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RESEARCH PAPER

Assessment of Physicochemical, Microbiological and Sensory Attributes of Probiotic Papaya Curd Prepared by inoculating *Lactobacillus brevis* UN and *Lactobacillus spicheri* G2

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Abstract

In the present investigation, two strains *Lactobacillus brevis* UN and *Lactobacillus spicheri* G2 were used to prepare flavored probiotic curd. Flavor was added in the curd by adding fresh papaya cubes. Four different sets of papaya curd were prepared by taking different combinations of probiotic inoculums during fermentation process. Control was also prepared by using native inoculum of curd. Fermentation of each set was carried out at 30 ˚C for 4h, product formed was kept at a refrigerated temperature upto 12 days. Sensorial, microbiological and physiochemical analysis of each prepared set of probiotic curd was performed during storage interval. Results obtained after sensorial evaluation revealed that different combinations of *L. brevis* UN and *L. spicheri* G2 exerted a significant influence on the sensory quality of the flavored curd. Microbiological evaluation of each set of product proved that there was no loss in total number of viable cells in the product during storage. Statistical analysis of physio-chemical attributes revealed that changes taking place during storage intervals in various sets of Probiotic curd were non-significant.

Keywords: Curd, Lactic acid bacteria, Probiotic, Sensory, Titratable acidity,papaya

During the past 20 years, junk food has attracted attention of an average consumer irrespective of age groups. These junk food contain no proteins or vitamins but are rich in salt, sugar and calories which can cause obesity and hypertension in people. Though, it is difficult to convince people mostly school and college going students against their junk habits, it is a big challenge for policy makers to replace these junk items with nutritionally rich diets. Probiotic health drinks could be a good alternative for these "high in calories-low in nutrition" junk food. "Probiotics" defined as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance (FAO, 2002).

Probiotics are also called "friendly bacteria" or "good bacteria" Saikhon and Jairath (2010). By replacing junk food or Ready-to-Eat (RTE) packed food with probiotic food products people not merely satisfy their taste rather they will add nutrition in to their diet. Apart from nutritional aspect, probiotics are the primary vehicle to prevent health chronic diseases viz., cancer, ulcers, gastroenteritis, Traveler's Diarrhea, inflammatory Bowel's disease, diabetes, pregnancy urogenital infections and HIV etc. (Petrick, 2012). People in U.S.A. and Europe have developed their interest towards the benefits associated with probiotic cultures and marketed many probiotic food and beverages. However, for Indian societies still there is a long way to go before these probiotic foods change the Indian mind set and reach the common man to explore their full health benefit. Nevertheless, Indians have been known to use probiotics in the form of many traditional fermented food products since ancient time but due to urbanization and modernization, consumption of these kinds of food have become limited (Gautam and Sharma, 2014), though, fermented food products viz., curd and *lassi* in daily diet is used by maximum consumers. Moreover, inoculum used to prepare these traditional fermented food products or dairy products are not tested scientifically for their probiotic attributes and hence, cannot be assured of their health benefits.

These traditional fermented foods and dairy products can be made as authentic probiotic products by fermenting them with potential probiotic tested strains at a suitable level and marketed to provide health benefits to the consumers. These probiotics could also be used in infant formulations.

Therefore, the present investigation was carried out to scientifically evaluate a probiotic flavored curd which is supplemented with papaya and fermented by tested potential probiotic microorganisms. In that context, this research aimed to study the physiochemical, microbiological and sensorial qualities of novel probiotic papaya curd prepared by using *L. brevis* UN and *L. spicheri* G2 in fermentation process. The results obtained are described in this paper.

MATERIALS AND METHODS

Preparation of curd

Papaya curd was prepared by using different combinations of food grade bacterial cultures *L. brevis* UN (JX046150) (Gautam *et al*. 2014) and *L. spicheri* G2 (JX481912) (Gautam, 2012). Each culture was maintained at -20 ˚C on de Man Rogosa (MRS) broth containing 40% glycerol (v/v) in deep freezer and the cultures were propagated three times prior to use (Gautam and Sharma, 2015).

