	4.06±0.05	4.04±0.05	3.93±0.02	4.09
Mean (P)	4.35	4.11	4.07	4.07	3.89	
Source		SEm	$CD_{(0.05)}$		CV%	
Period (P)		0.02	0.07			
Treatments (T)		0.01	NS		1.33	
P*T		0.03	NS			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

contaminations etc. are the factors which needs to be taken in considerations during deciding the shelf life of the product.

Effect of LABs and Corn-starch on SCFAs production in Fermented Milks During Storage Study at 5±1 °C

In the present objective, estimation of SCFAs production for both the types of milks throughout the storage study was carried out at 0, 7, 14, 21 and 28 days and data are presented in Table 7, 8 and 9 for acetic acid, propionic acid and butyric acid production, respectively.

Changes in acetic acid (µg/ml) production in fermented milks during storage

The mean score of acetic acid production by fermented milks is presented in Table 7. Significant difference (P<0.05) was observed among both the products with regards to acetic acid production. In general, the acetic acid production score for control product (2.93) was significantly lower (P<0.05) than the product T (8.14). The acetic acid production scores also did significantly increase during the storage periods (P<0.05). For developed product T, acetic acid production was significantly increased from 4.17 on 0 day to 11.0 µg/ml on 28th day.

Changes in propionic acid (µg/ml) production in fermented milks during storage

The mean score of propionic acid production for fermented milks is presented in Table 8. Significant difference (P<0.05) was observed among both the fermented milks with regards to propionic acid production. In general, the acetic acid production score for control(C) product (2.26) was significantly lower (P<0.05) than T (2.94). The propionic acid production scores also did significantly increase during the storage period (P<0.05). Product T had non-significant increase in propionic acid production up to 14th day, thereafter, it significantly increased up to (3.64) on 28th day, while for control, the propionic acid production remains non-significant throughout the storage study.

Changes in butyric acid production in fermented milks during storage

The mean score of butyric acid production for fermented milks is presented in Table 9. Significant difference (P<0.05) was observed among the products with regards to butyric acid production. In general, the butyric acid production for the product C (2.21) was significantly lower (P<0.05) than the developed product T (3.77). The butyric acid production scores also did significantly increase during the storage period (P<0.05). The butyric acid production

Table 5: Changes in log count of starter bacteria in fermented milks during storage

Treatments	Log count (CFU/ml) Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	9.53±0.02	8.69±0.2	8.53±0.5	8.29±0.1	7.26±0.1	8.46
T	9.57±0.01	8.76±0.09	8.66±0.1	8.36±0.2	7.36±0.2	8.54
Mean (P)	9.55	8.72	8.60	8.32	7.31	
Source		SEm	$CD_{(0.05)}$	CV%		
Period (P)		0.09	0.27			
Treatments (T)		0.05	NS	2.62		
P*T		0.13	NS			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

Table 6: Changes in titratable acidity of fermented milks during storage

Treatments	Acidity (% L.A.) Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	0.78±0.03	1.07±0.07	1.15±0.09	1.21±0.01	1.22±0.03	1.09
T	0.76±0.03	1.09±0.06	1.19±0.03	1.21±0.01	1.22±0.05	1.10
Mean (P)	0.77	1.08	1.17	1.21	1.22	
Source		Sem	$CD_{(0.05)}$		CV%	
Period (P)		0.02	0.06			
Treatments (T)		0.01	NS		5.0	
P*T		0.03	NS			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

in product T on 0 day was 3.18µg/ml, which increased significantly to 4.72 on 28th day, while for control it remained non-significant throughout the storage period.

Standardization of the sugar addition level for preparation of fermented milks

The addition of sugar did not have any significant effect on body, acidity, color and appearance. The score for color and appearance ranged from 8.73 to 8.78 and for body, it ranged from 8.55 to 8.72. The overall acceptability of sugar added products were better than the control product. Hence, the product with the lowest possible sugar addition (8%), was prepared and analysed for storage study attributes. The physico-chemical attributes in terms of pH and acidity did not change significantly with different rates of addition of sugar. Average pH of the product was 4.1 and the acidity was 1.08% L.A. The *Lactobacilli* count in the product did not change with the addition of the sugar, and

Table 7: Changes in acetic acid (µg/ml) production in fermented milks during storage

