Effect of storage on sensory quality, pH, wheying-off and probiotic count of lassi supplemented with Aloe barbadensis Miller juice

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Abstract Worldwide there is a huge demand for fermented dairy products incorporated with probiotics and herbs. The commercial success of these products mainly depends on their taste and appeal which are affected by storage conditions employed. In the present study, changes in sensory, physico-chemical characteristics and probiotic counts of Aloe barbadensis Miller supplemented probiotic lassi (APL) stored at 5±1°C were evaluated. During 12 days of storage period, probiotic counts decreased from 8.4 log cfu/mL on initial day to 8.0 cfu/mL on 12th day. pH of APL gradually decreased while wheying-off increased progressively throughout the storage. Scores of all sensory attributes decreased to below 7 after 9 days of storage, minimum level of acceptance set on nine-point hedonic scale. APL was rejected after 9 days of storage by the sensory panelists owing to its unacceptable sensory quality. PCA reduced eight original variables into two principal components which accounted for 99.937% of the total variations. Instrumental wheying-off (-ve) and pH (-ve) were loaded heavily on principal component 1 indicating strong relation among these variables. Correlation analysis also revealed that instrumental wheying-off and pH were strongly dependent on each other with highest Pearson's correlation coefficient (r=-0.962, p<0.01).

Keywords : Aloe vera, lassi, probiotics, shelf-life, principal component analysis

Introduction

Probiotics are the "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Health benefits attributed to intake of probiotics include alleviation of lactose intolerance, suppression of cancer, reduction in serum cholesterol concentrations, improved gastrointestinal immunity, prevention of urinary tract infections, management of food allergies and prevention of diabetes, etc. (Chapman et al., 2011). Probiotic cultures especially Lactobacillus group have had a long association with dairy products. Among the Lactobacillus group, species Lactobacillus paracasei is a common inhabitant of the human intestinal tract and also listed in the 'Inventory of microorganisms with documented history of use in human food' (Mogensen et al., 2002). Different strains of L. paracasei have been found naturally in fermented vegetables, milk and meat. Selected strains of this species are also used in Cheddar cheese, yoghurt, Kefir and other fermented milks (Chiang and Pan, 2012).

Aloe barbadensis Miller (Aloe vera), an herb of the family Aloaceae, is considered to be the most biologically active of the approximately 420 species of Aloe found to date. Aloe vera harbours many biologically active constituents including vitamins, minerals, saccharides, amino acids, anthraquinones, enzymes, lignin, saponins, phytosterols and salicylic acids (Foster et al., 2011). Several research reports credit Aloe vera with health benefits namely antitumor, antiabetic, healing wounds and burns, treatment of ulcers, hypolipidemic, immunomodulatory and antimicrobial properties, etc. (Hussain et al., 2013). A research conducted by Sinnott et al. (2007) proved that Aloe polysaccharides can be utilized better by human colonic bacteria indicating its potential as prebiotic. Increased interests of today's consumers towards "natural health" have propelled the consumption of Aloe vera and a considerable portion of today's functional food market consists of Aloe vera used as a supplement.

Besides their functionality and nutrition, shelf-life of the functional foods plays an important role in their marketing.
Upholding the viability of probiotics above "therapeutic minimum" i.e. $10^6$ cfu/mL till their consumption is an important quality criteria for probiotic foods to confer desired health benefits to the host (Kurmann and Rasic, 1991). In addition, it is imperative to ensure that added probiotics and herbs should not adversely affect the palatability of the food during the storage (Heller, 2001). In the present study effect of storage (at 5±1°C) time on the survival of probiotic strain L. paracasei ssp. paracasei, sensory attributes, pH and wheying-off of lassi (a traditional fermented milk beverage of India) supplemented with Aloe barbadensis Miller (APL) is delineated.