Papaya curd Ingredients

1	Powdered milk (Everyday make)	150 g
2	Water	500 ml3.
3	Papaya cubes	15 g
4	Sugar	5 g
5	*L. brevis* UN	10^6 cfu/ml
6	*L. spicheri* G2	10^6 cfu/ml
7	Native Inoculums	10^6 cfu/ml

The complete process of prepration of papaya curd was prepared as depicted in Fig. 1 In the present investigation an attempt has been made to prepare probiotic flavored curd by using different combinations of lactic acid bacteria viz., *L. brevis* UN (Accession no JX046150) and *L. spicheri* G2 (Accession no. JX481912). Flavored curd (Fig. 1) was prepared by using powdered milk (150 g) which was dissolved with water (500 ml) followed by boiling at 90 ˚C or 2 min and cooled to 37+ 1 ˚C.

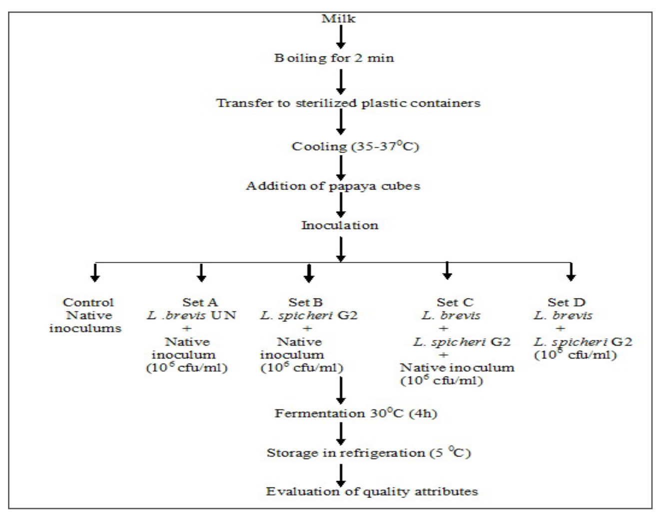

Fig. 1: Flow diagram of Preparation of Papaya curd

Papaya was mixed to improve nutritional value and taste of *Probiotic* preparation as these are considered the goldmines of nutrition**.** Each of product prepared in the present study was divided into 5 sets (100 ml) each with respect to different inocula used for fermentation Set A was inoculated with *L. brevis* UN+ native inoculum (1:1;10^6cfu/ml) , *L. spicheri* G2 +

native inoculum (1: 1; 10^6cfu/ml) were added to set B, *L. brevis* UN + *L. spicheri* G2 + native inoculum were added to set C and *L. brevis* UN + *L. spicheri* G2 added to set D (1:1:1; 10^6). In addition to all these sets control inoculated with native inoculum of curd (10^6 cfu/ml) was also prepared. After inoculation fermentation of each set was carried out at 30 ˚C for 4h and was kept at refrigerated temperature upto 12 days. Microbiological and physicochemical analysis of each prepared set of curd was performed at regular interval during storage.

Microbial evaluation during storage

The colony count was taken during storage period by standard spread plate method (Aneja *et al.* 2003). MRS agar was used to enumerate lactic acid bacteria while nutrient agar(NA), yeast extract agar (YEA), potato dextrose agar (PDA) were used to enumerate total aerobic mesophilic bacteria including yeast and mold.

Physioco-chemical changes during storage

pH: pH of each sample was measured using pH meter.

TSS: TSS was measured by placing 1-2 drops of sample on the prism of a hand refractometer. The results were expressed as °Brix (Ranganna, 1997).

Titratable Acidity: An aliquot of the sample prepared was diluted with recently boiled distilled water. 2-3 drops of 1% phenolphthalein solution was used as an indicator and titration was done with 0.1 N NaOH. Titer value was noted and calculations were done as percent anhydrous lactic acid as per the mentioned of AOAC (1995)

Titratable acidity (%) =

$$\frac{\text{Titre} \times \text{Normality of alkali} \times \text{volume made up} \times \text{equivalent weight}}{\text{Volume of sample taken} \times \text{Volume of aliquot taken} \times 1000} \times 100$$

Ascorbic acid : Ascorbic acid was determined as per (AOAC 1995) method.