Treatments	Acetic acid production (µg/ml) Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	1.27±0.1	1.75±0.2	2.79±0.2	3.44±0.1	5.37±0.1	2.93
T	4.17±0.1	6.44±0.2	8.7±0.2	10.4±0.2	11±0.1	8.14
Mean (P)	2.53	4.10	5.74	6.92	8.05	
Source		SEm	$CD_{(0.05)}$		CV%	
Period (P)		0.09	0.27			
Treatments (T)		0.05	0.17		4.1	
P*T		0.13	0.38			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

Table 8: Changes in propionic acid (µg/ml) production in fermented milks during storage

Treatments	Propionic acid (µg/ml) production Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	2±0.1	2.21±0.1	2.21±0.1	2.27±0.08	2.4±0.1	2.26
T	2.52±0.1	2.57±0.1	2.71±0.07	3.25±0.06	3.64±0.07	2.94
Mean (P)	2.26	2.39	2.49	2.84	3.02	
Source		SEm	$CD_{(0.05)}$		CV%	
Period (P)		0.04	0.13			
Treatments (T)		0.02	0.08		4.2	
P*T		0.05	0.18			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

Table 9: Changes in butyric acid (µg/ml) production in fermented milks during storage

Treatments	Butyric acid (µg/ml) production Storage period in days (P)					Mean (T)
	0th day	7th day	14th day	21st day	28th day	
C	2.11±0.1	2.23±0.08	2.23±0.08	2.25±0.05	2.25±0.1	2.21
T	3.18±0.2	3.46±0.2	3.55±0.06	3.94±0.1	4.72±0.1	3.77
Mean (P)	2.64	2.84	2.89	3.10	3.48	
Source		SEm	$CD_{(0.05)}$		CV%	
Period (P)		0.06	0.17			
Treatments (T)		0.03	0.11		4.95	
P*T		0.08	0.25			

C= Control product Probiotic milk prepared by replacing CS with SMP
T = Developed product Probiotic milk prepared using CS

the average count was observed to be 9.49 log CFU/ml. A carbonated probiotic fermented milk with *Lactobacillus helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5460 was prepared and standardized with respect to carbon dioxide pressure, sugar and salt concentrations based on sensory, physico-chemical and microbial parameters and the product was acceptable on 28th day of storage under refrigeration condition (Shah and Prajapati, 2014).

Assessment of biochemical characteristic and sensory attributes of fermented milk during refrigerated storage

Changes in flavour of fermented milks

The mean score for flavour remained above 7 (moderate liking preference) at day 14, thereafter significantly (P<0.05) decreased during storage. Usually, the lipolytic and proteolytic activity of LAB is generally responsible for causing off-flavours in fermented milk (Tamime and Robinson, 2007). Apart from these activities, in the present study, the flavour profile of corn-starch also contributes to overall flavour perception. El-Aidie et al. (2017) studied the sensory evaluation of cereal-based fermented milk product and reported that all products have good sensory acceptability.

Changes in body of fermented milks

The mean score for body (7.18) remained above 7 (moderate liking preference) on 14th day for the product T, thereafter significantly (P<0.05) decreased during storage. Viscosity and consistency of food system is usually affected by sugar and other macromolecules through their interaction with the solution or solvent (Zapsalis and Beck 1985). The level of addition of sugar and corn starch may have influenced the higher viscosity of our fermented milk supplemented with CS. Mohammad (2004) found that seven different stabilizers (pectin, guargum, carboxymethylcellulose (CMC), carrageenan, sodium alginate, corn starch and gelatin), storage time and total solids combined with the thickeners significantly affected the properties such as syneresis and texture of the yogurt samples. Textures (hand-felt and oral) of yogurts were significantly affected by starch concentrations and/or storage time. Williams et al. (2003) reported that the addition of starch has beneficial effects on yogurt properties.

Changes in sourness of fermented milks

The mean score for mouthfeel (7.48) remained above 7 (moderate liking preference) on 14th day for product T, thereafter significantly (P<0.05) decreased during storage. It was observed by Supavititpatana et al. (2010) that the acidity of the yoghurt samples increased during the 28days storage. This increase in acidity during storage agreed with the findings of for corn milk yoghurt. The increase in titratable acidity may be as a result of anaerobic microbial activities resulting in the formation of lactic and other organic acids. It has been reported that increase in titratable acidity and the extent of increase was influenced by the type of lactic acid bacteria present (Sanni et al. 1999; Bucker et al. 2008). However, as titratable acidity increased, the pH decreased as a function of fermentation time (Walia et al. 2013).

