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Assessment of transformation in biochemical parameters of Tulsi *(Ocimum sanctum Linn.*) and honey enriched herbal lassi during its storage

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Abstract
Milk is a very important and essential ingredient of our diet. Development of various products from milk is common in our country. One of the important issues with these dairy products is storage and is spoilage in a definite period. In this study, chemical changes during storage of herbal honey lassi was studied. 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. Storage study were done by keeping final product at 7°C at different days i.e. 0 day, 7 days, 14 days, 21 days, 28 days. Generally during storage titrable acidity and pH varied day by day and there is no any significant change in rest chemical attributes. Titratable acidity increased from 0.81 to 1.24% lactic acid. in control while in symbiotic lassi it increased from 0.77 to 1.38% L.A. A gradual decline in pH was also observed in normal lassi and herbal honey lassi viz., 4.53 to 3.91 and 4.57 to 3.89, respectively.

Keywords: Lassi, Tulsi, Honey, Herbal, Transformation, Storage

1. Introduction
India placed in top list among the world's milk producing country. Production of milk in our country during the period 2017-18, has increased to 176.4 million tonnes at an average annual growth rate of 4.5 percent. The per capita availability of milk in the country has increased to 374 gram per day in 2017-18. This represents sustained growth in the availability of milk and milk products for our growing population. Fermented milks were developed throughout the world as a means of preserving milk solids against spoilage. These fermented milks have persisted over the centuries in the developing world. Fermented milks are popular in view of their organoleptic and other properties such as the characteristic flavour, refreshing taste and improved digestibility. The composition of fermented milks can be easily tailored to meet various dietary requirements especially in the production of varieties like low fat fermented milk desserts. Fermentation is probably the ideal technology to preserve milk – a highly perishable commodity without any adverse effect on nutritive value. In fact, pre-digestion of the protein, fat and carbohydrates in milk during fermentation is a factor in the higher nutritive value of fermented dairy products as compared to the milk from which they are made. The demand for fermented milk products is increasing and it has been estimated that about 10.0 percent of total milk produced in India is used for preparation of traditional fermented milk products (Khurana and Kanawjia, 2007) [5]. Cultured dairy products with many therapeutic properties are an excellent medium to generate an array of products that fit into the current consumer demand for health-based foods. Fermented milk products are known for their excellent nutritional characteristics due to improved digestion and absorption of amino acids and the presence of easily assimilable proteins resulting from proteolytic activity of starter cultures and increased content of vitamins (Sarkar, 2008) [9]. 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) [3, 6]. Lassi is described as a fermented milk beverage obtained after the growth of selected culture, usually lactic streptococci, in heat treated or partially whole milk followed by sweetening with sugar (Aneja *et al.*, 2003) [1]. Lassi contains appreciable amounts of milk protein, phospholipids and nutritive value of fermented milk product that are derived from the nutrients in form of various metabolites produced by lactic

acid bacteria during fermentation. The reason to add herbs like Tulsi in lassi that it can address physical, chemical, metabolic and psychological stress through a unique combination of pharmacological actions and broad- spectrum antimicrobial activity. The antimicrobial activity in most honey is due to the enzymatic production of hydrogen peroxide. Several substances with antibacterial activity are found in honey in small quantities e.g. Pinocembrin, Terpenes, Benzyl alcohol, Syringic acid etc (Molan, 1992) [7]. With this background, this study was conducted in the department laboratory, Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) India.

2. Materials and methods

Fresh cow milk was procured from The Dairy Farm run and maintained by Institute of agricultural sciences, Banaras Hindu University. The milk was standardized to the required level of fat and SNF using appropriate amounts of cream/skim milk. Dahi cultures required for the study were obtained from the Market. Fresh leaves of medium size, matured and green colour Tulsi **(***Oscimum sanctum Linn***.)** were selected and cut from the plant and used for flavour extraction. "Sabour Honey" manufactured by "Bee keeping-cum-honey production unit" Bihar Agricultural University (BAU), Sabour Bhagalpur, Bihar was used for preparation of Lassi. Level optimization of honey and Tulsi for the preparation of herbal honey lassi with incorporation of honey at 6 levels, viz., 0%, 2%, 4%, 6%, 8%, 10% and 12% and Tulsi 2% v/v was tested. These were added during heating of milk for preparation of herbal honey lassi.

