International Journal of Fermented Foods: v.4.n.1-2 p. 23-33 June and Decemeber 2015

DOI: 10.5958/2321-712X.2015.00003.4

Probiotic lassi preparation and sensory evaluation using *L. paracasei* immobilized dry fruit pieces

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Abstract

The objective of the study was to formulate and study sensory acceptability and probiotic cell count of fresh and stored lassi prepared using probiotic cells immobilized on two selected dry fruits. Free cells of the probiotic microorganism and cells immobilized in alginate beads were used for control lassi preparation. The organism used was *L. Paracasei* and the dry fruits selected for immobilization were figs and dates.Sensory evaluation (using modified Hedonic score card) of the fresh and stored products was carried out and *L. paracasei* count (on RCABV Agar) was also enumerated.

Results indicated that for all storage days (0d, 7d, 15d & 21d) fig lassi followed by date- lassi obtained scores higher than 8. But as the number of days of storage increased acceptability showed a decrease in dates whereas in the case of free cells and alginate beads the product showed an increase in acceptability. For microbial analysis date - lassi showed higher *L. paracasei* count (above 9 log cfu/gm) in the fresh product and during the storage period. All the stored products showed a count above 7 log cfu/gm upto 21 days of storage.The study concludes that dry fruit – immobilized lassi is highly acceptable and may be stored upto a period of 21 days without a decrease in probiotic count.

***Keywords*:** Probiotic, immobilization, lassi preparation, sensory evaluation

In recent years an increased interest has developed in formulating novel foods containing probiotic micro-organisms such as *Lactobacillus* and *Bifidobacterium* spp.. Such functional foods have great potential for promoting human health by maintaining and improving the intestinal microbial balance (Mattila-Sandholm *et al*. 2002). Probiotic products should be provided with an adequate amount of live bacteria (at least 10^7 cfu/ g; Oliveira *et al*. 2002) to survive the acidic conditions of the upper gastrointestinal tract and proliferate in the intestine to obtain the health-promoting benefits. The probiotic concept yet remains ineffective partly as a result of viability losses in traditional probiotic products. Immobilization of probiotics in gel matrices, most notably calcium/sodium alginate and κ-carrageenan, is a mild process that is used to protect cells from storage and GI transit (Kailaspathy and Chin, 2000). Immobilization is the physical entrapment of microbial cells in or on a polymer matrix.

Immobilization supports of food grade quality should satisfy additional prerequisites such as purity, cost effectiveness, availability and positive contribution to the sensory characteristics of the final product.

Cell immobilization on a food grade support holds promise on the basis of food safety and flavor development in the product especially when fruits are used as support material.Apple pieces and pear were used as support material for immobilization of *Saccharomyces cerevisiae* yeast cells for wine production and also in probiotic fermented milk, cheese and lactic acid production (Kourkoutas 2001, 2002, 2005, and 2006)resulting in improved productivity, aroma and taste.

Fruits have been traditionally added to dairy products for flavour enhancement. Flavor is a key factor for food stuff acceptability by consumers. *Lassi* is a ready-to-serve, fermented dairy product obtained after the growth of selected culture, usually *Lactic streptococci*, in heat-treated milk followed by sweetening with sugar and / or fruit additives. It has a creamy consistency, sweetish rich aroma, and a mild to acidic flavour, which makes the product refreshingly palatable. It is consumed as a cold, refreshing therapeutic beverage in summers in India (Anjana *et al*. 2002). Manufacture of the indigenous dairy product *lassi* with probiotic micro-organisms along with dry fruit flavor could provide an alternate variety to the health conscious consumer.

The present study was designed with the aim to formulate and evaluate sensory acceptability and probiotic cell count of fresh and stored probiotic lassi prepared using probiotic cells immobilized on two selected dry fruits, namely figs and dates.

Materials and Methods

Material procurement

Lactobacillus paracasei 022 selected as the probiotic microorganism for immobilization and the yogurt strains *Streptococcus thermophilus* 074 and *Lactobacillus Bulgaricus* 09 were procured from NCDC at NDRI, Karnal. Dry fruits selected for immobilization were fig and date which were obtained from the local market of V.V. Nagar.