Changes in appearance of fermented milks

The mean score for appearance (7.62) remained above 7 (moderate liking preference) up to 14th day, thereafter significantly (P<0.05) decreased up to 28 days of storage. Similar to our observations, Yasni & Maulidya (2014) also found the color of corn yoghurt light yellow because of the yellowish pigment in sweet corn.

Changes in overall acceptability of fermented milks

The mean score for overall acceptability (7.01) remained above 7 (moderate liking preference) on 14th day. Alim et al. (2016) evaluated the sensory qualities based on different attributes for sweetened cow milk yoghurt (without homogenization) with incorporation of corn starch at different levels. They found that all the sensory scores of different samples were significantly different (p<0.01) and the samples showed various degrees of acceptability. However, they concluded that the highest overall acceptability was found at 2.0% of incorporation level for corn-starch.

Changes in pH of fermented milks

The effect of treatments and the combined effects of starter bacteria and storage periods, however, did not observe to influence (P>0.05) the changes in pH of fermented milks. Kpodo et al. (2014) reported that skimmed milk powder addition increases the concentration of lactose that could be degraded by the starter culture enzymes to produce lactic acid. This acid in turn increases the acidity and automatically reduces the pH. Onweluzo and Nwakalor (2009) reported similar relationship between pH and acidity. Ifediba & Ozoh (2018) reported that their product breadfruit-corn yoghurt contained corn protein (zein) that may lower the pH, the acidification effect of skim milk could bear more influence owing to faster utilization of lactose by the fermenting microorganisms, whereas, the commercial milk yoghurt used in their study equally contained corn starch as declared on the label, an indication that the zein protein may have equally contributed to the acidity. Supavititpatana et al. (2010) reported similar trend in pH reduction during 35 days of corn milk yoghurt and commercial milk yoghurt storage, with greater reduction found in corn milk yoghurt.

Changes in viability of LAB

Our results of loss of viability in prolonged refrigeration storage are in agreement with the previous reports (Dave and Shah1998~ Akalin et al. 2007~ Roseburg et al. 2010). However, the interaction effects of starter cultures during storage resulted in non-significant changes (P>0.05) in the total viable counts of starter bacteria. Imamoglu et al. (2017), reported that the amount of starter culture bacteria increased as storage time increased, which is typical for cultured yogurt (added with two types of starch: corn and kudzu) products and was to be expected. **Changes in titratable acidity of fermented milks:** Walia et al. (2013) reported that fermentation time had a positive effect on acidity but a negative effect on pH in mango soy fortified yoghurt. In our study, acidity of the fermented milks samples increased during the 28 days storage. This increase in acidity during storage agreed with the findings of Supavititpatana et al. (2010) and Adeiye et al. (2013) for corn milk yoghurt and groundnut milk respectively. Similarly, Yasni & Maulidya (2014) found the observation of the total titrated acid of corn milk yoghurt for 4 weeks at refrigerator temperature showed that the lactic acid percentage of corn milk yoghurt continually increased. In the initial week (0 week) the lactic acid percentage was 0.99% and after 4-week observation it increased to 1.22% ± 0.01.

Effect of LABs and Corn-starch on SCFAs Production in Fermented Milks During Storage Study at 5±1 °C

Changes in acetic acid (µg/ml) production in fermented milks during storage

There was significant increase in acetic acid production during every week of storage in both the products. Further, the acetic acid production in our CS added product was almost two to three times higher than the control product prepared without CS. The higher amount of acetic acid production seen in this product was difficult to explain as there are many factors which are playing role. One of the factors is use of toned milk instead of RSM used in phase one and two of the study. Similarly, Adhikari et al. (2001), showed that the diversity of organic acids in fermented dairy foods is due to the activity of the added probiotic bacteria such as *Bifidobacterium* (*B. longum* B6 and ATCC15708). By using HPLC method, they showed that the concentrations of acetic and lactic acid increased during storage.