**Materials and Methods**

Fresh buffalo milk and dahi culture NCDC-60 (Lactococcus lactis ssp. var diacetylactis) were obtained from Experimental Dairy and National Collection of Dairy Cultures (NCDC), respectively of the National Dairy Research Institute (NDRI), Karnal. Potential probiotic strain Lactobacillus paracasei ssp. paracasei was isolated from market samples of lassi. Prior to its use, a thorough biochemical analysis and genetic characterization of the strain Lactobacillus paracasei ssp. paracasei was carried out and the strain was then deposited in NCDC with accession number NCDC-627. Skim milk powder (medium heat classified) was procured from M/s Modern Dairies Ltd., Karnal. Food grade Aloe barbadensis Miller juice was procured from Mehta Herbs and Spices, Coimbatore. The juice had aloin content of 0.012%, mucopolysaccharides 20000 molecules in length, pH 5.1 and total solids content of 0.98%. Dehydrated media viz. MRS agar and MRS broth were procured from Hi-Media Laboratories, Bombay.

Maintenance of starter culture and probiotics

During the study period, NCDC-60 and NCDC-627 were maintained in sterilized reconstituted skim milk (11 g SMP in 100 mL distilled water) tubes. The propagation of the cultures (at 37°C) was done at weekly intervals to maintain their activity. The cultures were stored at 5°C between the transfers.

Preparation of APL

Fresh buffalo milk (standardized to 4% milk fat and 10% milk solids-not-fat) was heat treated to 90°C for 15 min followed by immediate cooling to 37°C and then added with sterilized (121°C for 16 min) Aloe vera juice at 16% level. This milk-Aloe vera mixture was inoculated with a culture combination comprising of NCDC-60 and NCDC-627 (1:1) at 2% level, followed by incubation at 37°C for 10 h to obtain a firm curd. Overnight refrigerated curd samples were added with sterilized (90°C for 5 min) and cooled (10°C) sugar syrup (50 Brix) at 14% level and subsequently broken-down using a hand blender (Type: DX 505, Leema Industries, India) to obtain APL. APL samples obtained were filled into LDPE pouches (200 mL capacity) using sachet filling machine (Kingpak, Chadha sales Pvt Ltd., New Delhi) and stored under refrigeration at 5±1°C.

Sensory evaluation, whey-ing-off and pH measurement

APL samples were evaluated for sensory characteristics using a 9 point hedonic scale suggested by Stone et al. (1974). Sensory evaluation panel consisted of ten judges having adequate knowledge about the sensory evaluation methods as well as product characteristics.

The pH of APL samples was measured at 20°C using a pH meter (pH Tutor, EUTECH Instruments, Malaysia) with combined glass electrode fitted in association with a temperature probe. Before experiment, the pH meter was standardized using standard buffers of pH 4.0 and 9.0 at 20°C.

Instrumental whey-ing-off (mL/10 mL of sample) in APL was measured by the centrifugation method described by Keogh and O’Kennedy (1998) with slight modifications.

Enumeration of L. paracasei ssp. paracasei

For the enumeration of L. paracasei ssp. paracasei, serial dilutions of APL were carried out in sterile normal saline (0.9% NaCl) and the appropriate diluents were plated on MRS agar plates followed by incubation at 37°C for 48-72 h. The counts of probiotic were expressed as log cfu/mL sample.

APL shelf-life was decided based on either deterioration in its sensory quality (over all acceptability score above 7 on 9 point hedonic scale) or decline in probiotic count below therapeutic minimum i.e. $10^6$ cfu/mL.

Statistical analysis

Data obtained from various experiments was statistically analyzed with the application of SPSS v.16.0 for Windows software (SPSS South Asia Pvt. Limited, Bangalore, India). The same software was used to apply principal component analysis (PCA) and calculate Pearson's correlation coefficients between sensory, physicochemical properties and probiotic count. All the experiments were carried out in duplicate.

**Results and Discussion**

Changes in sensory attributes

Fermented beverages undergo several chemical changes during storage due to the persistent microbial activity, which affects their palatability and hence acceptability. The flavour score of APL samples decreased gradually throughout the storage period (Fig. 1a). The analysis of variance (Table 1) indicated
that the flavour scores of APL samples differed significantly (p<0.01) within the storage days. On 12th day of APL storage, the flavour score obtained was 6.5 which was below the minimum acceptable limit fixed i.e. 7.0. The decrease in flavour score of APL with the progress in storage time could be ascribed to post-acidification phenomenon which is common in case of fermented milks. Increased perception of acidic flavour with the progress of APL storage could be due to the presence of prebiotic polysaccharides and other growth promoting substances of Aloe vera which might have helped the growth of probiotic L. paracasei ssp. paracasei leading to increased organic acid concentration. Donkor et al. (2007) also reported that presence of prebiotic substances in probiotic yoghurts altered the organic acid production, proteolysis patterns and flavour profile of probiotic yoghurts during storage.