Peparation of Tulsi (*Ocimum sanctum Linn.*) extract

50 g of Tulsi leaves were weighed and washed with water and dried with muslin cloth. It was then crushed in mixer by adding 50 ml of distilled water and collected extract by pressing the mass through muslin cloth. Total solid with distilled water (2.0%) was adjusted. This extract was used for preparation of herbal drink.

Flow diagram for the preparation of herbal honey lassi

Receiving of fresh cow milk
↓
Pre - heating (35 °C)
↓
Filtration / Clarification
↓
Standardization (at 4% fat and 9% Solid-not-fat (SNF)
↓
Homogenization (176 kg/cm)
↓
Pasteurization (63 °C/30 min)
↓
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 extracts (2%)
↓
Uniform mixing
↓
Pouring into earthen cups (100 ml)
↓
Storage at 7±1 °C

Preliminary trials

For the present study of chemical analysis following treatment combination was taken for study. Combinations which were taken for biochemical analysis are in Table 1.

Table 1: Various combinations taken for chemical analysis.

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 chemical analysis of various above treatments and it was found that lassi with 2% Tulsi leaf extract and 10% honey was found most suitable on the basis of sensory score.

Biochemical analysis of herbal honey lassi at different storage periods

Titratable acidity

Titratable acidity of herbal honey lassi was estimated by the procedure described in (IS: 1479, part I, 1960) [4]. 10 ml of thoroughly mixed herbal honey lassi was taken in to a porcelain dish and an equal volume of distilled water was added to it, then 1.0 ml of phenolphthalein indicator (0.5%) was added in to the sample. The contents of dish were titrated with 0.1 N NaOH till the appearance of light pink tinge, which persisted for 30 seconds in the solution. The acidity was expressed as per cent lactic acid. Titratable acidity was calculated by the following formula:

Acidity (% lactic Acid) = 9VN / X

Where,
V = Volume in ml of 0.1N NaOH required for titration
N = Normality of NaOH solution, and
X = Volume in ml of herbal honey lassi taken for the titration.

Determination of pH

pH of herbal honey lassi was determined by electronic pH meter, Model µ Ph System 361 manufactured by Systronics.

Storage study for herbal honey lassi

Based on preliminary trials on growth curve as well as the shelf-life of the product added with 10% honey designated as herbal honey lassi (T) was studied by storage at 7±1°C and compared with a control product prepared without honey, designated as Control (normal) lassi (C). The shelf- life was monitored by chemical transformation during 28 days of storage in a week interval (0 day, 7 days, 14 days, 21 days and 28 days respectively).

3. Results and discussion

Changes in titratable acidity

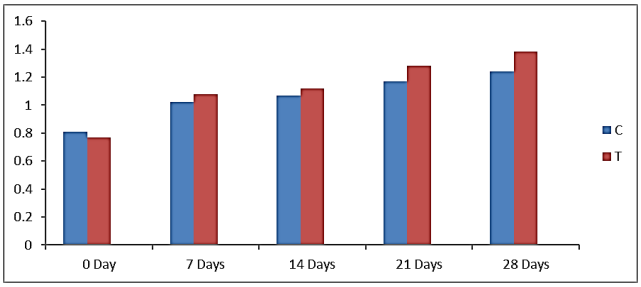
During refrigerated storage, titratable acidity gradually increased from 0.77 to 1.38% lactic acid (LA) in herbal honey

lassi whereas in control it increased from 0.81 to 1.24% lactic acid (LA). According to statistical analysis, control sample showed significant increase in titratable acidity till 14th day of storage followed by non-significant effect on 21st day and again showed a significant increase on 28th day. Herbal honey lassi showed significant increase in titratable acidity throughout the storage period till 28th day. The average acidity increase over the entire storage period was significantly higher (P<0.05) in herbal honey lassi (1.09) as compared to control (1.02). Patidar and Prajapati (1998) [8] reported that titratable acidity showed an increase in the probiotic lassi. A similar trend in increase in acidity of Lassi was reported by Bagal *et al.* (2007) [2] during storage period. The changes in titrable acidity (% Lactic acid) of control (C) and herbal honey lassi (T) stored at refrigerated temperature are shown in Table 2 and Figure 1.

