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MILK FERMENTATION EFFICACY OF IMMOBILIZED *L. PARACASEI* CELLS ON SELECTED FRESH FRUIT PIECES

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ABSTRACT

The objective of the present study was to immobilize *Lactobacillus paracasei* cells on selected fruit pieces viz. sapota, banana, mango and pineapple to assess the milk fermentation efficacy and storage stability using different parameters namely pH, acidity, lactose and viable count. *L. paracasei* was immobilized on fruit pieces. Such biocatalysts were washed twice using distilled water and reused for fresh milk fermentation (reactivation of biocatalysts). Parameters were analyzed for both fresh and stored [(0,7,15,30 and 60 days after storage (DAS)] fermented milk samples at refrigeration temperature as well as reactivated fermented milk samples on the next day of each storage period. Results showed that all fruit biocatalysts (fresh and stored) could successfully ferment milk, could be stored up to 60 days without contamination and reactivated on the successive day of the storage period. A decrease in pH, lactose content and viable count and an in increase in acidity were observed for stored fermented milk samples whereas higher lactobacilli count was observed for fruit pieces even after the 60th day of reactivation compared to the initial day (day0). Among the fruits, pineapple pieces showed maximum milk fermentation efficacy whereas mango pieces showed the least.

Key words: Immobilization, fruit pieces, *L. paracasei*, milk fermentation efficacy,

INTRODUCTION

The concept of probiotics (which means, "for life") was introduced in early 20thcentury by Elie Metchnikoff [1], however now probiotic bacteria are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' [2]. Currently, the standard for any food sold with added probiotics with health clamis should follow the FAO/WHO recommendation that it must contain, per gram, at least 10^6 to 10^7 cfu of viable probiotic bacteria [2].

Even now the probiotic approach remains ineffective to some degree because of loss of viability in traditional probiotic products. Immobilization of probiotics in gel matrices is utilized to protect cells from storage and GI transit [3].

Cell immobilization in fermentation is an exciting as well as quickly growing research area due to their technical and affordable benefits in comparison with the free cell system [4-6]. For application in the food industry, immobilization carrier should provide adequate flavor to the product with food grade purity, should be minimal in cost and readily available. Fruits utilized as carriers, fulfill all the above expectations [6]. Immobilization on fruit support is undertaken by adsorption process, whereby microorganisms are held on to porous and inert carrier materials which is similar to the adsorption of colloid particles [7].

Milk products serve as important delivery vehicles for probiotic bacteria. Probiotic bacteria have a long history of association with dairy products[8],and fruits have been traditionally added to dairy products for flavour enhancement.

Hence the present study was designed with the aim of immobilizing probiotic bacterial cells i.e *L. paracasei* NCDC 022 on selected fruit matrices (sapota, banana, mango and pineapple) and studying their milk fermentation efficacy.

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METHODOLOGY

A. Material procurement:

Lactobacillus paracasei 022 was selected as the probiotic microorganism for immobilization and procured from NCDC at NDRI, Karnal. Fresh fruits selected for immobilization viz. sapota, banana, mango and pineapple were obtained from the local market of V.V. Nagar. Low-fat milk (double toned, 1.5% fat) was procured from the AMUL retail outlet.

B. Immobilization of fruit pieces

Immobilization of fruit pieces was carried out as described by Kourkoutas et al. [9]. Briefly for immobilization, cells were grown on a synthetic medium [9], sterilized at 121°C for 15min. Flasks were inoculated with 1% of the activated probiotic microorganisms and incubated at 37°C without agitation. About 100 g of each of the autoclaved selected fresh fruit pieces were introduced in 250 ml of liquid culture of *L. paracasei*. All fruit pieces were incubated in the liquid culture for 24 h at 37°C. When the immobilization was complete, liquid culture was decanted and the supported biocatalyst was washed twice with sterile distilled water. The fresh biocatalysts were then used for fermentation of milk.