1 ml of curd sample was diluted with 10 ml of 3% HPO_3 followed by centrifugation. Aliquot of the HPO_3 of the sample was titrated with the standard dye to a pink end point which persisted for 15 sec. Titration was done rapidly and preliminary determination of the titre was done and using the title , the ascorbic acid contents was measured and expressed as mg/100ml

CALCULATION

Sensory Evaluation

Sensorial evaluation of each sample was done in terms of appearance, texture, flavor and overall acceptability. Nine point hedonic scale method as given by (Amerine *et al*. 1965) was followed for conducting the sensory evaluation of each set of probiotic product. The panel of 7 judges comprising the students of Department of Basic Sciences (Microbiology section), Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, H. P. India were selected to evaluate the products for sensory parameters outlined earlier. Efforts were made to keep the same panel for sensory evaluation throughout the course of study. The sample was presented to the judges and plain water was given to them to rinse their mouth in between the evaluation of samples. No discussion during evaluation was allowed.

Statistical analysis

Data on sensorial evaluation of probiotic products were analyzed by using randomized block design (RBD) described by (Mahony, 1985).

Data pertaining to the physicochemical attributes of probiotic product was analyzed by completely randomized design (CRD) factorial.

RESULTS AND DISCUSSION

Nutritional Analysis

Nutritional facts of fresh papaya curd have been presented in Table 1. The attributes marked with (*) were evaluated in the laboratory where as attributes marked with (**) have been taken from literature for the sake of comparison.

Table 1: Nutritional chart of probiotic papaya curd

* Lactic acid (%)	0.12-0.21
* pH	4.04 - 4.20
* Total soluble sugar (^{0}B)	5.03– 6.03^{0} B
*Ascorbic acid (mg)	3.0-3.4
** Protein (g)	14.47
** Dietary fiber (g)	1.5 g
** carbohydrate (g)	7.86 g
** Fat (g)	0.2 g
** Mineral	0.724 g

*Values evaluated in lab; ** Values taken from USDA SR-21

Sensorial evaluation

A positive response of panelists was observed for acceptability of this prepared product (Table 2 and Fig. 2). The sensorial quality of papaya curd was found to be the best as each set of treatment of this curd scored above 80% except set D. Papaya added to this curd sample seemed to enhance its taste. Statistically analysis of the sensorial evaluation data was carried out by Randomized Block Design (RBD). Statistically results were significant so it could be said that the quality of a fermented product depends upon inoculated bacterial strains as well as on the properties of type of additives added in it.

Table 2: Sensorial evaluation of papaya curd

Parameter	Appearance	Texture	Flavor	Overall acceptability
Control♦	8.42	8.14	8.00	8.18
Set A♦♦	8.71	8.57	9.00	8.76
Set B♦♦♦	8.71	9.00	8.00	8.57
Set C^	8.42	8.57	7.71	8.22
Set D ^^	8.00	7.42	7.14	7.52
CD $_{0.05}$	0.49	0.51	0.41	0.27

♦Native inoculum; ♦♦*L.brevis* +native inoculum; ♦♦♦ *L.spicheri* + native inoculum; ^ *L.brevis* UN + *L.spicheri* G2+ native inoculum; ^^ *L.brevis* UN + *L.spicheri.*

Microbiological analysis of papaya curd during storage

Data regarding changes in microbial count have been presented in Table 3. The viable count for *Lactobacilli* was maximum in set D (26.08 log cfu/ml) and minimum for set B (14.28 log cfu/ml) at initial h after fermentation which was increased during storage period and became maximum for set A i.e. 31.21 and minimum for control i.e. 20.64. Though, there was a variation in number of viable cells of different sets of papaya curd during storage but statistically the variation was non-significant. The viable count for total aerobic mesophiles were however below the