An optimum consistency is desirable in case of fermented beverages to achieve maximum consumer acceptability. In the present study, consistency score of APL samples decreased gradually throughout the storage period (Fig. 1b). Till 9th day the consistency score was more than 7.0, however, after that on 12th day it decreased to 6.8 which was below the minimum acceptable score. During storage, starter cultures continue to grow and release proteolytic enzymes in fermented milks which results in breakdown of protein network leading to syneresis (Shihata and Shah, 2002). The wheying-off score of APL decreased at higher rate when compared with the remaining sensory attributes during the advancement of storage period.

The wheying-off scores on 0th and 12th day were 8.4 and 6.5, respectively (Fig. 1c). Analysis of variance (Table 1) showed that wheying-off scores of APL differed significantly (p<0.01) between storage days. The decrease in APL wheying-off score could be ascribed to the persistent metabolic activity of starter culture which might have destabilized the whey protein and casein networks leading to oozing out of water. The colour and appearance score of APL was 7.83 on the 0th day of storage and it was gradually decreased (Fig. 1d) to 6.9 on 12th day, which was below the minimum score of acceptance set. The lower scores of colour and appearance as judged by sensory panellists could be attributed to the addition of Aloe vera juice which was a greenish yellow coloured liquid. Similar trend was observed by Shuwu et al. (2011) for honey added lassi during their storage at refrigeration temperature.

The overall acceptability score of APL decreased with the advancement of storage time (Fig. 1e). With prolongation of storage time, increased acidity and wheying-off were resulted in decreased acceptability scores of APL. On 12th day of storage, the overall acceptability score of APL was 6.5, which was below the acceptable limit set i.e. 7.0. The results obtained in the present study are in agreement with those obtained by Çakmakci et al. (2012) for banana mix fortified probiotic yogurt.

Changes in pH

The pH of APL on initial day of manufacturing was 3.95 and decreased to 3.44 on 12th day of storage (Fig. 1f). The analysis of variance (Table 1) showed that storage days significantly (p<0.01) affected the pH of APL. The persistent growth and metabolic activity of lactic acid bacteria results in accumulation of organic acids and causes reduction in pH of fermented milks (Ruggeri et al., 2008). Presence of prebiotic polysaccharides and other growth promoting substances of Aloe vera might have sustained the metabolic activity of lactic acid bacteria leading to decrease in pH of APL.

Changes in wheying-off

The wheying-off in APL increased with the increase in storage
period (Fig. 1g). The difference in wheying-off of APL on 0th day (2.21 mL/10mL) and 12th day (3.2 mL/10 mL) of storage was significant. The increased wheying-off in APL with the progress of the storage period could be ascribed to the bacterial growth leading to protein network destabilization resulting in oozing out of water from the casein micelle which was previously observed in case of yoghurt by Peng et al. (2009).

Changes in probiotic *L. paracasei ssp. paracasei* counts

In the present study, there was no significant decrease in the probiotic count of APL during the 12 days of its storage. The viability of *L. paracasei ssp. paracasei* in APL ranged from 8.4±0.06 log cfu/mL on the initial day of its manufacture to 8.0±0.12 cfu/mL on 12th day of storage (Fig. 1h). The concentration of probiotics at the end of 12th day was slightly higher than the recommended therapeutic level i.e. 6 log cfu/mL. The survivability of *L. paracasei ssp. paracasei* in APL during storage could be attributed to the better resistance of probiotics to high acidic conditions. Donkor et al. (2007) also reported that *L. paracasei* L26 remained viable above therapeutic level of 10⁶ cfu/g and was resistant to constant decline in pH of yoghurt. Prebiotic potential of *Aloe vera* as it was reported by Sinnott et al. (2007) could also be responsible for nourishing the growth and metabolic activity of *L. paracasei ssp. paracasei* in APL. Makras et al. (2005) also advocated that prebiotics have modified the negative environmental impact created by the stress factors in food matrix by providing additional nutrients for better sustainability of probiotic bacteria.