Assessment of milk fermentation efficacy

Milk fermentation efficacy of fresh and stored immobilized biocatalysts and fermentation efficacy of the same after reactivation at the end of each storage period (4°C) was carried out as given below:

Fifteen gram of freshly immobilized fruit pieces (biocatalysts) were inoculated per 100 ml of previously heated [95°C for 5 min] and then cooled [42°C] milk samples and incubated overnight. Fermented milk samples thus obtained were used for fresh analysis and were also stored under refrigeration for specific time periods (0, 7th, 15th, 30th & 60th day). At the end of each storage period, immobilized pieces from each fermented milk sample were collected, washed twice with 100 ml of sterile distilled water and again introduced in 100 ml of fresh milk sample for fermentation (reactivation of stored biocatalysts). Fermented milk sample at the end of each storage period and fermented milk sample on each of the reactivated day (i.e. next day) were analyzed for fermentation efficacy parameters. *Lactobacilli* counts of the immobilized biocatalyst recovered from the reactivated fermented milk was also carried out. Milk sample fermented using free cells (1%) was considered as the control.

C. Parameters studied

The following parameters were analyzed from fermented milk – fresh and stored, before and after reactivation:

a) Physico-chemical parameters

pH was measured using a pH meter for food testing (Eutech PHSPEAR/ 01X366920/ Oakton 35634-40) after pH calibration with standardized buffer solutions of pH 4 and 7. Titratable acidity in % lactic acid was measured as described by BIS [10]. Lactose content was estimated according to Tele's method [11].

b) Microbial parameters:

L. paracasei count was carried out from fermented milk samples on each of the specific storage day and from the immobilized fruit pieces on the successive day after the reactivation process by plating 0.1ml of each serial dilution in DeMan-Rogosa-Sharpe agar (MRS agar) followed by incubation at 37°C for 48 h. Colonies were counted and the *L. paracasei* count results were expressed in logarithm of colony forming units per gram of product (log cfu/g).

c) Statistical analysis:

Data were expressed as Mean ± SD of three repetitions. One-way analysis of variance (ANOVA) and Duncan test (p 0.05) were used to analyze the results. Statistical analysis was performed using SPSS version 17.

RESULTS AND DISCUSSION

The pH values of fermented milk samples for each storage day and after reactivation, the next day were analyzed (Table 1). pH value of fresh and stored fermented milk samples ranged between 4.07-4.80 for control and 3.57- 5.29 for fruit immobilized samples. Analysis of stored fermented milk samples showed that pH value decreased significantly on the 60th day compared to the initial days of storage for all samples. Among fruits, mango pieces showed a significantly ($p< 0.01$) higher pH (4.13-5.29) compared to other samples throughout the storage period while pineapple pieces showed a significantly lower pH (3.57-4.55) as the storage period progressed.

Table 1: pH of stored and reactivated fermented milk samples

Sample	Day 0	Day 7	Day 15	Day 30	Day 60	F- value
Control	$4.80 \pm 0.01^{c,2}$	$4.75 \pm 0.0^{1,c,d,2}$	$4.51 \pm 0.01^{a,b,1,2}$	$4.30 \pm 0.01^{a,1,2}$	$4.07 \pm 0.02^{c,1}$	246.02**
C- Reactivated	$4.23 \pm 0.01^{a,1}$	$4.03 \pm 0.3^{a,1}$	$5.24 \pm 0.28^{c,3}$	$4.79 \pm 0.01^{d,2}$	$4.61 \pm 0.09^{e,,2}$	236.3**
Sapota	$4.59 \pm 0.06^{b,3}$	$4.51 \pm 0.01^{c,2}$	$4.54 \pm 0.01^{a,b,2,3}$	$4.55 \pm 0.04^{c,2,3}$	$3.86 \pm 0.04^{a,1,}$	259.2**
Sap-Reactivated	$4.68 \pm 0.10^{b,c,1,2}$	$4.53 \pm 0.01^{c,1}$	$4.61 \pm 0.01^{a,1,2}$	$4.98 \pm 0.04^{f,3}$	$4.59 \pm 0.01^{e,1}$	20.30**
Banana	$4.59 \pm 0.05^{b,2}$	$4.57 \pm 0.00^{c,2}$	$4.59 \pm 0.02^{b,2}$	$4.50 \pm 0.02^{b,2}$	$3.85 \pm 0.02^{a,1}$	553.76**
BA-Reactivated	$4.69 \pm 0.02^{b,c,3}$	$4.47 \pm 0.00^{b,c,2}$	$4.59 \pm 0.02^{a,b,2}$	$4.91 \pm 0.02^{e,4}$	$4.40 \pm 0.06^{d,1}$	153.11**
Mango	$5.29 \pm 0.28^{d,3}$	$4.88 \pm 0.32^{d,2}$	$5.50 \pm 0.07^{c,3}$	$4.58 \pm 0.03^{c,2}$	$4.13 \pm 0.21^{c,1}$	22.48**
MA-Reactivated	$4.56 \pm 0.05^{b,1}$	$4.50 \pm 0.32^{c,1}$	$5.33 \pm 0.07^{c,3}$	$4.90 \pm 0.03^{e,2}$	$4.48 \pm 0.15^{d,e,1}$	109.7**
Pineapple	$4.55 \pm 0.04^{b,5}$	$4.43 \pm 0.01^{b,c,4}$	$4.39 \pm 0.01^{a,3}$	$4.30 \pm 0.02^{a,2}$	$3.57 \pm 0.04^{a,1}$	861.8**
PA-Reactivated	$4.38 \pm 0.13^{a,2,3}$	$4.41 \pm 0.01^{c,2}$	$4.41 \pm 0.01^{a,2}$	$4.50 \pm 0.02^{b,2}$	$4.16 \pm 0.09^{c,1}$	19.6**
F- value	27.19**	8.26**	71.11**	150.24**	47.64**	

