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To Study the Microbial Changes of Herbal *Lassi* (Enriched with Honey and *Tulsi (Ocimum sanctum Linn.*) During Storage

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A B S T R A C T

Milk production of India has increased significantly that it out spaced the global milk production. 7.0 percent milk produced is converted to fermented dairy products. In our present study, we prepared herbal *Lassi* enriched with honey and *Tulsi* extract. Honey is an important and unique food product containing bioactive compounds derived from bees and plants. Bioactive components in honey which confers health benefits includes a number of flavonoids, phenolic acids, ascorbic acid, tocopherols, alkaloids, number of aromatic acids and carotenoids. *Ocimum sanctum* popularly known as *'Tulsi'* in Hindi and 'Holy basil' in *English* is one of the sacred herb for Hindu in Indian subcontinent. It has versatile role to play in traditional medicine. The combination of 2.0 percent *Tulsi* extract and 10.0 percent honey was considered to be the most appropriate level for manufacturing of herbal honey *Lassi*. During storage studies average lactobacilli count, streptococci count, yeast and mold count was found 9.06 log CFU/g, 8.27 log CFU/g and 2.18 log CFU/g respectively for herbal *Lassi.* Coliform count was found absent during entire storage period.

Introduction

India as the 'Oyster' of the global dairy industry that provides opportunities galore for the entrepreneurs globally. Since last 15 years, India continues to be the largest producer of milk in the world. India has outpaced the global milk production with an annual growth rate of 5.53 percent compared with the 2.09 percent achieved globally. Milk production has increased significantly to 164 million tones in 2016-17. India's milk production in 2016-17 rose by 18.81 percent as compared to 2013-14. Similarly, the per capita availability of milk increased to 351 gin 2016-17.*Lassi*, is a popular beverage in the western and northern states of India. It is popular for its pleasant taste, cooling and thirst quenching properties and therapeutic value. It has creamy consistency, sweetish rich aroma and mild to acidic flavour, which makes the product refreshingly palatable. On an average *Lassi* may have fat from 3.0-3.5 percent, total solids 16-18 percent and acidity varying from 0.75 - 0.88 percent (De, 2004; Mathur *et al.*, 2005). *Lassi* has been mentioned as one of the best among milk products due to its immense therapeutic and nutritional value (Sarkar, 2008). Regular use of *Lassi* immensely helps the jaundice and

alcoholic liver patients to regain normal appetite and digestion (Trivedi, 1971; Shashtri, 1974; and Anon, 2003).

In the process of development of Herbal *lassi*, we have enriched it with *Tulsi* (*Ocimum sanctum Linn*.) and Honey as sweetner. *Ocimum sanctum* popularly known as *'Tulsi'* in hindi and 'Holy basil' in *English* is one of the sacred herb for hindu in Indian subcontinent. It has versatile role to play in traditional medicine. Within Ayurveda, *Tulsi* is known as "The Incomparable One" "Mother Medicine of Nature" and "The Queen of Herbs" and is revered as an "Elixir of life" that is without equal for both its medicinal and spiritual properties. A number of active constituents responsible for the medicinal actions of *Tulsi* (*Ocimum sanctum Linn.)* have been isolated and are being characterized. *Tulsi* (*Ocimum sanctum Linn.)* leaves contain 0.7 percent volatile oil comprising about 71.0 percent Eugenol and 21.0 percent Methyl Eugenol. These studies reveal that *Tulsi* has a unique combination of actions that include: Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), mosquito repellent, anti-diarrheal, anti-oxidant, anti-cataract, anti- inflammatory, chemo preventive, radioprotective, hepato-protective, neuro-protective, cardio-protective, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive, anti-carcinogenic, analgesic, anti-pyretic, anti-allergic, immunomodulatory, central nervous system depressant, memory enhancement, anti-asthmatic, anti-tussive, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti- arthritic, adaptogenic, anti-stress, anti-cataract, anti-leukodermal and anti-coagulant activities. These pharmacological actions help the body and mind cope with a wide range of chemical, physical, infectious and emotional stresses and restore physiological and psychological function.

Honey is an important and unique food product containing bioactive compounds derived from bees and plants. Bioactive components in honey which confers health benefits includes a number of flavonoids, phenolic acids, ascorbic acid, tocopherols, alkaloids, number of aromatic acids and carotenoids. It is composed primarily of fructose and glucose but also contains 4 to 5 percent fructo-oligosaccharides which serve as prebiotic agents (Chow, 2002). It contains more than 180 substances, including amino acids, vitamins, minerals and enzymes (White, 1979). Honey is a supersaturated sugar solution with a low water activity (a_w), which does not support the growth of bacteria and yeast. Natural acidity of honey inhibits many pathogens.