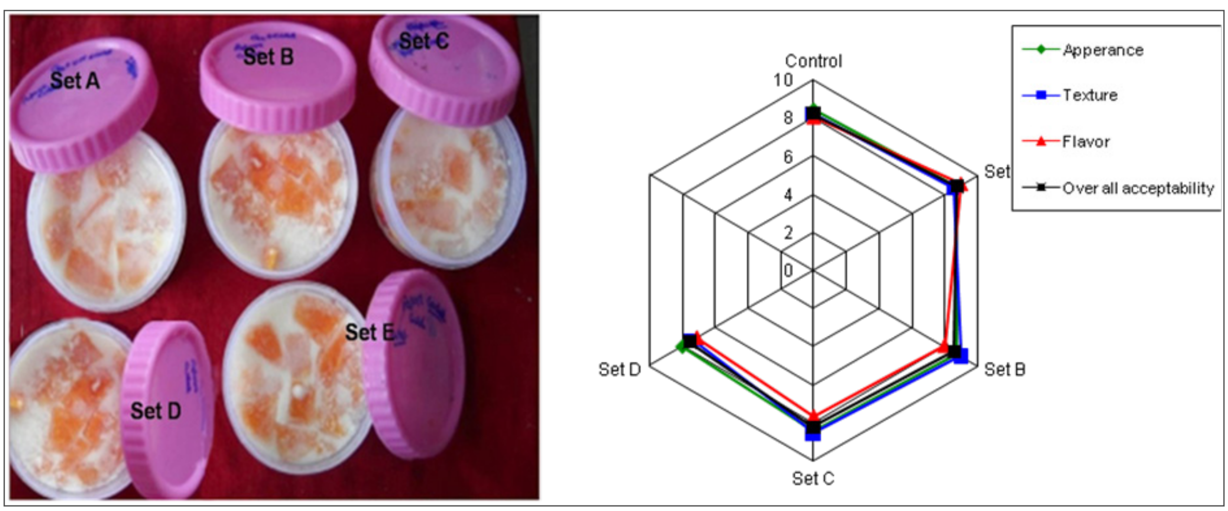

Fig. 2: Role of *L. brevis* UN and *L. spicheri* G2 in preparation papaya curd and sensory evaluation of papaya curd

detection limit (<10 log cfu/ml) as presented in Fig. 3 and it is desirable

Table 3: Microbiological examination of papaya curd

Sl. No.	Name of treatment	Storage days (Log cfu/ml)					
		0	3	6	9	12	Mean
1	Control ⬩	18.36	23.98	27.63	20.04	20.64	22.13
2	Set A ⬩⬩	17.16	22.61	26.53	29.49	31.21	25.40
3	Set B ⬩⬩⬩	14.28	20.45	27.63	28.86	29.19	24.08
4	Set C^	27.71	26.33	29.24	28.98	28.13	28.08
5	Set D ^^	26.08	29.29	29.64	26.63	26.23	27.57
	Mean	20.72	24.53	28.14	26.80	27.08	
		Treatment (0.052) Days (0.052)					
		$CD_{0.05}$ T× D = (0.12)					

⬩ Same as in Table 2; ⬩⬩ Same as in Table 2; ⬩⬩⬩ Same as in Table 2; ^ Same as in Table 2; ^^ Same as in Table 2.

Physicochemical characteristics of papaya curd during storage

Perusal of data regarding pH of papaya curd has been shown in Table 4. The initial pH values for different sets of papaya curd were in the range of 4.92 to 4.04. It was noticed that during storage spam pH values kept on decreasing and on 12th day of storage pH came down to 3.77 for control, 3.76 for set A and B, 3.75 to set C and 4.0 to set D. In case of TSS, difference were not appreciable in ^{0}B values of different sets of papaya curd at 0 hr except for control and set D where TSS was 6.03. On 12th day TSS for set A and C did not show any change while TSS lowered down from 5^0 B to 3.10^0 B in set B and from 6.10^0 B to 4.0 ^{0}B. In contrast to all TSS increased in control from 6.03 to 6.40^0 B. The lactic acid was maximum in control, i.e. 0.21 % at initial h while 0.17 % for rest of the treatments. An increase was observed in lactic acid content of all the treatments during storage. On 12th day of storage lactic acid was maximum i.e. 1.0 % for control and set B while minimum for set D. Ascorbic acid content for different treatments on initial h was in a range of 3.0 to 3.4 mg/ml which increased in the range of 3.4 to 3.5 mg/100 ml, during storage interval. Statistically study revealed that the result were non-significant for in various quality attributes during storage conditions of papaya curd.