Immobilization procedure

Alginate bead immobilization

Alginate bead immobilization was carried out as described by King (1983). Cell suspension of OD 1 was prepared and mixed in 1:1 ratio with sterile 2% sodium alginate solution. Resulting 1% alginate - bacteria mixture was extruded using a sterile syringe with needle. The droplets were introduced into 4% sterile calcium chloride solution at room temperature and stored in the solution for 30 min to allow complete gelation (Care was taken to see that every bead is of equal size.). Discarded the supernatant and washed the beads with D/W and transferred to 0.4% sterile calcium chloride solution. The beads were stored in this solution at 4^{o} C until use on the next day (approx 135-140 no. of beads were prepared from 1ml of culture + 1ml of sodium alginate mixture).

Immobilization of fruit pieces

Immobilization of fruit pieces were carried out as described by Kourkoutas *et al*. (2005). For immobilization, cells were grown on a synthetic medium as described by Kourkoutas *et al*. (2005). This medium was sterilized at 121°C for 15min. Flasks were inoculated with 1% of the activated probiotic micro-organisms and incubated at 37 ^{0}C without agitation. About75g of each of the auto claved dry fruit pieces were introduced in 250ml of liquid culture of *L. paracasei.* Both dry fruits were incubated in the liquid culture for 24 h at 37^{o}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 preparation of lassi. 15 g of immobilized fruit was inoculated per 100 ml of milk for lassi preparation.

Preparation of Lassi

100ml of UHT treated milk was coagulated using *S. thermophilus, L. Bulgaricus* and the probiotic organism *L.paracasei* as free cell or in the immobilized form (alginate bead immobilized, date - immobilized or fig immobilized). After coagulation, cooled to 4 ^{o}C, then added 15% (15 ml) of water and 15% (15 gm) of sugar and mixed using a hand blender. In the case of alginate-immobilized sample the beads were added after blending.

List of samples prepared

Sample 1: Probiotic control lassi (ST + LB + free cells of *L. paracasei*),

Sample 2: Alginate bead lassi (ST +LB + alginate bead(immobilized *L. paracasei*))

Sample 3: Fig lassi (ST + LB + immobilized fig pieces)

Sample 4: Date lassi (ST + LB + immobilized date pieces)