Materials and Methods

The experiment studies were conducted in the department laboratory, Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) India. The objective of the present study is to increase the functional value of *Lassi* by incorporation of honey and *Tulsi* (medicinal herb) that make it more useful and acceptable to undertake its commercial production.

Preliminary trials

The treatments used for present study of sensory evaluation

T_0	100 % *Lassi* + 0 %honey+ 0% *Tulsi* extract
T_1	100 % *Lassi* + 6 %honey+ 2% *Tulsi* extract
T_2	100 % *Lassi* + 8%honey+ 2% *Tulsi* extract
T_3	100 % *Lassi* + 10 % honey + 2% *Tulsi* extract
T_4	100% *Lassi*+ 12 % honey + 2% *Tulsi* extract

Optimization was done on the basis of sensory score and Lassi with 2% Tulsi and 10% honey was found most suitable on the basis of sensory score.

Microbial analysis of herbal honey *lassi*

Preparation of herbal honey *Lassi* Samples for microbial analysis: 11g of Herbal honey *Lassi* sample was aseptically pipetted out and transferred to 99 ml phosphate buffer dilution blank to obtain 1:10 dilution. Subsequently, 1 ml of above dilution was used for making further dilutions in 9 ml phosphate buffer tubes. Suitable dilutions were prepared and poured in a set of sterile Petri dishes in duplicates (Fig. 2).

Lactobacilli count: Petri plates with one ml of appropriate serial dilutions of the samples were prepared as described above and 1 ml from selected dilutions was poured in duplicate plates and mixed with tempered MRS medium. After setting of the agar, another layer of the same medium (5-7 ml) was poured. The plates were then incubated at 37+2 ^{o}C for 72 h. After incubation, plates were removed for counting the colonies. The lactobacilli count was expressed as CFU/g.

Streptococci count: For streptococci count, serial dilutions of the samples were prepared as described above and 1 ml from selected dilutions was poured in duplicate plates and mixed with tempered M17 medium. After setting of the agar, another layer of the same medium (5-7 ml) was poured. The plates were then incubated at 37+2^{o}C for 48 h. After incubation, plates were removed for counting the colonies. The Streptococci count was expressed as CFU/g (IS: 1479, Part III,1962).

Yeast and mould count: The petri plates containing the diluted samples were poured with melted and cooled potato dextrose agar medium at around 45^{o}C (adjusted to pH 3.5 using 10 % sterile tartaric acid solution, within 30 min of addition to the sample) mixed properly, and allowed to solidify. The plates were incubated at temperature 22-25^{o}C for 3-5 days before count were recorded (IS: 5403, 1969).

Coliform count: Serial dilutions of the samples were prepared as described above and 1 ml from selected dilutions was poured in duplicate plates and mixed with tempered VRBA medium. After setting of the agar, another layer of the same medium (5-7 ml) was poured. The plates were then incubated at 37+2 ^{o}C for 24 h. After incubation, plates were removed for counting dark red colonies having a diameter of >0.5 mm size. The coliform count was expressed as CFU/g (IS: 5401, 1969).

Statistical analysis

All the parameters under study were analyzed by statistical methods. Optimization study data were analyzed by Completely Randomized Design as per the methods described by Steel and Torrie (1980). Storage study data were examined using Factorial CRD. The values for microbial counts were log transformed before analysis.

Result and Discussion

Storage study for herbal honey *Lassi*

Based on preliminary trials on growth curve as well as overall higher sensory scores in terms of flavor, colour and appearance and overall acceptability, we decided to take further trials for shelf-life study with a product incorporated with 10% honey and 2% *Tulsi* extract (Fig. 1). Even though non-significant, the count of lactobacilli and streptococci were also relatively lower in 10% honey and 2% *Tulsi* extract added samples. The shelf-life of the product added with 10% honey, designated as herbal honey *Lassi* (T) was studied by storage at 7±1^{o}C and compared with a control product prepared without honey, designated as Control (normal) *Lassi* (C). The shelf- life was monitored by chemical, microbiological and sensory attributes.