Mean ±SD of three trials
Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column wise comparison while numericals indicate row wise comparison
**indicates significant difference ($p<0.01$)

pH values ranged between 5.24 to 4.03 for the reactivated control samples and 5.33 to 4.16 for the reactivated fruit immobilized samples. Significantly lower pH values were found for the fruit based reactivated milk samples after 60-days of storage compared to the initial reactivation days in most of the cases indicating that 60-days of storage did not affect the viability of the immobilized cells. pH values on reactivation increased between the 15th day (control and mango) and 30th day (sapota, banana and pineapple) for the samples as the storage period progressed. Values were close to each other for control and immobilized samples.

On comparing the storage day samples with reactivated samples, control samples showed significantly lower pH between day-0 to day-7 which increased significantly between day15 to day60 indicating increased viability on reactivation in the initial period compared to the later period. Mango pieces also showed similar results as control. On day15, no significant differences were observed between values of stored and reactivated fruit immobilized fermented milk samples, whereas compared to stored samples reactivated fruit immobilized samples showed 7 - 9.5 % higher pH on day 30 and 8-19% higher pH on day 60, indicating lower viability from day 30 onwards compared to initial days on reactivation, for all fruits.

Oliveira et al. [12] observed the pH of milk fermented by *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Bifidobacterium lactis* in co-culture with *Streptococcus thermophilus* to range from 4.32 to 4.48 on day1 of fermentation and to range from 4.15- 4.37after 7 days which is similar to the present study except for mango sample. Birollo et al. [13] observed that the pH of fermented milk was primarily 4.7, diminishing to 4.25 at the end of 45 days.

Table 2 shows the acidity of the stored and reactivated fermented milk samples. Acidity of stored fermented milk samples ranged from 0.56 to 0.99 % and reactivated fermented milk samples ranged from 0.53 to 1.51%, respectively.

