Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 4767-4777

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Special Issue-7 pp. 4767-4777
Journal homepage: http://www.ijcmas.com

Original Research Article

Studies on Microbial Profile of Probiotic Low Fat Kokum Lassi by Using Honey as Sweetner

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Keywords

Microbial Profile of Probiotic, Lassi , Honey as Sweetner

A B S T R A C T

The effect of kokum syrup and honey on microbial qualities of lassi during storage were investigated. Kokum syrup and honey was added at different levels *viz.*,K_1 H_1: Addition of Kokum syrup @ 5%+ Honey@ 8 % of curd, K_1 H_2: Addition of Kokum syrup @ 5%+ Honey @10 % of curd, K_1 H_3: Addition of Kokum syrup @ 5%+ Honey @12% of curdK_2 H_1: Addition of Kokum syrup @ 10%+ Honey @8% of curd, K_2 H_2: Addition of Kokum syrup @ 10%+ Honey @10% of curd, K_2 H_3: Addition of Kokum syrup @ 10%+ Honey @12% of curd, K_3 H_1: Addition of Kokum syrup @ 15%+ Honey @8% of curd,K_3 H_2: Addition of Kokum syrup @ 15%+ Honey @10% of curd, K_3 H_3: Addition of Kokum syrup @ 15%+ Honey @12% of curd. Addition of kokum syrup and honey improved sensory quality and acceptability of the product. The most acceptable quality lassi could be prepared by using kokum syrup @ of 10 per cent and honey @12 per cent of the curd and it contained, average *L. acidophilus* count 6.45× 10^6cfu/g, *L. casei*count 6.27× 10^6cfu/g standard plate count, 14.65 x 10^4cfu/g, Psychrophilic count 0.55 x 10^2cfu/g and *E. coli* were absent in lassi. The most acceptable quality lassi was found to have desired probiotic count (> 10^6cfu/ml) up to 12 days of storage at refrigerated temperature(9±1°C).

Introduction

Probiotic fermented milk is one of the major segment amongst fermented milks that has tremendous potential for growth and development. Milk is an excellent medium to carry or generate live and active cultured dairy products. The technology of applications of probiotic organism in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in nutrition, healthy and desirable product for the consumer. Probiotic has been therapeutically to modulate immunity, improve digestive process, prevent cancer, improve lactose intolerance etc. Lactic acid bacteria decreases serum cholesterol level and increases vitamin-D content of the product (Grill *et al.*, 2000). Lassi is an ancient refreshing beverage for quenching thirst.lassi is an indigenous milk product obtain by lactic fermentation of milk to base product "Dahi" followed by its breaking, mixing and/ or addition of water, sugar, flavouring and colouring agents to make homogenous liquid mass. In rural India lassi is also known as buttermilk. Lassi is creamy viscous fluid with rich aroma and mildly acidic in taste. On an average Lassi contains

79 per cent water, 3 per cent fat, 2.8 per cent protein, 4.5 per cent lactose and 12 per cent sugar (Sharma, 2006).

Now a days, the consumer's trend has been moved towards the foods with more natural antioxidants, dietary fibers, natural colourants, minerals, vitamins, low calories, low cholesterols and low fat and free of synthetic additives etc. Consumer would need to ingest considerably less medicine and artificially produced vitamin and mineral supplements if fermented milk products were enriched with vitamins, proteins, essential fatty acids and trace elements of natural origin. A simple way of attaining this goal is the use of cyanobacteria in the manufacture of cultured dairy foods. Kokum and honey is one of the good natural sources of these functional components.

Milk fat is composed of higher concentration of saturated fat and cholesterol to add the problems of calorie conscious people. Preparing low fat lassi i.e. from low fat buffalo milk restricts the calorie intake. Milk fat is the main contributor to the rich flavour and mouthful. Low fat lassi is bound to affect the rich taste and pleasant flavour of milk fat, however, it was hypothecated that addition of natural flavour may suppress this problem. Kokum (*Garciniaindica* Choisy), fruit has got dietary importance and it is utilized in preparation of chatni, solkadhi, fish curry and sharbat. The hydroxycitric acid (H.C.A) is present in kokum which prevents conversion of sugar into fat which is claimed to prevent bodyweight increase and fattiness can be avoided that's why some nutrition scientist considered kokum as 'antiobesity drug'. Ripe kokum fruits contain substantial amount of malic acid, little tartaric or citric acid, which has an acceptable acidic taste. It is anthelmintic, cardiotonic and useful against dysentery, pains and heart complaints. Thus all parts of kokum plant are useful in both economical and medicinal point of view (Lachake, 2006).