Microbiological changes during shelf-life study

Change in lactobacilli count

Lactobacilli count was comparatively higher (P<0.05) in normal *Lassi* (9.06 log CFU/g) as compared to herbal honey *Lassi* (8.72 log CFU/g). For normal *Lassi* the count varied from 9.24 to 8.65 log CFU/g while herbal honey *Lassi* showed a decline from 8.87 to 8.46 log CFU/ml during storage for 28 days. According to statistical analysis, control sample showed significant increase in Lactobacilli count till 7th day of storage. Subsequently, up to 14th day, effect was significant followed by non-significant effect on 21st and 28th day. Herbal honey *Lassi* showed non- significant increase in lactobacilli count followed by significant decrease in count and thereafter the changes were non-significant till 28th day of storage. The changes in pH of control (C) and Herbal honey *Lassi* (T) stored at refrigerated temperature are depicted in Table 1 and figure 3.

Changes in Streptococci count

Streptococci count of control *Lassi* (C) was marginally higher than that of herbal honey *Lassi* (T).

For control *Lassi*, the count gradually decreased from 8.56 to 7.98 log CFU/g during storage for 28 days whilst herbal honey *Lassi* showed a decline in count from 8.46 to 7.43log CFU/g, upto 7th day and thereafter again a reduction was observed from 7thday to 28thday i.e., from 7.43 to 7.83 log CFU/g. The changes in pH of control (C) and Herbal honey *Lassi* (T) stored at refrigerated temperature are depicted in Table 2 and figure 4.

Table.1 Changes in Lactobacilli count of control sample (Normal *Lassi*) and optimized herbal honey *Lassi* during storage at 7± 1ºC

Treatment	0 Day	7 Days	14 Days	21 Days	28 Days	Treatment mean
T	8.87	9.04	8.72	8.46	8.46	**8.72**
C	**9.24**	**9.46**	**9.05**	**8.81**	**8.65**	**9.06**

*C Represents control sample (Normal *Lassi*). *T represents treated sample (i.e., 100 ml *Lassi* + 10% honey + 2% *Tulsi*)

Table.2 Changes in Streptococci count of control sample (Normal *Lassi*) and treated sample (Herbal honey *Lassi*) during storage at 7± 1ºC

Treatment	0 day	7 days	14 days	21 days	28 days	Treatment mean
C	8.56	8.46	8.39	8.16	7.98	**8.34**
T	8.46	8.43	8.35	8.06	7.83	**8.27**
Period mean	**8.61**	**8.44**	**8.37**	**8.11**	**7.99**	

*C Represents control sample (Normal *Lassi*). *T represents treated sample (i.e., 100 ml *Lassi* + 10% honey + 2% *Tulsi*)

Table.3 Changes in yeast and mold count of Control *Lassi* and herbal honey *Lassi* during storage at 7± 1 ^{o}C

Treatment	0 Days	7 Days	14 Days	21 Days	28 Days	Treatment mean
C	2.18	2.28	3.41	3.46	2.93	**2.85**
T	**1.59**	**1.98**	**2.64**	**2.60**	**2.09**	**2.18**

*C Represents control sample (Normal *Lassi*). *T represents treated sample (i.e., 100 ml *Lassi* + 10% honey + 2% *Tulsi*)

Figure.1 Flow diagram for the preparation of herbal extract

Method of *Tulsi* leaf extraction

Weighed 50 g of *Tulsi* leaves

↓

Washed with water and dried with muslin cloth

↓

Crushed in mixer by adding 50 ml of distilled water

↓

Collected extract by pressing the mass through muslin cloth

↓

Adjusted TS with distilled water (2.0 %)

↓

Used the extract for preparation of herbal drink

Figure.2 Flow diagram for the preparation of herbal honey *Lassi*

Preparation of Herbal honey *Lassi*

Receiving of fresh cow milk

↓

Pre - heating (35 °C)

↓

Filtration / Clarification

↓

Standardization(at 4% fat and 9% SNF)

↓

Homogenization
↓
Heating (85 °C/15 min)
↓
Cooling (35±2 °C)
↓
Inoculation with starter cultures
↓
Incubation (42 °C) till desired acidity obtained
↓
Breaking of *Dahi*and cooling (< 7 °C)
↓
Mixing with mechanical stirrer
↓
Addition of honey (10%)
↓
Addition of *Tulsi* leaf extract (2%)
↓
Uniform mixing
↓
Pouring into earthen cups (100 ml)
↓
Storage at 7 ±1^{o}C

Figure.3 Changes in Lactobacilli count of control sample (Normal *Lassi*) and optimized herbal honey *Lassi* during storage at 7± 1ºC