Table: 2 Acidity(% lactic acid) of stored and reactivated fermented milk samples

Samples	Day 0	Day 7	Day 15	Day 30	Day 60	F- value
Control	$0.88 \pm 0.02^{d,1}$	$0.88 \pm 0.03^{c,1}$	$0.86 \pm 0.03^{e,f,1}$	$0.85 \pm 0.03^{d,1}$	$0.89 \pm 0.01^{d,1}$	1.825*
C- Reactivated	$0.88 \pm 0.02^{d,1}$	$1.12 \pm 0.22^{d,2}$	$0.88 \pm 0.05^{f,1}$	$1.51 \pm 0.02^{g,3}$	$0.87 \pm 0.02^{d,1}$	30.18**
Sapota	$0.73 \pm 0.01^{c,1}$	$0.77 \pm 0.01^{c,b,a,2}$	$0.82 \pm 0^{c,d,3}$	$0.85 \pm 0.01^{d,e,4}$	$0.86 \pm 0.03^{d,4}$	42.091**
Sap-Reactivated	$0.67 \pm 0.02^{b,2}$	$0.71 \pm 0.01^{a,b,3,4}$	$0.74 \pm 0.01^{a,4}$	$0.68 \pm 0.03^{b,2,3}$	$0.61 \pm 0.02^{a,b,1}$	24.18**
Banana	$0.74 \pm 0.02^{c,1}$	$0.77 \pm 0.01^{b,c,a,2}$	$0.83 \pm 0.00^{d,e,4}$	$0.81 \pm 0^{c,d,e,3,4}$	$0.79 \pm 0.02^{c,2,3}$	23.117**
Ba-Reactivated	$0.66 \pm 0.01^{b,1}$	$0.87 \pm 0.01^{c,4}$	$0.82 \pm 0.01^{c,d,1}$	$0.65 \pm 0.01^{a,b,2}$	$0.53 \pm 0.01^{a,b,1}$	719.55**
Mango	$0.56 \pm 0.05^{a,1}$	$0.66 \pm 0.07^{a,2}$	$0.76 \pm 0.01^{b,3}$	$0.80 \pm 0.01^{c,d,3}$	$0.65 \pm 0.07^{b,2}$	42.57**
Ma-Reactivated	$0.73 \pm 0.02^{c,3}$	$0.79 \pm 0.02^{b,c,3}$	$0.77 \pm 0.01^{a,b,3}$	$0.61 \pm 0.02^{a,2}$	$0.54 \pm 0.08^{a,b,1}$	31.075**
Pineapple	$0.75 \pm 0.02^{c,1}$	$0.82 \pm 0.01^{c,b,1}$	$0.79 \pm 0^{b,c,1}$	$0.99 \pm 0.01^{f,2}$	$1.02 \pm 0.11^{e,2}$	24.071**
PA-Reactivated	$0.88 \pm 0.01^{d,3}$	$0.87 \pm 0.03^{c,3}$	$0.87 \pm 0.03^{f,3}$	$0.78 \pm 0.07^{c,2}$	$0.65 \pm 0.04^{b,1}$	21.104**
F-value	89.32**	11.59**	33.12**	292.01**	40.23**	

Mean ±SD of three trials
Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column wise comparison while numericals indicate row wise comparison
*indicates significant difference ($p<0.05$) while**indicates significant difference ($p<0.01$)

Control sample showed stable acidity during the storage period (0.85-0.89%), pineapple samples showed slightly higher values (0.75-1.02%) while mango pieces showed slightly lower values (0.56-0.80%). Stored fermented milk samples with sapota and pineapple showed significantly higher values on day 30 and 60 compared to the initial days indicating increasing microbial activity as the storage progressed.

During the reactivation period acidity of control milk samples ranged between 0.87- 1.12% and showed significantly higher values on the 7th and 30th day indicating no major trend. Reactivation data for fruit immobilized samples showed a range between 0.53 to 0.88 %. Reactivated sapota showed a range of 0.61- 0.74, banana showed a range of 0.53-0.87 and mango showed a range of 0.54- 0.77%. Pineapple showed a range of 0.65-0.88 and showed significant decreases on day 30 and day 60 compared to the initial days, indicating decreased viability on reactivation as the storage period increased. All fruits showed increased activity on day-7 and day-15 compared to initial day (day 0) and final days (day 30 and day 60).

Comparing storage and reactivated data, control samples on reactivation days did not differ from storage days in general, except on day 7 and day 30 where significantly higher values were observed for reactivated samples. Acidity values for sapota samples showed significant decreases for the reactivation days (0.61 to 0.57 %) compared to the storage days (0.73-0.86 %) and were significantly lower as the storage period increased. Similarly for banana on reactivation (0.53 – 0.87 %), values showed significant decrease compared to storage days (0.74-0.83 %) as the storage period progressed, both fruits indicating lower viability on reactivation. For mango on reactivation (0.79-0.54 %), values were significantly higher compared to storage days (0.56-0.80 %) but values decreased significantly as storage period increased, especially on the 30th and 60th day. For pineapple samples on reactivation, acidity values were significantly higher (0.65 – 0.88 %) compared to the storage days in the initial period and significantly lower in the later period i.e. 30th day and 60th day and the values decreased significantly as the storage period increased.

Data indicates that in the treatment, free cell as control showed almost similar acidity on reactivation even until 60 days of storage, but none of the immobilized fruit pieces were able to maintain acidity after reactivation, indicating lower viability compared to free cells. From 0 day to the 15th day all fruits (except sapota) showed significantly higher values on reactivation day compared to the respective storage day indicating increased viability on reactivation but on day 30 and day 60 significantly decreased values which indicate a decrease in viability. Banana and mango samples showed the least activity on day 60 compared to sapota and pineapple, although no significant differences were observed.