Table 2: Changes in Titratable acidity (% lactic acid) of Control (Normal lassi) and Optimized herbal honey lassi during storage at 7± 1 0C.

Treatments	0 Day	7 Day	14 Day	21 Day	28 Day	Treatment Mean
C	0.81	1.02	1.07	1.17	1.24	1.06
T	0.77	1.08	1.12	1.28	1.38	1.13
Period Mean	0.79	1.05	1.09	1.22	1.31	

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



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

Fig 1: Changes in Titratable acidity (% lactic acid) of Control (Normal lassi) and Optimized herbal honey lassi during storage at 7± 1 ^{0}C.

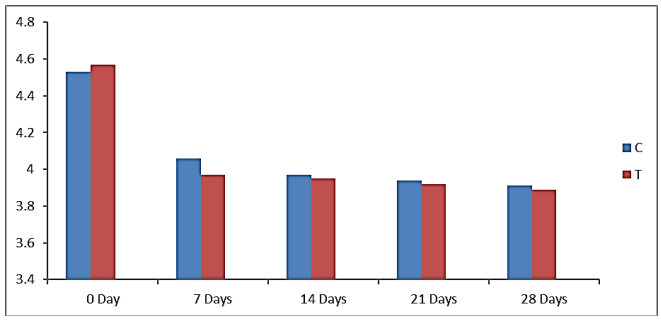
Change in pH

A gradual decline in pH in both, normal lassi as well as herbal honey lassi viz., 4.53 to 3.91 and 4.57 to 3.89, respectively was recorded during storage at 7±1^{o}C. Significant decrease in pH of control sample (normal lassi) was observed from 0th day to 21st day and then on 28th day of storage, the pH change was non-significant. In Herbal honey lassi, till 7th day, the change was significant followed by non-significant change on 14th day. Later, on 21st day of storage, pH significantly decreased and again on 28th day the change in pH was non-significant. Statistically, when two treatments were compared, they were significantly different (P<0.05). The changes in pH values of control (C) and herbal honey lassi (T) stored at refrigerated temperature are presented in Table 3 and represented graphically in Figure 2.

Table 3: Changes in pH of control sample (normal lassi) and treated sample (herbal honey lassi) during storage at 7± 1 oC.

Treatment	0 Day	7 days	14 days	21 days	28 days	Treatment Mean
C	4.53	4.06	3.97	3.94	3.91	4.08
T	4.57	3.97	3.95	3.92	3.89	4.06

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



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

Fig 2: Changes in pH of control sample (normal lassi) and treated sample (herbal honey lassi) during storage at 7± 1 ^{o}C.

4. Conclusion

During storage of herbal honey lassi mainly two biochemical attributes get varied day by day. These are titrable acidity and pH of the products. The storage study for 28 days was carried out for lassi added with 10% honey (herbal honey lassi) as well as control without honey (normal lassi) stored at 7±1 ^{o}C which were assessed on the basis of sensory, chemical and microbiological changes taking place during the storage. The average acidity increase over the entire storage period was significantly higher (P<0.05) in herbal honey lassi (1.13) as compared to control (1.06). Titratable acidity increased from 0.81 to 1.24% L.A. in control while in symbiotic lassi it increased from 0.77 to 1.38% L.A. A gradual decline in pH was also observed in normal lassi and herbal honey lassi viz., 4.53 to 3.91 and 4.57 to 3.89, respectively.

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