It was observed from the storage study of papaya curd that no doubt there was an increase or decrease in values of quality attributes (pH, TSS, titratable acidity, ascorbic acid) interval but statistically these changes were non-significant that means these slight variations in quality attributes had nil effect on product quality. This slight change in quality is due to many reasons as discussed here. Curd is a product

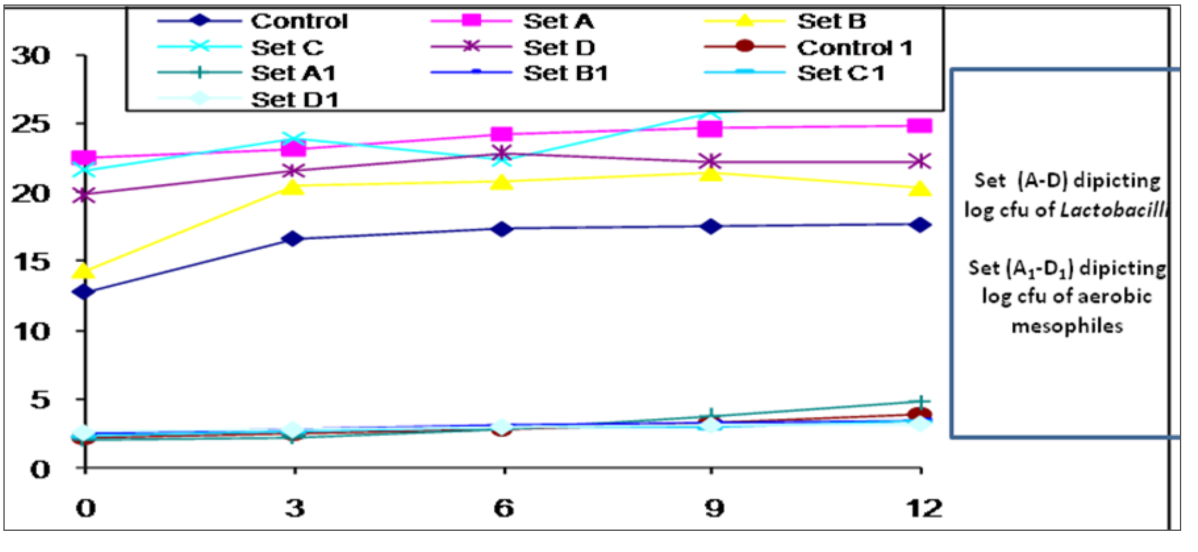

Fig. 3: Total viable count of Lactic acid bacteria and Aerobic mesophilies in papaya curd

Table 4: Physicochemical characteristics of papaya curd during storage

Treatment (T)	pH						TSS (^{0}B)						Titratable acidity (%)						Ascorbic acid (mg/100ml)					
	Storage Interval (days)					Mean	Storage Interval (days)					Mean	Storage Interval (days)					Mean	Storage Interval (days)					Mean
	0	**3**	**6**	**9**	**12**		**0**	**3**	**6**	**9**	**12**		**0**	**3**	**6**	**9**	**12**		**0**	**3**	**6**	**9**	**12**	
Control♦	4.04	3.82	3.80	3.80	3.77	**3.84**	6.03	6.06	6.06	6.06	6.40	**6.12**	0.21	0.26	0.44	0.44	1.0	0.47	3.4	3.5	3.5	3.4	3.5	3.46
Set A♦♦	4.72	4.20	3.86	3.75	3.76	**4.06**	5.03	4.96	5.00	5.00	5.00	**5.00**	0.17	0.35	0.44	0.44	0.71	**0.42**	3.2	3.5	3.5	3.5	3.5	3.44
Set B♦♦♦	4.80	3.80	3.82	3.91	3.76	**4.02**	5.00	5.00	5.00	4.10	3.10	**4.44**	0.17	0.35	0.44	0.44	1.0	**0.48**	3.1	3.5	3.5	3.5	3.5	3.42
Set C^	4.92	3.92	3.94	3.86	3.75	**4.07**	5.03	5.03	5.00	6.10	5.03	**5.23**	0.17	0.35	0.44	0.46	0.53	**0.39**	3.0	3.5	3.5	3.4	3.4	3.36
Set D^^	4.20	4.09	4.08	4.00	4.00	**4.07**	6.10	5.96	5.00	4.00	4.00	**5.01**	0.12	0.35	0.44	0.46	0.50	0.37	3.1	3.5	3.5	3.5	3.4	3.40
Mean	4.53	3.96	3.90	3.86	3.80		5.44	5.40	5.21	5.05	4.70		0.17	0.33	0.44	0.48	0.74		3.16	3.50	3.50	3.46	3.47	
$CD_{0.05}$																								
Treatment (T)						0.038						0.042						0.042						0.15
Storage interval (S)						0.038						0.042						0.042						0.15
TxS						0.084						0.093						0.094						0.34