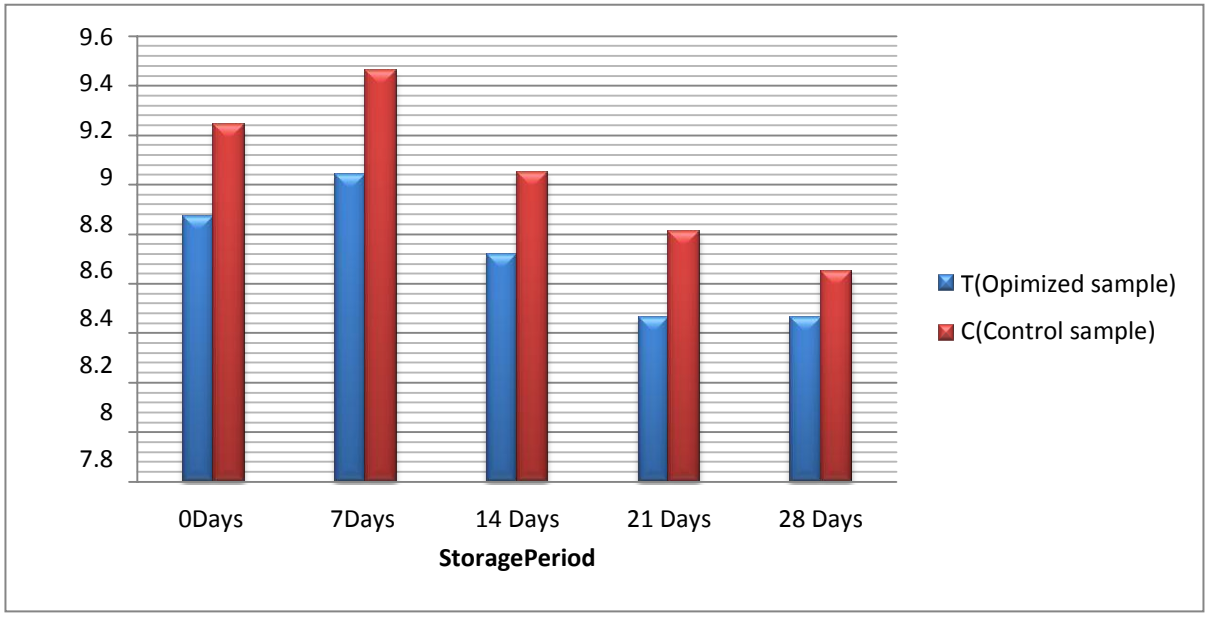


Figure.4 Changes in Streptococci count of Control sample (Normal *Lassi*) and Treated sample (Herbal honey *Lassi*) during storage at 7± 1ºC