Ozer et al. [14] observed a production of lactic acid ranging from 9.9 to 11.8 mg/g after 1 day of fermentation, and from 13.3 to 15.8 mg/g after 14 days of storage. Oliveira et al. [12] reported lactic acid production ranging from 8.1 to 10.3 mg/g on day 1 of fermentation and from 9.0 to 12.4 mg/g after 7 days of fermentation which is similar to the present study in the case of control and pineapple fermented milk samples. But mango samples, showed significantly lower acidity in our study. Kopsahelis et al. [15] showed that in thermally dried immobilized cells of *Lactobacillus delbrueckii subsp. bulgaricus* on apple pieces stored at 4–6°C, reactivation in whey was immediate after 165 days of storage and the immobilized biocatalyst was able to produce up to 51.7 g/L of lactic acid at 37 °C. Shekher et al. [16] noted that in the case of dairy products there is no proportional relationship between pH and acidity because of buffering capacity of milk solids. In the results for dahi preparation from cow milk he found a pH range between 4.11 to 4.20 whereas acidity of the same ranged between 0.65 to 0.79 % in which 0.65 % acidity corresponded to 4.11 pH and 0.79% acidity corresponded to 4.20 pH.

Table 3 shows the lactose content of stored and reactivated fermented milk samples. Lactose content of stored and reactivated fermented milk samples ranged between 1.68 to 5.08 and 2.03 to 5.08%, respectively.

Table:3 Lactose content (g%) of stored and reactivated fermented milk samples

	Day 0	Day 7	Day 15	Day 30	Day 60	F- value
Control	5.08 ±0.06^{f,3}	3.9 ±0.36^{e,f,1,2}	3.47 ±0.11^{b,1,2}	4.11 ±0.16^{e,2}	3.21 ±1.11^{c,d,1}	7.41**
C- Reactivated	5.08 ±0.06^{f,4}	3.05 ±0.52^{c,d,2}	2.40 ±0.07^{a,1}	4.17 ±0.23^{e,3}	4.11 ±0.32^{d,3}	51.01**
Sapota	4.22 ±0.26^{d,e,3}	3.07 ±0.40^{c,d,1,2}	4.25 ±0.07^{f,3}	3.69 ±0.23^{d,2,3}	2.79 ±0.85^{a,b,c,1}	8.74**
Sap-Reactivated	4.2 ±0.54^{d,e,2}	3.41 ±0.36^{d,e,1}	4.00 ±0.07^{c,2}	3.66 ±0.07^{d,1}	3.36 ±0.11^{b,c,d,1}	8.3**
Banana	3.54 ±0.17^{b,c,3}	1.67 ±0.38^{a,1}	4.15 ±0.04^{d,e,f,4}	3.29 ±0.08^{c,3}	2.65 ±0.47^{a,b,2}	44.54**
BA-Reactivated	3.30 ±0.34^{b,c,1}	3.48 ±0.49^{d,e,1}	4.05 ±0.04^{c,d,2}	3.54 ±0.14^{d,1}	3.46 ±0.28^{b,c,d,1}	4.68*
Mango	4.74 ±0.55^{e,f,5}	1.67 ±0.11^{a,1}	4.12 ±0.02^{c,d,e,4}	3.00 ±0.11^{b,2}	3.54 ±0.05^{c,d,3}	84.36**
MA-Reactivated	4.24 ±0.09^{d,e,4}	2.50 ±0.36b^{,c,1}	4.22 ±0.02^{e,f,4}	3.49 ±0.03^{c,d,3}	3.20 ±0.12^{b,c,2}	65.4**
Pineapple	2.55 ±0.43^{a,1}	4.26 ±0.29^{f,3}	3.51 ±0.19^{b,2}	2.58 ±0.08^{a,1}	2.29 ±0.42^{a,1}	28.19**
PA-Reactivated	3.97 ±0.69^{c,d,3}	2.03 ±0.35^{a,b,1}	3.82 ±0.19^{c,2,3}	3.29 ±0.23^{c,2}	2.84 ±0.49^{a,b,c,2}	11.81**
F- value	17.27**	23.5**	206.33**	38.75**	3.99**	

Mean ±SD of three trials
Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column wise comparison while numericals indicate row wise comparison
*indicate significant difference ($p<0.05$) while **indicates significant difference($p<0.01$)

For the storage days lactose content ranged between 5.08-3.21% for control and from 4.74 to 1.67 % for fruit immobilized samples. Except day 15 all immobilized fruit-fermented milk samples showed significantly ($p\leq0.01$) lower lactose content than control, indicating higher utilization. Lactose content of pineapple immobilized samples was observed to be significantly lower compared to control and other fruit immobilized pieces on 0, 30 and 60th day for the storage day values. Lactose content of all samples showed 10-36 % decrease at the end of the storage period compared to initial day (Day 0) indicating lactose utilization in general.