Changes in propionic acid (µg/ml) production in fermented milks during storage

The mean score for propionic acid production (2.84) after 21 days of storage significantly (P<0.05) increased to (3.02) on 28th day of storage. Similarly, an increase in the amount of propionic acid during storage in plain yogurt was reported by Fernandez-Garcia and McGregor (1994). Adhikari et al. (2001) reported that no particular pattern was observed for the stirred yogurts. However, the propionic acid content increased by about 10 mg/100 g yogurt for the non-encapsulated *B. longum* B6 treatment and by about 20 mg/100 g yogurt for the encapsulated *B. longum* ATCC 15708 treatment.

Changes in butyric acid production in fermented milks during storage: The mean score for butyric acid production (2.89) significantly differ after 14 days of storage (P<0.05) which significantly increased to (3.1) and (3.48) on day 21 and 28, respectively. Adhikari et al. (2001) reported that in fermented dairy foods the treatments containing non-encapsulated *bifidobacteria* or encapsulated *B. longum* B6 showed comparable values and they were stable throughout the storage period. Yadav et al. (2007), studied the production of fatty acids and conjugated linoleic acid (CLA) in the ordinary and probiotic yogurt dahi (prepared with buffalo milk) containing *L. acidophilus* and *L. casei*, during fermentation and after 10 days storage at 5 °C. They reported that an increased level of fatty acids especially butyric acid during fermentation and storage in the probiotic yogurt samples is mainly due the lipolysis of milkfat which was higher in the presence of probiotic bacteria.

Conclusion

The findings of present study suggested that fermented milk prepared with V3 and M5 cultures (T) in combined showed superior sensory and better storage stability with increased SCFAs production up to 28 days as compared to the control (C). Proximate compositions of developed product were 9.84 percent total solids, 2.48 percent fat; 2.9 percent protein, 3.62 percent carbohydrate and 0.84 percent ash. These two promising Lactobacillus cultures could be used for the development of fermented milks with health beneficial properties. The validation of the health claim needs to be conducted on human subjects.

References

Adeiye OA, Gbadamosi SO and Taiwo AK (2013) Effects of some processing factors on the characteristics of stored groundnut milk extract. African J Food Sci 7(6):134-142, doi: 10.5897/AJFS12.149.

Adhikari K, Grun IU, Mustapha and Fernando LN (2001) Changes in the profile of organic acids in plain set and stirred yogurts during manufacture and refrigerated storage. J Food Quality 25(5):435-51, doi.org/10.1111/j.1745-4557.2002.tb01038.x.

Akalin AS, Tokuşoglu O, Gonç S and Aycan S (2007) Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. Int Dairy J 17(9):1089-95, doi.org/10.1016/j.idairyj.2007.02.005.

Alim MA, Wadehra A and Singh AK (2016) Effect of various plant starches on the quality characteristics of starch-based sweetened cow milk yoghurt. J Bangladesh Agric University 14(1):119-26, doi.org/10.3329/jbau.v14i1.30606.

AOAC (Association of Official Analytical Chemists) (2006) Official methods of analysis, 18th edition. AOAC, Arlington, Virginia, doi.org/10.1093/jaoac/92.1.61.

Arunachalam K, Gill HS and Chandra RK (2000) Enhancement of natural immune function by dietary consumption of *Bifidobacterium lactis* (HN019). European J Clin Nutr 54(3):263-7, doi.org/10.1038/sj.ejcn.1600938.

Bigliardi B and Galati F (2013) Innovation trends in the food industry: The case of functional foods. Trends Food Sci Technol 31(2):118-29, doi.org/10.1016/j.tifs.2013.03.006.

Bucker JR, Mitchell JH and Johnson MG (2008) Lactic acid fermentation ofpeanut milk. J Food Sci 44(5) 1534-1538, doi.org/10.1111/j.1365-2621.1979.tb06481x.

Canani RB, Di Costanzo M, Leone L, Pedata M, Meli R, et al (2011) Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol 17(12):1519, doi: 10.3748/wjg.v17.i12.1519.

Chakraborti CK (2015) New-found link between microbiota and obesity. World J Gastrointestinal Pathophysiol 6(4):110, doi: 10.4291/wjgp.v6.i4.110.