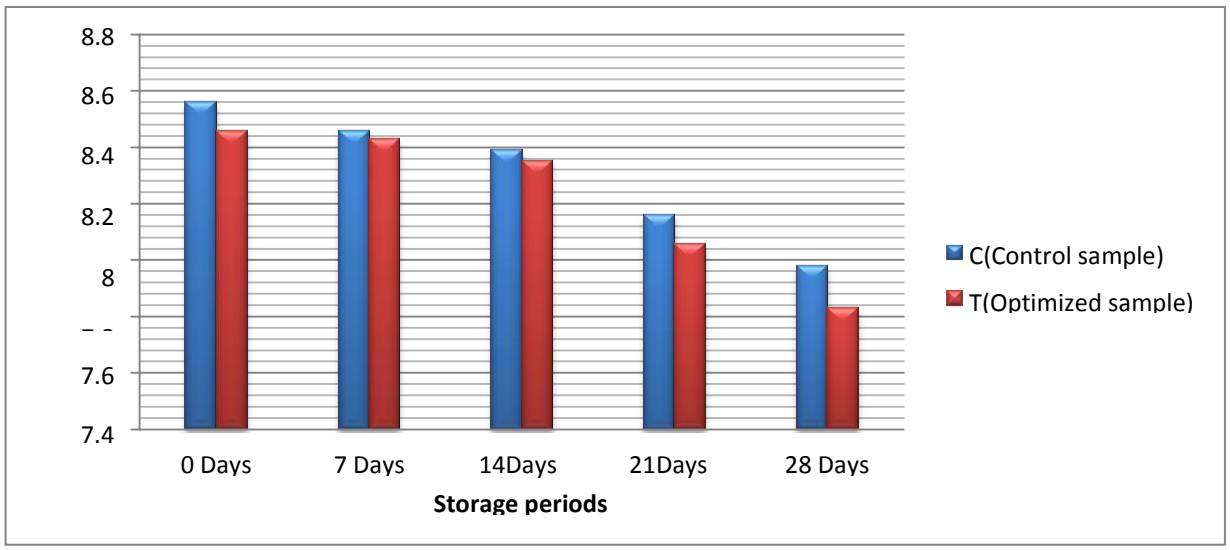
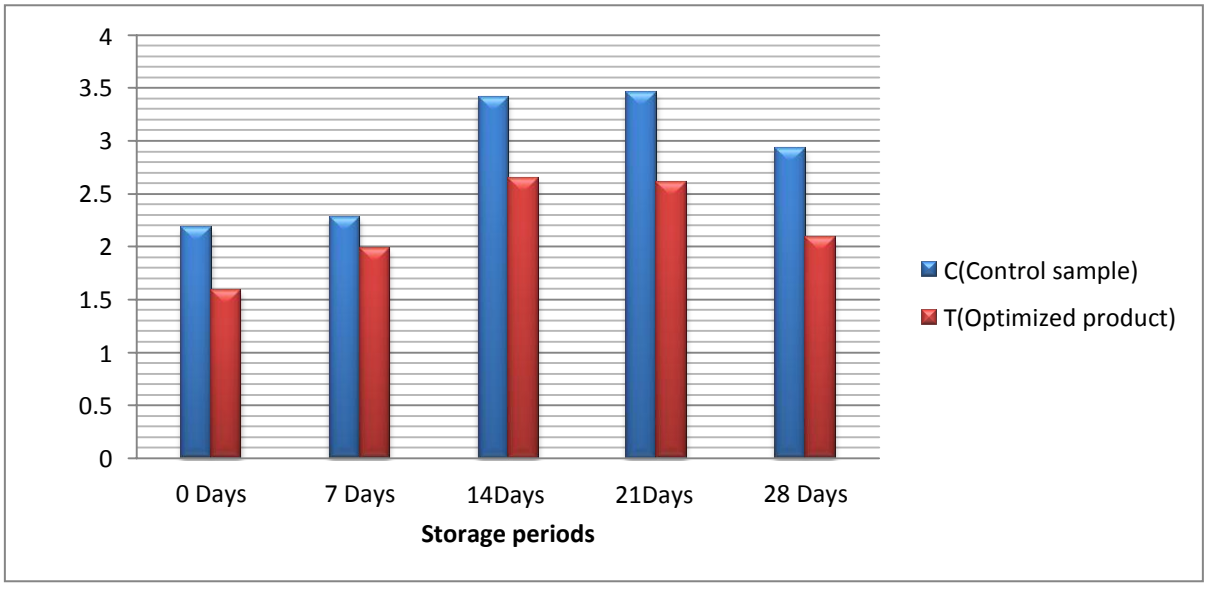


Figure.5 Changes in yeast and mold count of Control *Lassi* and herbal honey *Lassi* during storage at 7± 1 ºC



Changes in yeast and mold count

Yeast and mold count shows a significant increase from 7th day to 21st day i.e., 2.18 to 3.46 log CFU/g and thereafter count decreases upto 28th day i.e. 3.46 to 2.93 log CFU/g. While the herbal *Lassi* shows gradual increase in yeast and mold count from 0th to 21st day. And after that decreases significantly to 2.09 log CFU/g. On an average control lassi having yeast and mold count 2.85 log CFU/g during entire storage period and herbal lassi having 2.18 log

CFU/g during entire storage period. The changes in yeast and mold of control (C) and Herbal honey *Lassi* (T) stored at refrigerated temperature are depicted in Table 3 and figure 5.

Changes in coliform count

Coliforms are opportunistic pathogens, which generally indicate hygiene and post pasteurization contamination. Their presence in fermented milk depends on the type of culture used for product manufacturing as well as the initial acidity of the product. Coliform was found to be absent (in 1g) in both control sample (normal *Lassi*) and herbal *Lassi* throughout the storage study of 28 days.

In conclusion, the present study was attempted to develop Herbal *Lassi* by incorporation of honey and *Tulsi* extract. The combination of 2.0 percent *Tulsi* extract and 10.0 percent honey was considered to be the most appropriate level for manufacturing of herbal honey *Lassi*. The experiment studies were conducted in the department laboratory, Department of Animal Husbandry and Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.), India. The findings of present study are summarized as under:

During microbial study, it was found that, Total viable lactobacilli count was 8.87 to 8.46 logCFU/g in control *Lassi* while in herbal honey *Lassi* it varied from 9.24 to 8.65 log CFU/g, respectively at the end of storage. Streptococci count varied from 8.46 to 7.83 log CFU/g in herbal honey *Lassi* whereas a decrease from 8.56 to 8.98 log CFU/g was observed in control. The yeast and mold count was significantly lower in herbal honey *Lassi* than control during storage. Coliforms were absent (in 1 g) in both control (normal) and herbal honey *Lassi* throughout storage period.

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