For the reactivation days, lactose content ranged between 5.08 – 2.40 % for control and from 4.24 - 2.03 %

for fruit immobilized samples. For control least values of lactose were found on day15 and day30 (significant) after reactivation as compared to other days. After reactivation of samples, on day7 minimum lactose content was observed for all fermented milk samples followed by day 60 indicating fluctuation in values. For sapota, mango and pineapple, there was a significant decrease in lactose content on the 7th day in the reactivated samples compared to 0 day indicating 7th day samples to be more active than all other days of storage. Reactivated pineapple sample showed significantly lower lactose content compared to all other reactivated fermented milk samples.

Comparing the reactivated samples with stored samples, on the initial day (day0) reactivated sapota, banana and mango fermented milk samples showed lower lactose content (0.5 to 10.55 %) than day 0 samples whereas pineapple sample showed 55% higher lactose content because stored pineapple samples showed lower values on day 0. For control samples and fruit immobilized samples except banana, day0 values showed the highest lactose content whereas during the storage period lactose values were lower on reactivation, indicating higher utilization of lactose on reactivation during the storage period which indicates that viability of the biocatalysts is not lost during storage.

Saccaro et al. [17] observed that after the fermentation period, lactose content of all the fermented milk samples averaged 4.12 ± 0.05 %.Values found in the present study were similar to these. Similar observations were reported by [18], [19] and [20] which was mainly attributed to the metabolic activity of the starter cultures and probiotic organisms. Similar experiment was carried out by Kourkoutas et al. [9] who immobilized *L.casei* cells on apple pieces and used it for probiotic fermented milk production. Lactose content of stored and reactivated fermented milk on 98 days of storage and 129 days of storage was found to be 19.1g/l and 28.8g/l, respectively, for stored fermented milk and 23.8 and 27.53 g/l, respectively, for reactivated fermented milk in their study. These values are higher than the 60th day results of the present study. But higher lactose content in reactivated samples compared to stored ones is also found in the study by [9].Teh et al. [21] immobilized cells on agro wastes and the results of the study showed that soymilk containing immobilized cells showed greater reduction of soy sugars such as stachyose, raffinose, sucrose, fructose, and glucose compared to the control.

Lactobacilli count of stored fermented milk samples and reactivated biocatalysts ranged between 3.95 to 8.15 and 5.06 - 9.79 log cfu/g, respectively (Table 4).

Table: 4 *Lactobacillus* count of stored fermented milk and fruit pieces after reactivation (log cfu/g)

	Day 0	Day 7	Day 15	Day 30	Day 60	F-value
Control	5.05 ±0.04^{a,2}	5.99 ±0.03^{b,c,d,3}	5.18 ±0.03^{a,b,2}	3.95 ±0.07^{a,1}	5.03 ±0.2^{b,2}	227.58**
C- Reactivated	5.06 ±0.12^{a,2}	6.27 ±0.84^{c,d,3}	5.16 ±0.05^{a,b,2}	4.35 ±0.15^{b,1}	6.33 ±0.09^{d,3}	19.35**
Sapota	5.34 ±0.04^{b,1}	5.29 ±0.93^{a,b,1}	6.47 ±0.7^{d,2}	7.48 ±0.16^{b,c,3}	8.15 ±0.09^{g,3}	23.44**
Sap-Reactivated	7.08 ±0.17^{d,e,2}	6.62 ±0.22^{a,b,1,2}	6.66±0.7^{b,c,1}	8.78 ±0.16^{e,3}	6.08 ±0.06^{b,1}	73.82**
Banana	5.82 ±0.42^{c,1}	5.75 ±0.61^{a,b,1}	7.23 ±0.18^{d,2}	7.07 ±0.05^{b,2}	7.44 ±0.07^{f,2}	22.37**
BA-Reactivated	7.42 ±0.08^{f,2}	7.36 ±0.19^{c,d,2}	7. 3 ±0.18^{e,2}	9.00 ±0.62^{e,3}	6.37 ±0.12^{c,1}	78.14**
Mango	5.45 ±0.03^{b,1,2}	5.06 ±0.75^{a,1}	5.72 ±0.21^{a,2}	7.33 ±0.26^{b,c,4}	6.47 ±0.16^{d,3}	23.12**
MA-Reactivated	7.20 ±0.12^{e,f,3}	6.13 ±0.2^{c,2}	5.87 ±0.21^{c,2}	8.11 ±0.56^{d,4}	5.13 ±0.12^{a,1}	115.83**
Pineapple	5.93 ±0.04^{c,d,1}	6.78 ±0.08^{d,2}	6.74 ±0.13 ^{d,e,3}	7.83 ±0.58^{c,d,3}	6.99 ±0.03^{e,2}	32.92**
PA-Reactivated	7.89 ±0.04^{g,1}	7.86 ±0.12^{d,1}	8.74 ±0.13^{d,1}	9.79 ±0.33^{f,3}	8.07 ±0.17^{g,2}	202.8**
F- value	59.72**	6.12 **	32.6**	110.39**	517.12**	