Natural honey is also one of the most widely sought products due to its unique nutritional and medicinal properties which are attributed to the influence of the different groups of substances it contains. Codex Alimentarius Commission (CAC) defined honey as the natural sweet substance produced by honey bees. (Buba *et al.,* 2013). Honey has been a common sweetner for foods and powerful medicinal tool for centuries. Honey is highly nutritious. It has traces of minerals and vitamins not to mention the antioxidants which destroy free radicals and delay ageing. In short, it is a safe and wholesome food for old, children and adults. Although not an herb, honey is a plant by-product and used medicinally around the world. Furthermore, some studies suggest that honey may be effective in increasing the populations of probiotic bacteria in the gut, which may help to strengthen the immune system, improve digestion, lower cholesterol and prevent colon cancer.

Hence, considering the nutritional and medicinal properties of kokum syrup and use of low fat milk in restricting the caloric intake, the present research project entitled "Probiotic low fat kokum lassi by using honey as sweetner was conducted.

Materials and Methods

For preparation of lassi, buffalo milk was collected from dairy farm of College of Agriculture, Dapoli and low fat milk was obtained by centrifugal cream separation method. Kokum syrup, salt and honey were purchased from local market.

Freeze dried cultures *viz., Lactobacillus acidophilus* (015) and *Lactobacillus casei*

(017) were procured from the National Collection of Dairy Cultures, NDRI, Karnal (Haryana).

Lassi preparation

Lassi was prepared as per the procedure described by Kadlag (1982) with partial modifications while mixing kokum syrup and honey. The probiotic lassi was analyzed for Lactobacilli count, Standard Plate Count (SPC), *Escherichia coli* count and psychrophilic count.The pour plate method was adopted for enumeration of different groups of microorganism. *Lactobacilli* count was carried out as per the procedure described by the IDF standards (117A: 1989). Rogosa SL Agar M130 was used to enumerate *Lactobacillus acidophilus.* MRS Broth Agar MRS369 was used to isolate *Lactobacillus casei*.Plate count Agar M1396 was used to enumerate standard plate count. Violet Red Bile Agar M049 was used to enumerate *E. coli* count.

Appropriate dilutions of the respective samples were transferred to sterile petriplates in duplicate. Plates were poured with respective media. Most aseptic condition was observed to avoid contamination during plating.

The plates were incubated at optimum growth temperatures and period mentioned in respective procedures. After incubation period, the colonies were counted. All *lassi* samples were stored at 9± 1°C in refrigerator for shelf life studies. The samples of stored *lassi* were analyzed for microbiological qualities on 4^{th}, 8^{th} and 12^{th} day of storage.

The experiment was laid out in Factorial randomized block design with three replications. The experimental data were analyzed using the statistical method of Snedecor and Cochran, (1994).

Treatment Details

K_1 H_1: Addition of Kokum syrup @ 5%+ Honey@ 8 % of curd

K_1 H_2: Addition of Kokum syrup @ 5%+ Honey @10 % of curd

K_1 H_3: Addition of Kokum syrup @ 5%+ Honey @12% of curd

K_2 H_1: Addition of Kokum syrup @ 10%+ Honey @8% of curd

K_2 H_2: Addition of Kokum syrup @ 10%+ Honey @10% of curd

K_2 H_3: Addition of Kokum syrup @ 10%+ Honey @12% of curd

K_3 H_1: Addition of Kokum syrup @ 15%+ Honey @8% of curd

K_3 H_2: Addition of Kokum syrup @ 15%+ Honey @10% of curd

K_3 H_3: Addition of Kokum syrup @ 15%+ Honey @12% of curd.

The trial was conducted with three replications.