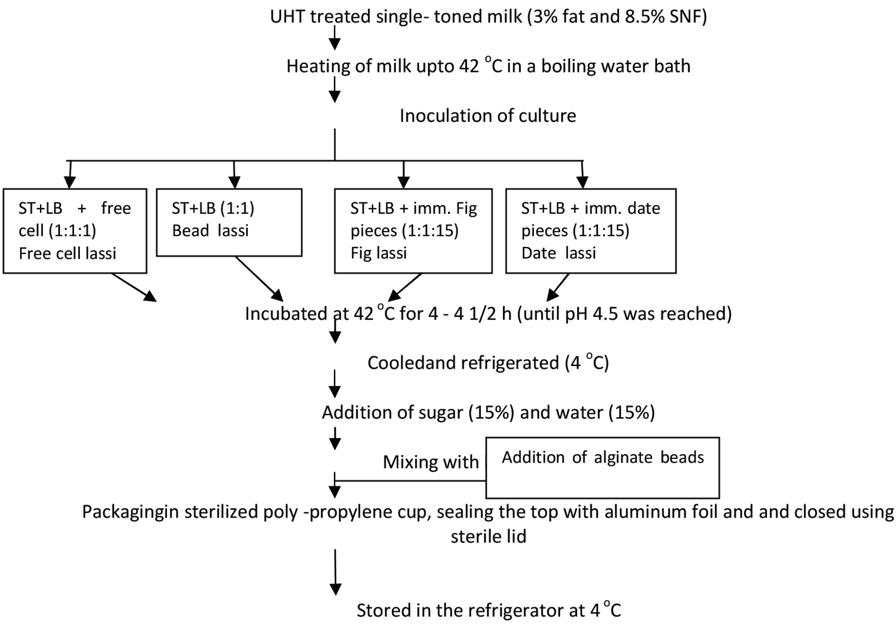

Fig 1: Flow chart for lassi preparation

D. Parameters Studied

Sensory evaluation

All the four samples were evaluated for organoleptic characteristics (such as color/appearance, odor, texture, taste, acceptability scores based on sourness, and sweetness etc.) and overall acceptability by 8 panelists who comprised of M.Sc and Ph.D students of the P.G. Department of Home Science; using a nine point hedonic scale ranging from excellent (score = 9) to very poor (score = 0) as extremes (Obi *et al*., 2010).

Microbial Analysis

RCA (Reinforcel Clostridial Agar) with bromocresol green and vancomycin (RCABV)was used for enumerating *L. paracasei* (Phillips *et al*. 2006)for all of the four samples. On each sampling day, portions of 5 g samples were collected aseptically and blended in a stomacher with 45 ml of sterile 0.1% peptone water and submitted to serial dilution with the same diluents. For bead enumeration 5 g bead lassi sample was blended with 45 ml of 2% citric acid solution.

The plates were incubated for 48 h at 37 °C prior to observation,the colonies were counted and the results were expressed in logarithm of colony forming units per gram of product (log cfu /g). All plate counts were carried out in duplicates.

Storage of products

The shelf-life of the formulated *lassi* samples were studied for a period of 21 days. At every 7 day- interval, the stored (refrigerated) samples were examined for overall acceptability using sensory evaluation and micro-biological analysis. Thus evaluation was carried on four occasions namely 0day, 7thday, 14thday and 21st day. Minimum three repetitions were carried out for the parameters studied.

Statistical analysis

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

Table1: Color and appearance of control and experimental lassi

Product	Day 1	Day 7	Day 14	Day 21	F- Value
Free cell	8.25 ± 1.26 $^{a, 1}$	8.25 ± 0.79 $^{a, 1}$	8.50 ± 1.07 $^{a, 1}$	8.50 ± 0.92 $^{a, 1}$	0.246
Bead	8.25 ± 1.33 $^{a, 1}$	8.25 ± 1.08 $^{a, 1}$	8.25 ± 1.77 $^{a, 1}$	8.53 ± 0.95 $^{a, 1}$	1.155
Fig	8.38 ± 0.65 $^{a, 1}$	8.50 ± 0.52 $^{a, 1}$	8.50 ± 1.54 $^{a, 1}$	7.88 ± 0.86 $^{a, 1}$	1.345
Date	8.25 ± 0.69 $^{a, 1}$	8.50 ± 1.13 $^{a, 1}$	8.38 ± 0.86 $^{a, 1}$	7.88 ± 0.99 $^{a, 1}$	1.005
F- Value	0.373	0.259	0.506	0.170	

Mean ±SEM of three trials

Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column –wise comparison
Numericals indicate row –wise comparison.