Correlation between variables

Correlation shows the relation between pairs of variables. An able correlation analysis can lead to a greater understanding of the research data. In the present study, linear association between the variables was calculated using Pearson’s correlation coefficient (Table 2). Colour and appearance was highly positively correlated with pH (r=+0.918, p<0.01) and negatively correlated with instrumental wheying-off (r=-0.823, p<0.01). Decrease in pH led to aggregation of casein micelle and whey separation in APL thus resulted in low colour and appearance score. Flavour was highly positively correlated with overall acceptability (r=+0.939, p<0.01) and weakly correlated with probiotic count (r=+0.616, p<0.05) indicating the importance of flavour on the acceptability of APL by sensory panellists and non-significant effect of reduction in probiotic count on flavour of APL. Recently, Raju and Pal (2012) also reported positive correlation (r=+0.846, p<0.05) between overall acceptability and flavour scores of misti dahi. Consistency and overall acceptability scores were highly negatively correlated with instrumental wheying-off (r=-0.923, p<0.01 and r=-0.872, p<0.01, respectively) thus indicating wheying-off had a negative impact on the acceptability of the APL samples.

Principal component analysis

Principal component analysis (PCA) is mathematical approach used to reduce the dimensionality of the data set containing a large number of variables into a small set while losing only a small amount of information (Fievez et al., 2003). PCA transforms a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible. Principal component analysis (PCA) method is advantageously applied for the evaluation in food products analyses (Arvanitoyannis et al., 2005). PCA reduced eight original variables (colour and appearance, flavour, consistency, wheying-off, overall acceptability, pH, instrumental wheying-off and probiotic count) into two principal components which accounted for 99.937% of the total variations (Table 3). Principal component 1 with Eigen value more than 1 (Fig. 2) accounted for highest i.e. 98.784% of the variations whereas component 2 accounted for 1.153% variations with Eigen value less than 1. Eight variables were loaded on two dimensions. According to

### Table 1  Analysis of variance for storage related changes in *Aloe barbadensis* Miller supplemented probiotic lassi

<table>
<thead>
<tr>
<th>Product characteristics</th>
<th>F value</th>
<th>Mean sum of squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory attributes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour and appearance</td>
<td>29.41**</td>
<td>0.28</td>
</tr>
<tr>
<td>Flavour</td>
<td>14.65**</td>
<td>0.73</td>
</tr>
<tr>
<td>Consistency</td>
<td>28.87**</td>
<td>0.49</td>
</tr>
<tr>
<td>Wheying-off</td>
<td>36.36**</td>
<td>1.81</td>
</tr>
<tr>
<td>Overall Acceptability</td>
<td>20.65**</td>
<td>0.90</td>
</tr>
<tr>
<td>pH</td>
<td>118.8**</td>
<td>0.15</td>
</tr>
<tr>
<td>Instrumental whey-off</td>
<td>23.72**</td>
<td>0.47</td>
</tr>
<tr>
<td>Probiotic count (log cfu/mL)</td>
<td>2.74**</td>
<td>0.08</td>
</tr>
</tbody>
</table>

ns Non-significant; **p<0.01;
Stevens (1992) an attribute/variable is considered to load heavily on a given component if the factor loading was greater than 0.72. Instrumental wheying-off (-ve) and pH (-ve) were loaded heavily on principal component 1 indicating strong relation between these variables. It can also be seen from the correlation matrix (Table 2) that these two variables obtained highest Pearson's correlation coefficient ($r$=-0.962, $p<0.01$) when compared with the remaining attributes indicating their strong dependence on each other. Principal component 2 was heavily loaded with probiotic count (+ve) and sensory wheying-off (-ve). Correlation analysis also revealed a good amount of correlation ($r$=+603; $p<0.05$) between these two variables. Except wheying-off, loadings of the remaining sensory attributes did not meet Stevens (1992) directives (<0.72) and loaded imperceptibly on the biplot (Fig. 3).