Cheng HH and Lai MH (2000) Fermentation of resistant rice starch produces propionate reducing serum and hepatic cholesterol in rats. J Nutr 130(8):1991-5, doi.org/10.1093/jn/130.8.1991.

Contor L (2001) Functional Food Science in Europe. Nutrition, metabolism, and cardiovascular diseases 11(4 Suppl):20-3.

Dave RI and Shah NP (1998) Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J Dairy Sci 81(11):2804-16, doi.org/10.3168/jds.S0022-0302(98)75839-4.

Di Bartolomeo F, Startek JB and Van den Ende W (1998) Prebiotics to fight diseases: reality or fiction? Phytotherapy Res (10):1457-73, doi.org/10.1002/ptr.4901.

El-Aidie SA, El-Dieb SM, El-Nawawy M, Emara E and Sobhy H (2017) Nutraceutical food based on cereal and probiotic fermented milk. Int J Dairy Sci 12(6):377-84, DOI: 10.3923/ijds.2017.377.384.

Fernandez-Garcia E and McGregor JU (1994) Determination of organic acids during the fermentation and cold storage of yogurt. J Dairy Sci 77(10):2934-9, doi.org/10.3168/jds. S0022-0302(94)77234-9.

Fuller R, editor 1997 Probiotics 2: applications and practical aspects. Springer Science & Business Media.

Hamer HM, Jonkers DM, Venema K, Vanhoutvin SA, Troost FJ, et al (2008) The role of butyrate on colonic function. Alimentary Pharmacol Therapeutics 27(2):104-19, doi.org/10.1111/j.1365-2036.2007.03562.x.

Hasan MN, Sultan MZ and Mar-E-Um M (2014) Significance of fermented food in nutrition and food science. J Scientific Res 6(2):373-86, doi.org/10.3329/jsr.v6i2.16530.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, et al (2014) The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Rev Gastroenterol Hepatol 11(8):506-14, doi.org/10.1038/nrgastro.2014.66.

Ifediba DI, Ozoh CN (2018) Effects of Storage on Physicochemical Properties and Microbiological Qualities of African Breadfruit-Corn Yoghurt. European Scientific J 14(6):172-91, doi: 10.19044/esj.2018.v14n6p172.

Imamoglu H, Coggins PC and Rowe DE (2017) Influence of storage time and starches on texture attributes of conventional milk yogurt using response surface methodology. Int Food Res J 24(4):1721.

Jaiswal AK and Sharma S (2016) Enzymes in synthesis of novel functional food ingredients. Enzymes in Food Beverage Process 2016:381-400.

Joint FAO, WHO Expert Committee on Food Additives (2002) World Health Organization. Evaluation of certain food additives and contaminants: fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization.

Kpodo FM, Afoakwa EO, Amoa BB, Budu AS and Saalia FK (2014) Effect of Ingredient Variation on Microbial Acidification, Susceptibility to Syneresis, Water Holding Capacity and Viscosity of Soy-Peanut-Cow Milk Yoghurt. J Nutr Health Food Engg 1(2): 00012, DOI: 10.15406/jnhfe.2014.01.00012.

Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, Chakraborty C, Singh B, Marotta F, Jain S, Yadav H (2012) Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Experimental Diabetes Res doi:10.1155/2012/902917.

Makwana M (2019) Evaluation of probiotic cultures for production of short-chain fatty acids and its effect on cholesterol reduction. PhD Dissertation. Anand Agricultural University, Anand-388110, Gujarat, India.

McDonough FE, Hitchins AD, Wong NP, Wells P and Bodwell CE (1987) Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons. The American J Clin Nutr 45(3):570-4, doi.org/10.1093/ajcn/45.3.570.

Mohammad, A (2004) Influence of different types of milk and stabilizers on sensory evaluation and whey separation of yoghurt. Pakistan J Sci Industrial Res 47(5): 398-402, DOI: 10.3923/pjbs.2000.1336.1338.

Official Methods of Analysis (1995) 16th Ed., AOAC INTERNATIONAL, Arlington,VA, sec. 935.29, doi.org/10.1093/jaoac/92.1.61.

Onweluzo JC and Nwakalor C (2009) Development and evaluation of vegetable milk from Treculiaafricana (Decne) seeds. Pakistan J Nutr 8(3):233-8, DOI: 10.3923/pjn.2009.233.238.