Same as in Table 2

of the acidic fermentation of milk. It was observed that there is a decrease in pH with an increase in lactic acid content of curd during storage and it is an established relation between pH and acid. pH is lower down during storage due to remaining microbial activity that results in continuous lactose fermentation (Rasic *et al*. 1978). Lactose present in milk is continuously converted to lactic acid, which lowers down the pH (Zourari *et al*. 1992). Low pH not only contributing to the texture of curd but it ionizes calcium and thus, facilitate its absorption in intestine. Acidic pH also reduces the inhibitory effect of phytic acid on calcium bioavailability and improve lactose absorption. Our results are in accordance with study of (Cakmakci *et al.* 2012) on probiotic properties of banana yogurt when a decrease in pH value during storage period of 14 days was documented. A significant difference in pH of control and other yogurt was also documented. Ozturk and Oner (1999) also reported a decline in pH value of juice flavoured yogurt during 14 days of storage.

In contrast to pH, total soluble solid content in papaya curd either decreased or remained the same during the storage period which was according to the reports published in literature. It has been already reported that total soluble content of the product decreased with the passage of time (Manimegalai *et al.* 2001).

It was also found in present investigation that papaya curd had good amount of ascorbic acid content which aid to increase the nutritional properties of these functional foods. Ascorbic acid is a very important component of diet and dietary supplements. It is found naturally in many fruits and vegetables synthetic form of the vitamin C and is also added to the food to increase its antioxidant activity (Bjelakovic *et al.* 2012). It is reported that lactic acid bacteria synthesize certain synthetic vitamin content during curdling process. The common antioxidant and nutritional value of ascorbic acid can help ward off a variety of health problems. Vitamin C helps white blood cells to fight infectious bacteria by phagocytosis (Coombes and Fassett, 2012).

The lactic acid content was increased in prepared functional foods during storage. This increase in titratable acidity is due to post-acidification of curd that has going to increased even at refrigerated temperature. The facts of post-acidification have been suggested by many authors in literature according to them some species of *Lactobacilli*, viz. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are responsible for post-acidification of yogurt during cold storage. From the present study it can be said that *L. brevis* and *L. spicheri* also take part in post acidification process. Post acidification also may be due to lactose fermentation. As lactic acid bacteria actively convert lactose into lactic acid which may help lactose intolerant individuals to tolerate more lactose than they would otherwise have tolerated (Sanders *et al.* 2003). Similar results have been reported by Ozturk and Oner, 1999. Stating that the titratable acidity of concentrated grape juice flavored yogurts and control yogurts increased after 7 days of storage at 4 °C.

CONCLUSION

It is concluded that probiotic strains *L. brevis* UN and *L. spicheri* G2 accomplished successful fermentation of milk supplemented with papaya cubes. Fermentation of milk product in combination with papaya cubes is useful because papaya has enhanced taste and nutritive value of the product. In addition to this, probiotic papaya curd is a potential functional food with beneficial health properties as product is prepared by using efficient probiotic strains. No single bacterial strain can easily fulfill all requirements of probiotics therefore, we can say that combinations of bacteria species as used in the present investigation is appropriate to make probiotic supplements that could be commercialized in near future.

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