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Physicochemical and biochemical characterization in process optimisation of Tulsi (*Ocimum sanctum* Linn.) and honey enriched herbal honey lassi

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Abstract

Dairy products are important ingredient of our daily diet either in any form such as milk or product like dahi, lassi, khoa, srikhand. Of these dairy products maximum share of milk conversion is for fermented products. Lassi is one of the main products of dahi and its therapeutic value increases with incorporation of honey and Tulsi. This increases its therapeutic uses in our country. Incorporation of honey at different percentage i.e. 0%, 2%, 6%, 8%, 10%, 12% and 2% Tulsi was analysed chemically in different treatments of sample for its chemical stability and relatively beneficial for microbial growth and its physical characteristics. Herbal honey lassi was analysed during its optimisation process for its fat content, protein content, carbohydrate, total solid, Titrable acidity and its ash content. Effect of different levels of honey along with 2 percent Tulsi is analysed and it was revealed that addition of honey at 10% level showed relatively high score for chemical acceptability as compared to control, 8% and 12% honey addition. Best combination was prepared as final product i.e. herbal honey lassi.

Keywords: Dahi, lassi, honey, Tulsi, herbal

Introduction

Our country India placed in top list among the world's milk producing country since 1998. 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 compared with world's 2.09 percent. FAO reported 1.46% increase in world milk Production from 800.2 million tonnes in 2016 to 811.9 million tonnes in 2017. The per capita availability of milk in the country has increased to 374 gram per day in 2017-18 as against the world estimated average consumption of 294 grams per day during 2017. This represents sustained growth in the availability of milk and milk products for our growing population.

Fermented milk products occupies a good place in Indian diet such products may include products like dahi, lassi, shrikhand etc. It is estimated that about 7.0 percent of milk produced is converted into fermented milks. Dahi occupies an important place in the diet of average Indian who is mainly a vegetarian as it serves as an important source of animal protein. Fermented milk products are highly recommended to the lactose intolerance individuals because of the reduced lactose content. Besides this, lactic acid also helps in the absorption of calcium and phosphorous in the intestine (Renner, 1986; Kaup, 1988) ^{[1], [9]}. The physico-chemical properties of milk and milk products affect most of the unit operations used during their processing. These operations include fluid flow, heat transfer processes, mixing and churning, emulsification and homogenisation. Some of the rheological properties are also used for assessing and monitoring the quality of products such as cream, dahi, butter and cheese. 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) ^{[2], [10]}. 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. 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. Lassi has been praised as an effective tool/medicine for treatment of several diseases.

Tulsi leaves contain 0.7 percent volatile oil comprising about 71.0 percent Eugenol and 21.0 percent Methyl Eugenol.

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in has been found to be largely responsible for the therapeutic potentials of Tulsi. 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.

Incorporation of honey at different levels was tested. They were added during heating of milk for preparation of herbal honey lassi. Evaluation of product was done by carrying out chemical (acidity and pH) analysis. Based on above tests, best level was selected for preparation of herbal honey lassi.

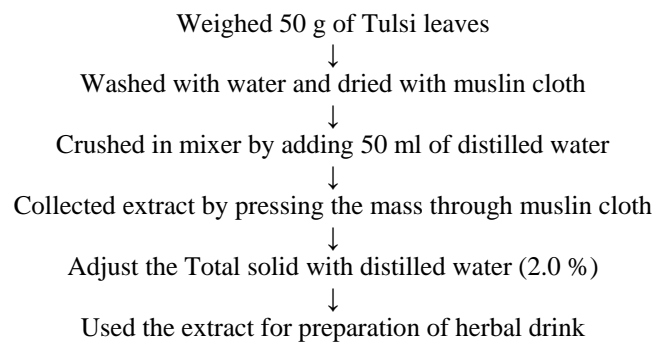
Materials and Methods

The experiment studies were conducted in the department

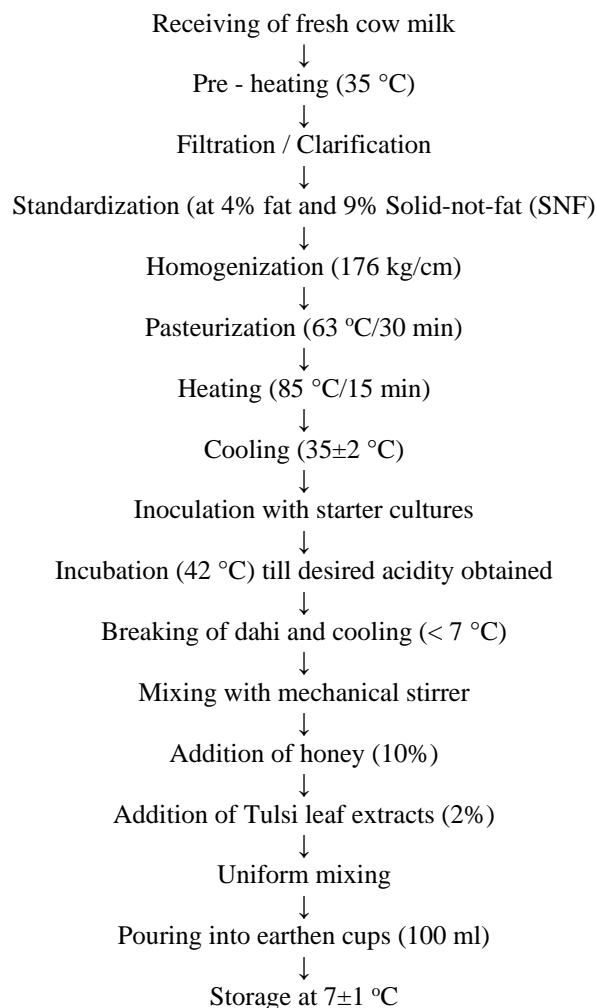
laboratory, Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) India.