Patidar SK and Prajapati JB (1998) Standardisation and evaluation of lassi prepared using Lactobacillus acidophilus and Streptococcus thermophilus. J Food Sci Technol (Mysore), 35(5):428-31.

Perry JC, D'Almeida V, Souza FG, Schoorlemmer GH, Colombari E, et al. (2007) Consequences of subchronic and chronic exposure to intermittent hypoxia and sleep deprivation on cardiovascular risk factors in rats. Respiratory Physiol Neurobiol 156(3):250-8, doi.org/10.1016/j.resp.2006.10.004.

Prajapati JB, Khedkar CD, Chitra J, Suja S, Mishra V, Sreeja V, Patel RK, Ahir VB, Bhatt VD, Sajnani MR, Jakhesara SJ. (2011) Whole-genome shotgun sequencing of an Indian-origin Lactobacillus helveticus strain, MTCC 5463, with probiotic potential. J Bacteriol 193:4282-4283, doi.org/10.1128/jb.05449-11.

Pranckute R, Kaunietis A, Kuisiene N and Citavicius D (2014) Development of synbiotics with inulin, palatinose, α-cyclodextrin and probiotic bacteria. Pol J Microbiol 63(1):33-41, DOI: 10.33073/pjm-2014-005.

Roopashri AN and Varadaraj MC (2014) Hydrolysis of flatulence causing oligosaccharides by α-d-galactosidase of a probiotic Lactobacillus plantarum MTCC 5422 in selected legume flours and elaboration of probiotic attributes in soy-based fermented product. European Food Res Technol 239(1):99-115, doi.org/10.1007/s00217-014-2207-y.

Roseburg V, Boylston T, White P (2010) Viability of Bifidobacteria strains in yoghurt with added oat beta glucan and corn starch during cold storage. J Food Sci 75(5): C439-C444, doi.org/10.1111/j.1750-3841.2010.01620.x.

Sanni AI, Onilude AA, Adeleke EO (1999) Preparation and characteristics of lactic acid fermented cowpea milk. Zeitschriftfür Lebensmitteluntersuchung und-Forschung A 208(3):225-9, doi.org/10.1007/s002170050408.

Shah N, Prajapati JB (2014) Effect of carbon dioxide on sensory attributes, physico-chemical parameters and viability of Probiotic L. helveticus MTCC 5463 in fermented milk. J Food Sci Technol 51(12):3886-93, doi.org/10.1007/s13197-013-0943-9.

Stone H, Bleibaum R and Thomas HA (2020) Sensory evaluation practices. Academic press, DOI: 10.1016/B978-0-12-672480-6.50011-1.

Supavititpatana P, Wirjantoro TI and Raviyan P (2010) Characteristics and shelf-life of corn milk yogurt. J Natural Sci 9(1):133-47.

Tamime AY and Robinson RK (2007) Tamime and Robinson's yoghurt: science and technology. Elsevier.

Walia A, Mishra HN, Kumar P (2013) Effect of fermentation on physicochemical, textural properties and yoghurt bacteria in mango soy fortified yoghurt. African J Food Sci 7(6):120-127, DOI: 10.5897/AJFS08.049.

Williams RP, Glagovskaia O and Augustin MA (2003) Properties of stirred yogurts with added starch: effects of alterations in fermentation conditions. Australian J Dairy Technol 58(3):228.

Yadav H, Jain S and Sinha PR (2007) Production of free fatty acids and conjugated linoleic acid in probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei during fermentation and storage. Int Dairy J 7(8):1006-10, doi.org/10.1016/j.idairyj.2006.12.003.

Yasni S and Maulidya A (2014) Development of corn milk yoghurt using mixed culture of Lactobacillus delbruekii, Streptococcus salivarus, and Lactobacillus casei. Hayati J Biosci 21(1):1-7, doi.org/10.4308/hjb.21.1.1.

Zambell KL, Fitch MD and Fleming SE (2003) Acetate and butyrate are the major substrates for de novo lipogenesis in rat colonic epithelial cells. J Nutr 133(11):3509-15, doi.org/10.1093/jn/133.11.3509.

Zapsalis C and Beck RA (1985) Food chemistry and nutritional biochemistry, Wiley, doi.org/10.1002/9781118688496.ch9.