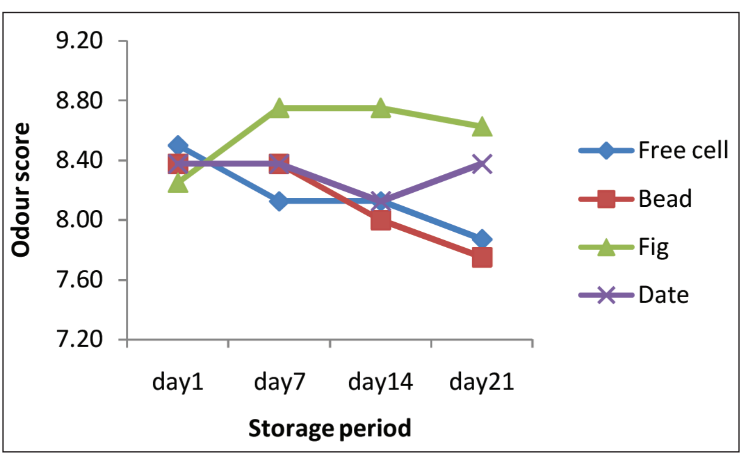

Fig. 2: odor of control and experimental lassi

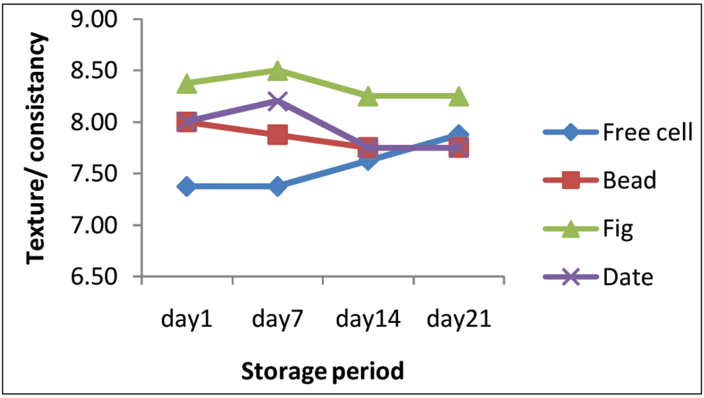

Fig. 3: texture/consistency of control and experimental lassi

Table 2 : Taste of control and experimental lassi

Product	Day 1	Day 7	Day 14	Day 21	F- Value
Free cell	8.38 ± 1.42 [a, 1]	8.00 ± 1.23 [a, b, 1]	8.00 ±0.89 [a, b, 1]	8.00 ± 0.78 [a, 1]	0.462
Bead	8.25 ± 1.49 [a, 1]	7.38 ± 1.31 [a, 1]	7.88 ±0.91 [a, 1]	7.75 ± 0.83 [a, 1]	1.081
Fig	8.75 ± 0.66 [a, 1]	8.50 ± 0.78 [b, 1]	8.25 ±1.73 [a, b, 1]	8.38 ± 0.91 [a, 1]	0.939
Date	8.38 ± 0.77 [a, 1]	8.75 ± 1.44 [b, 1]	8.75 ±1.23 [b, 1]	8.13 ± 1.44 [a, 1]	1.824
F- Value	0.672	3.862*	0.514	1.390	

Mean ±SEM of three trials

Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column –wise comparison
Numerical indicate row –wise comparison
*indicate significant difference($p<0.05$)