Mean ±SD of three trials
Reactivated values indicate the microbial count of immobilized fruit pieces.
Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column –wise comparison while numericals indicate row –wise comparison
**indicates significant difference (p<0.01)

Stored control milk samples showed almost no change in *lactobacillus* count as days of storage progressed which ranged between 5.99 - 5.03 log cfu/g showing a decrease only on day30. For stored fruit immobilized samples values ranged between 5.06-8.15 log cfu/g during the storage period and were higher than stored control values. Sapota fermented milk showed significantly higher *lactobacillus* count (5.29-8.15) compared to all other biocatalysts during the storage period.

For the reactivated biocatalysts, *lactobacillus* count ranged 4.35-6.33 log cfu/g for control and from 5.13 – 9.79 log cfu/g for immobilized fruit pieces. After reactivation of fruit biocatalyst, *lactobacillus* count was observed to be significantly higher in pineapple pieces (7.86-9.79 cfu/g) compared to other biocatalysts. Banana pieces showed moderate count (6.37-9.00 cfu/g) whereas mango pieces (5.13-8.11cfu/g) showed the least count. On the 60th day after reactivation, except pineapple, all other fruit biocatalysts namely sapota, banana and mango showed significant (14 to 28%) decreases in count.

On comparing the reactivation data with the storage data, all samples, control and fruit-immobilized showed increased count on reactivation for almost all days indicating increased viability on reactivation.

Oliveira et al. [12] observed that after day 0, probiotic counts ranged from 7.37 to 9.13 log cfu/ml whereas after 7 days of fermentation, the viability of the probiotic bacteria, *L. acidophilus* and *B. lactis* decreased by only ~0.04 and ~0.15 log cfu/mL, respectively in fermented milk. However, the counts remained stable for *L. rhamnosus* and *L. bulgaricus* cultures. In the study by Krasaekoopt and Suthanwong [22] on vacuum impregnation of probiotics in fruit pieces followed by partial drying of fruits and storage at 4°C for four weeks, for guava and papaya, fresh count was found to be 8.52 and 8.85 log cfu/g respectively, whereas at the end of storage it decreased to 7.17 and 7.52 log cfu/g respectively which was similar to the results obtained for mango and sapota pieces in the present study. In this study initially count increased during storage up to 3 weeks and dropped in the 4th week for both the partially dried fruits.

Around $3x10^6$ yeast cells were attached per gm of watermelon fruit pieces in the study by Reddy et al. [23]. Every time the biocatalyst was washed and reused (reactivation) for wine fermentation it showed 1 log decrease in count than the initial day.

Conclusion:

The results clearly showed that *L. paracasei* cells could successfully survive for extended storage time periods on immobilization in fruit pieces especially pineapple pieces. Pineapple showed pH and acidity values closest to control during the storage as well as the reactivation period whereas for lactose content pineapple and banana showed lower values and better results than control. *Lactobacillus* count was higher for pineapple, sapota and banana biocatalysts as compared to control. The fact that no loss of activity was observed on reactivation after various storage time periods, strengthens further the possibility for survival of immobilized *L. paracasei* on fruits pieces for a long period. The study indicates that selected fruit pieces showing increased viability of cells can be used for probiotic food product development.

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