Results and Discussion

There was significant increase in Lactobacilli count, Standard Plate Count (SPC), and psychrophilic count of fresh lassi with the increase in the levels of kokum syrup and honey.

L. acidophilus count during storage

The results in respect of *L. acidophilus* count of lassi during storage presented in Table 1. From critical perusal of data of Table1, it was observed that initial count of

L. acidophilus i.e. count of lassi (4.48×10^6cfu/g) was reduced to 2.16×10^6cfu/g on 12th day of storage period. It means *L. acidophilus* showed 44.74 per cent survival rate till 12th day of storage period.

Statistical interpretation of data showed that the effect of different levels of kokum syrup and honey is highly significant means there is positive impact of kokum syrup and honey on *L. acidophilus* count on 12th day of storage period. Statistical Interaction effect during storage showed non-significant on *L. acidophilus* count. In all the cases there was almost 50 per cent reduction in *L. acidophilus* count in 12 day of storage. However, there is no wonder in it because *L. acidophilus* grow well at temperature range of 37 to 42^{o}c however, in present study lassi was stored at 9±1°C. This marked reduction in storage temperature may be main cause for their reduction.

Guldas and Irkin (2010) reported that the survival of *L. acidophilus* was higher than the others during the whole storage period. *L. acidophilus* showed higher counts when compared to *L. bulgaricus* and *S. thermophilus*. Similar result related with *L. acidophilus* was also found in reconstituted sweet whey by Matijević *et al., (*2009). They found that *L. acidophilus* better grew during fermentation in comparison with *Bifidobacterium animalis* subsp. *lactis* regardless to the added amount of lactulose. Manuka Krishna *et al.,* (2013) reported the log count of viable *L. acidophilus* was 8.36, 8.53, 8.39 and 8.46 cfu/ml replacement of sugar 25, 50, 75 and 100 per cent sucralose in lassi.

*L. casei*count during storage

The results in respect of *L. casei*count of lassi during storage presented in Table 2.

From results, it can be observed that during storage, the highest count (7.28×10^6cfu/g) was observed when Kokum syrup and honey was used at the rate of 15 per cent and 12 per cent proportion. The count showed reducing trend with advanced storage periods. On 12th day of storage, the reduction in count ranges between 2.8-3.9 per cent as compared to initial count of lassi (Table 2). Statistical interpretation of data indicates that, effect of kokum syrup and honey was significant. Interaction effect was also found statistically significant.

In all the cases there was almost 50 per cent reduction in *L. casei* count in 12 day of storage. However, there is no wonder in it because *L. casei* grow well at temperature range of 37 to 42^{o}C however in present study lassi was stored at 9±1°C. The storage temperature may be main cause for reduction in *L. casei* count.

Standard Plate Count

The results in respect of SPC count of lassi during storage presented in Table 3. From results, it can be observed that on 0 day of storage, the highest count (15.653×10^4cfu/g) was observed when kokum syrup and honey was used at the rate of 15 per cent and 12 per cent proportion. The count showed reducing trend with advanced storage periods. On 12th day of storage, the reduction in count ranges between 15.7-12.6 per cent as compared to initial count of lassi (Table 3). Statistical interpretation of data indicates that, effect of kokum syrup and honey was significant. Interaction effect was also found statistically significant.

Singh *et al.,* (2014) while studying effect of storage periods on quality of ice cream reported that Standard Plate Count (SPC) of all the samples decreased significantly ($p<0.01$) during storage period of 60 days.

Table.1 Effect of different levels of kokum syrup and honey on *L. acidophilus* count of probiotic kokum lassi during storage (10^6cfu/g)