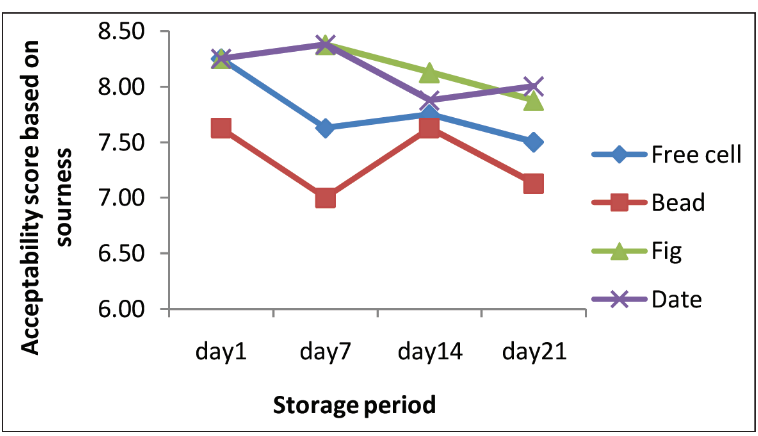

Fig. 4: Acceptability score based on sourness of control and experimental lassi

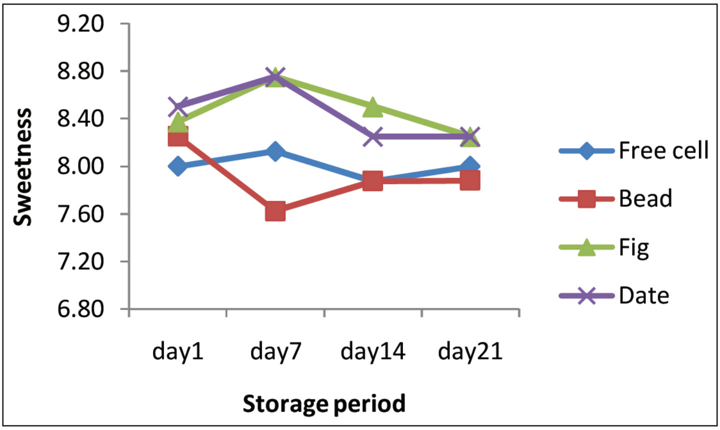

Fig. 5: Sweetnessscore of control and experimental lassi

Table 3: Overall Acceptability control and experimental lassi

Product	Day 1	Day 7	Day 14	Day 21	F- value
Free cell	7.62 ± 1.04 [a, 1]	8.17 ± 0.94 [a, b, 1]	7.79 ± 0.97 [a, 1]	7.93 ± 0.92 [a, 1]	0.393
Bead	7.54 ± 1.13 [a, 1]	7.67 ± 0.89 [a, 1]	7.70 ± 0.78 [a, 1]	7.71 ± 1.07 [a, 1]	0.768
Fig	8.75 ± 0.66 [b, 1]	8.50 ± 0.67 [b,1]	8.38 ± 1.40 [a, 1]	8.25 ± 0.92 [a, 1]	0.793
Date	8.38 ± 0.66 [a, b, 1]	8.75 ± 0.80 [b, 1]	8.13 ± 1.19 [a, 1]	8.00 ± 0.86 [a, 1]	1.210
F- value	4.120*	3.990*	0.28	0.374	

Mean ±SEM of three trials

Means carrying similar superscripts within a column/row are not significantly different
Alphabets indicate column –wise comparison
Numerical indicate row –wise comparison.
*indicate significant difference($p<0.05$)

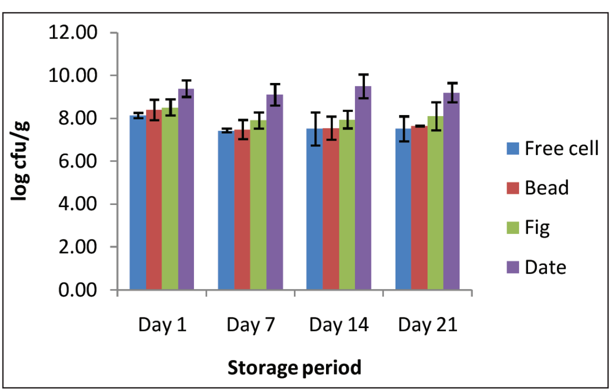

Fig. 6: *L. paracasei* count of control and experimental lassi

Results and Discussion

Table 1 shows the colour and appearance of the different lassi samples. Scores ranged between 7.88 - 8.50 during the study period. No significant differences were observed upto 14 days of storage of lassi, whereas on day 21 a slight dullness in colour was observed in fig and date -based lassi. Karasaekoopt *et al*. (2008) noted that the addition of probiotic beads significantly ($p<0.05$) increased the intensity of yellow color in yogurt.

Figure 2 shows the odour of the different lassi samples. Scores ranged between 7.75 - 8.75 during the study period. No differences were observed for odour values for the different lassi samples upto day 14 (i.e score ranged between 8.00 – 8.75) whereas on day 21 free cell and bead- immobilized samples showed significantly lower values. It is possible that the fruit based lassi sample showed higher values due to a fruity flavour.