**Conclusions**

In the present study, it was observed that *L. paracasei ssp. paracasei* counts in APL were not significantly affected during the 12 days of storage period (at 5±1°C). *Polysaccharides* and other growth factors present in *Aloe vera* might have sustained *L. paracasei ssp. paracasei* growth. However, post-acidification of APL during storage has led to deterioration in sensory quality and physico-chemical parameters. Owing to its unacceptable sensory quality, APL was rejected after 9 days of refrigerated storage. Principal component analysis reduced the original variables into two components which explained 99.937% of the total variations. Correlation analysis revealed that pH is highly negatively correlated with instrumental wheying-off and flavour is highly positively correlated with probiotic count.

**Table 2** Pearson's correlation coefficients between sensory, physico-chemical and probiotic count

<table>
<thead>
<tr>
<th></th>
<th>C&amp;A</th>
<th>Flavour</th>
<th>Consistency</th>
<th>Wheying-off</th>
<th>OA</th>
<th>pH</th>
<th>Instrumental whey-off</th>
<th>Probiotic count</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&amp;A</td>
<td>1.00</td>
<td>0.814**</td>
<td>0.759*</td>
<td>0.859**</td>
<td>0.709*</td>
<td>0.918**</td>
<td>-0.823**</td>
<td>0.523</td>
</tr>
<tr>
<td>Flavour</td>
<td>1.00</td>
<td></td>
<td>0.819**</td>
<td>0.908**</td>
<td>0.930**</td>
<td>0.867**</td>
<td>-0.832**</td>
<td>0.616*</td>
</tr>
<tr>
<td>Consistency</td>
<td>1.00</td>
<td></td>
<td></td>
<td>0.903**</td>
<td>0.906**</td>
<td>0.937**</td>
<td>-0.923**</td>
<td>0.652**</td>
</tr>
<tr>
<td>Wheying-off</td>
<td>1.00</td>
<td></td>
<td></td>
<td>0.941**</td>
<td>0.933**</td>
<td>-0.8082**</td>
<td>0.603*</td>
<td>0.641*</td>
</tr>
<tr>
<td>OA</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>0.902**</td>
<td>-0.872**</td>
<td>0.641*</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>-0.962**</td>
<td>0.699**</td>
</tr>
<tr>
<td>Instrumental whey-off</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td>0.635*</td>
</tr>
<tr>
<td>Probiotic count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
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* Correlation is significant at 0.05 level; ** Correlation is significant at 0.01 level;
C&A - colour and appearance; OA - overall acceptability

**Fig. 2: Scree plot showing the Eigen values for different components**

**Fig. 3: Scatter plot of the first two principal components PC1 and PC2 explaining sensory, physico-chemical and probiotic count (CA-colour and appearance; F-flavour; CS-consistency; Woff-sensory wheying off; OA-overall acceptability; InstrWoff-instrumental wheying off and PC-probiotic count)**
Acknowledgements

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References


Table 3 Total variance explained by principal components

<table>
<thead>
<tr>
<th>Component</th>
<th>Total</th>
<th>Percent variance</th>
<th>Cumulative %</th>
<th>Extraction Sum of Squared loadings</th>
<th>Total</th>
<th>Percent variance</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.939</td>
<td>98.784</td>
<td>98.784</td>
<td>4.939</td>
<td>98.784</td>
<td>98.784</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.058</td>
<td>1.153</td>
<td>99.937</td>
<td>0.058</td>
<td>1.153</td>
<td>99.937</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.002</td>
<td>0.037</td>
<td>99.974</td>
<td>0.002</td>
<td>0.037</td>
<td>99.974</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td>0.024</td>
<td>99.998</td>
<td>0.001</td>
<td>0.024</td>
<td>99.998</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.000</td>
<td>0.002</td>
<td>100.00</td>
<td>0.000</td>
<td>0.002</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Correlated with overall acceptability. These mathematical techniques demonstrated that decrease in pH increased the wheying-off and decreased the flavour scores of APL which led to its rejection by sensory panellists.