Storage period→ Treatments↓	**0day**	**4^{th} day**	**8^{th} day**	**12^{th} day**
Kokum Syrup(K)				
K_1	5.076	4.387	3.537	2.387
K_2	6.267	5.312	4.572	3.534
K_3	7.367	6.688	5.824	4.677
S.E.±	0.088	0.088	0.088	0.088
CD P<0.01	0.263	0.263	0.263	0.263
Honey(H)				
H_1	6.032	5.089	4.349	3.310
H_2	6.219	5.530	4.680	3.531
H_3	6.458	5.768	4.904	3.757
S.E.±	0.088	0.088	0.088	0.088
CD P<0.01	0.263	0.263	0.263	0.263
Interaction(K×H)				
K_1 H_1	4.827	4.160	3.310	2.160
K_1 H_2	5.080	4.380	3.530	2.380
K_1 H_3	5.320	4.620	3.770	2.620
K_2 H_1	6.120	4.657	4.137	3.320
K_2 H_2	6.230	5.530	4.680	3.533
K_2 H_3	6.450	5.750	4.900	3.750
K_3 H_1	7.150	6.450	5.600	4.450
K_3 H_2	7.347	6.680	5.830	4.680
K_3 H_3	7.603	6.933	6.043	4.900
Mean	**6.236**	**5.462**	**4.644**	**3.533**
S.E.±	0.152	0.152	0.152	0.152
CD P<0.01	NS	NS	NS	NS

Table.2 Effect of different levels of kokum syrup and honey on *L. casei*count of probiotic kokum lassi during storage (10^6cfu/g)

Storage period→ Treatments↓	0day	4th day	8th day	12th day
Kokum Syrup(K)				
K_1	5.250	4.343	3.467	2.246
K_2	6.082	5.337	4.386	3.140
K_3	7.078	6.380	5.393	4.244
S.E.±	0.015	0.015	0.015	0.015
CD P<0.01	0.046	0.046	0.046	0.046
Honey(H)				
H_1	5.883	5.173	4.191	2.937
H_2	6.086	5.347	4.389	3.242
H_3	6.441	5.540	4.666	3.451
S.E.±	0.015	0.015	0.015	0.015
CD P<0.01	0.046	0.046	0.046	0.046
Interaction(K×H)				
K_1 H_1	4.887	4.140	3.190	2.043
K_1 H_2	5.097	4.350	3.387	2.240
K_1 H_3	5.767	4.540	3.823	2.453
K_2 H_1	5.880	5.140	4.190	2.727
K_2 H_2	6.090	5.340	4.390	3.243
K_2 H_3	6.277	5.530	4.577	3.450
K_3 H_1	6.883	6.240	5.193	4.040
K_3 H_2	7.070	6.350	5.390	4.243
K_3 H_3	7.280	6.550	5.597	4.450
Mean	**6.137**	**5.353**	**4.415**	**3.210**
S.E.±	0.027	0.027	0.027	0.027
CD P<0.01	0.080	0.080	0.080	0.080

Table.3 Effect of different levels of kokum syrup and honey on Standard Plate Count of probiotic kokum lassi during storage (10^4cfu/g)

Storage period→ **Treatments↓**	**0day**	**4^{th} day**	**8^{th} day**	**12^{th} day**
Kokum Syrup(K)				
K_1	13.357	12.357	11.280	10.358
K_2	14.437	13.439	12.451	11.440
K_3	15.338	14.337	13.340	12.453
S.E.±	0.0006	0.0006	0.0006	0.0006
CD P<0.01	0.0019	0.0019	0.0019	0.0019
Honey(H)				
H_1	13.943	12.946	11.973	11.058
H_2	14.460	13.460	12.371	11.467
H_3	14.728	13.727	12.727	11.727
S.E.±	0.0006	0.0006	0.0006	0.0006
CD P<0.01	0.0019	0.0019	0.0019	0.0019
Interaction(K×H)				
K_1 H_1	12.690	11.690	10.743	9.680
K_1 H_2	13.500	12.500	11.207	10.517
K_1 H_3	13.880	12.880	11.890	10.877
K_2 H_1	14.120	13.127	12.147	11.127
K_2 H_2	14.540	13.540	12.567	11.537
K_2 H_3	14.650	13.650	12.640	11.657
K_3 H_1	15.020	14.020	13.030	12.367
K_3 H_2	15.340	14.340	13.340	12.347
K_3 H_3	15.653	14.650	13.650	12.647
Mean	**14.377**	**13.377**	**12.357**	**11.417**
S.E.±	0.0011	0.0011	0.0011	0.0011
CD P<0.01	0.0033	0.0033	0.0033	0.0033

Table.4 Effect of different levels of kokum syrup and honey on Psychrophilic count of probiotic kokum lassi up to 12thday of storage (10^2cfu/g)