Figure 3 shows texture/consistency of the different lassi samples. Values ranged between 7.38 - 8.50. Comparing texture/consistency of control and immobilized lassi samples on day 1 and day 7, fig lassi showed significantly higher values compared to other samples but on day14 and day 21 although values were higher no significant differences were observed. Free cell lassi showed values between 7.38 - 7.88 but no significant differences were observed during the storage period. A slight non significant increase in values on day 14 and day 21 was observed. According to Patidar and Prajapathi (1998) there is a slow coagulation effect caused by micro-organisms during storage. Karasaekoopt *et al.* (2008) also noted that the presence of probiotic bead affected the texture characteristics of plain yogurt, such as viscosity and difficulty of swallowing. In their study plain yogurt containing probiotic bead demonstrated a significantly ($p<0.05$) higher viscosity (7.4) than the yogurt without bead (control) (5.3). Increasing viscosity paralleled the difficulty of swallowing for the yogurt. Yogurt containing probiotic bead significantly ($p<0.05$) presented higher difficulty of swallowing (7.3) than that without beads (5.3). In the present study also consistency scores were slightly higher in bead lassi compared to free cell lassi.

Table 2 shows the taste scores of the different lassi samples. Scores ranged between 7.38 - 8.75 during the study period. Taste scores were lower throughout the storage period for bead- immobilized lassi samples while fig and date based lassi samples showed higher scores because of the fruity. Kourkoutas *et al*. (2006) found similar results for the prepared probiotic cheese using *L.casei* cells immobilized on apple and pear pieces where preliminary sensory evaluation showed the fruity taste of cheese containing immobilized cells on fruit pieces.

Figure 4 shows the acceptability scores based on sourness for the different lassi samples. Scores ranged between 7.00 - 8.38 during the study period. No significant differences were observed for acceptability scores based on sourness in any of the lassi samples except on day 7. Bead lassi showed a slightly lower score due to high sourness compared to other lassi samples.

Figure 5 shows the sweetness scores of the different lassi samples. Scores ranged between 7.63 - 8.75 during the study period. On day 1 no significant differences were observed for sweetness but as days of storage progressed Figure and Date-based lassi samples showed significantly higher sweetness compared to free cell and bead lassi samples.

Table 3 shows the overall acceptability scores of the different lassi samples. Scores ranged between 7.54 - 8.75 during the study period. Bead lassi sample showed significantly lower overall acceptability score followed by free cell sample whereas fig sample showed higher overall acceptability followed by date-based lassi sample. Except for colour and appearance scores, fruit based lassi samples secured higher scores for sensory attributes like odor, texture and consistency, taste, acidity, sweetness and overall acceptability and the results were found to be similar to the findings of Hingmire *et al*. (2008) and Shuwu *et al*. (2011).

Figure 6 shows the *L. paracasei* viable countof the different lassi samples. Viable count ranged between 7.43 - 9.19 log cfu/g during the study period. *L. paracasei* viable count of date sample showed a significantly higher*L. paracasei* count compared to all other lassi samples and this sample maintained a count above 9 log cfu/g through out the storage period although no significant differences were observed. Fig-lassi shows approximately 0.5 log cfu/g higher count than free cell and bead lassi samples. Free cell and bead sample *L. paracasei* count showed significantly lower counts compared to other samples but between these two samples no significant differences were observed. Kourkoutas *et al.* (2006) reported an initial count of 10^7 to 10^8 cfu/g for fruit immobilized cheese which is similar to the results obtained in the present study. Karasaekoopt *et al*. (2006) noted the viability of encapsulated *L. acidophilus*in yogurt prepared from UTH and conventionally treated milk. Yogurt sample showed an initial count of 9.6 log cfu/g on day 1 which reduced 1 log at the end of 4 weeks of storage to 8.4 log cfu/g. In thepresent study similar results were observed on day 1 where bead lassi shows an initial count of 8.39 log cfu/g which reduced to7.48 log cfu/g at the end of 21 days of storage. According to Oliveira *et al*. (2002) for a probiotic product a minimum of 10^7cfu/ml or cfu/gm count is recommended. In the present study none of the samples showed a count below 10^7 cfu/g sample throughout the storage period.

Conclusion

The study concludes that dry fruit – immobilized lassi is highly acceptable and can be stored upto a period of 21 days without significant differences in sensory score or decreases in the probiotic count. Thus dry fruits show promise as a medium for the maintenance of the probiotic micro-organism thus enhancing viability and also imparting flavor to the food product.

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