Storage period→ **Treatments↓**	**0day**	**4th day**	**8th day**	**12th day**
Kokum Syrup(K)				
K_1	0.000	0.788	1.343	1.677
K_2	0.000	0.618	1.243	1.563
K_3	0.000	0.546	1.171	1.473
S.E.±	0.000	0.000	0.000	0.000
CD P<0.01	NS	NS	NS	NS
Honey(H)				
H_1	0.000	0.712	1.283	1.617
H_2	0.000	0.659	1.247	1.567
H_3	0.000	0.580	1.228	1.530
S.E.±	0.000	0.000	0.000	0.000
CD P<0.01	NS	NS	NS	NS
Interaction(K×H)				
K_1 H_1	0.000	0.860	1.380	1.740
K_1 H_2	0.000	0.787	1.340	1.660
K_1 H_3	0.000	0.717	1.310	1.630
K_2 H_1	0.000	0.673	1.280	1.600
K_2 H_2	0.000	0.627	1.230	1.550
K_2 H_3	0.000	0.553	1.220	1.540
K_3 H_1	0.000	0.603	1.190	1.510
K_3 H_2	0.000	0.563	1.170	1.490
K_3 H_3	0.000	0.470	1.153	1.420
Mean	0.000	0.650	1.253	1.571
S.E.±	0.000	0.000	0.000	0.000
CD P<0.01	NS	NS	NS	NS

Flow Diagram

Buffalo whole milk
↓
Pre-heating (35-40°C)
↓
Filtration
↓
Separation of cream
↓→ Cream
Low fat milk
↓
Pasteurization (80°C±2/10 min)
↓
Cooling (37°C)
↓
Inoculation with mixed probiotic culture (1.5 %)
↓
Incubation at 42 ±1°C for 8 hrs.
↓
Cooling of low fat milk curd in refrigerator at (9±1°C for 3-4 hrs.)
↓
Breaking of coagulum and sampling
↓
Addition of @10 % kokum syrup and@12 %honey of curd (w/w)
↓
Addition of water @ 10%
↓
Mixing with curd beater
↓
Addition of salt @ 1% of lassi
↓
Mixing cum churning in mixer
↓
Probiotic kokum lassi
↓
Storage (9±1°C)

Pardhi *et al.*, (2014) reported Standard Plate Count of finger millet lassi for T_0, T_1, T_2 and T_3 was 13.20, 14.00, 10.80 and 15.80×10^2cfu/ml, respectively. Pawar *et al.*, (2010) while studying effect of nisin on shelf life of lassi reported that Standard Plate Count (SPC) on zero day control samples (T_0) had highest microbial load 16.32×10^7cfu/ml than rest of treatments it was followed by treated sample T_1 (15.88×10^7cfu/ml), T_2 (15.64×10^7cfu/ml), T_3 (15.45×10^7cfu/ml) and (14.97×10^7cfu/ml).

Treatment showed lowest microbial load than T_0, T_1, T_2, and T_3 due to level of nisin increased microbial load decreased.

E. coli count

Presence of coliform in any dairy product indicates unhygienic condition prevailing during production and packaging. In the present study *E. coli* was found absent in all fresh as well as stored samples during storage at 9 ±1°C. This indicates that proper hygienic precautions were taken during the production and storage of probiotic kokum lassi.

Psychrophilic count

The results in respect of psychrophilic count of lassi up to 12thday of storage presented in Table 4.

Statistical analysis of data indicates that effect of kokum syrup, honey and their interaction showed is also non-significant.

The increase in psychrophilic count may be attributed to the lower storage temperature. Psychrophills grow best between temperature range of 4 to 11^{o}c. However, presence of kokum syrup and honey may have prevented their growth rate. So psychrophilic proliferation was not awesome but even below normal standards. The storage temperature for lassi was 9±1°C.

From the results of the present investigation, it may be concluded that kokum syrup and honey could be successfully utilized for preparation of lassi. The most acceptable quality lassi can be prepared by using 10 per cent kokum syrup and 12 per cent honey. The microbial quality of lassi remained within the standard limit during storage period of 12 days.

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