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 used as starter culture required for the study were obtained from the Market. Fresh leaves of Tulsi (*Ocimum sanctum Linn.*) medium size, matured and green colour were selected and cut from the plant and used for flavour extraction. "Sabour honey" was used for preparation of lassi. Sabour honey is manufactured by "Bee keeping-cum-honey Production Unit" Bihar Agricultural University (BAU), Sabour, Bhagalpur, Bihar.

The flow diagram for preparation of Tulsi (*Ocimum sanctum Linn.*) extract



Flow diagram for the preparation of herbal honey lassi



Level optimization of honey and Tulsi for the preparation of herbal honey lassi done by incorporation of honey at 6 levels, viz., 0%, 2%, 4%, 6%, 8%, 10% and 12% and level of Tulsi leaf extract at 2% v/v was tested. They were added during heating of milk for preparation of herbal honey lassi.

Treatment details

For the preparation of herbal honey lassi by incorporating Tulsi and honey, various treatment combination comprising different levels of honey and Tulsi extract was taken for study. Some of the combinations which were taken for chemical 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

Details of experimental technique for chemical analysis during process optimisation

Estimation of total solids

The moisture and total solids content in milk was determined by the method given in IS: 9585 (1980) [6].

Determination of titratable acidity

Titratable acidity of herbal honey lassi was estimated by the procedure described in (IS: 1479, part I, 1960) [3]. 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:

$$\text{Acidity (\% lactic Acid)} = 9VN / X$$

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.

Estimation of Moisture

Moisture content of sample was determined according to the procedure described in (IS: 1479, part II, 1961) [4]. In a dry and clean previously weighed stainless steel dish, 5 ml of the sample was poured and the weight of sample was recorded. The duplicate dishes were placed on hot plate till the moisture got evaporated (indicated by a slight brown colour). The dishes were then transferred to the oven maintained at a temperature of 102 ± 2 °C for 1h or till the constant weight between two consecutive readings was recorded. The dishes were then transferred to desiccators for 5 min. To obtain the percentage moisture content of the sample, the following formula was used:

$$\text{Moisture (\% by weight)} = (W1-W2) / (W1-W)$$

Where,

W1 = Weight in g of the dish with material before heating to

constant weight.

W2 = Weight in g of the dish with material after heating to constant weight

W = Weight in g of the empty dry dish.

Estimation of Fat Content

Fat content of sample was determined in duplicate as per the by Gerber method given in (IS: 1224, Part I, 1981) [7]. Exactly 10.75 ml of well mixed herbal honey lassi sample was transferred in butyrometer followed by addition of 10 ml Gerber Sulphuric acid (sp.gr. 1.820- 1.825) and 1 ml of iso-amyl alcohol. The butyrometer was locked by stopper and the contents were vigorously shaken to digest non-fat substances. The butyrometer was then placed in the Gerber centrifuge and centrifuged for about 10 min at 1100 rpm. After completion of centrifugation, fat content was read out from the fat column.

Estimation of Total Protein

Protein content of product was estimated by Kjeldahl method as described in (IS: 9617, 1980) [5]. In a digestion flask, 0.5-1.0 g of sample was added and then 2.4 g of digestion mixture (Potassium sulphate: Copper sulphate: Selenium dioxide, 1:0.1:0.1) and 10 ml of nitrogen free concentrated sulphuric acid were added. The flasks were transferred to the digestion hood. The contents were digested for 60-90 min till the contents in the flask became free from yellow colour. When the contents in the flask were like clear liquid (light greenish blue) boiling was stopped, and the flasks were allowed to cool. The flask having cooled digested content was assembled in the distillation unit. A fixed volume of alkali (80 ml of 40 % Sodium hydroxide) was added to the sample. The digestion flask was heated to liberate the ammonia. The liberated ammonia was condensed and collected in 50 ml of saturated boric acid solution containing 3 drops of mixed indicator [equal volume of saturated solution of methyl red and 0.1 % Methylene blue solution, both made in 95 % (v/v) Ethanol]. The distillate (around 200 ml) in boric acid was titrated against 0.05 N Sulphuric acid. A reagent blank was simultaneously run using all the above chemicals except the sample and its reading was subtracted from the experimental reading.

The percentage of total nitrogen was calculated by using the following formula:

$$\text{Total Nitrogen (\%)} = 0.07[(\text{Burette Reading}-\text{Blank Reading})/W]$$

Where,

W = Weight of sample

For converting the amount of total nitrogen to percent total protein, the values were multiplied by a factor of 6.38.

Estimation of total carbohydrates

Total carbohydrates of sample were determined according to the procedure described in (IS: SP: 18, part XI, 1981) [8]. The total carbohydrates were determined through subtraction of the percentage of moisture, total milk protein, milk fat and total ash.

$$\text{Total carbohydrates} = 100 - (\text{moisture \%} + \text{total milk protein \%} + \text{milk fat \%} + \text{total ash})$$

Estimation of Ash Content

As per procedure given in (IS: 1479, Part II, 1961) [4], 5 g of lassi sample was weighed in a pre-weighed, dry silica

crucible, in duplicate. Then the samples were evaporated to dryness at 102 ± 2 °C overnight in a hot air oven. The crucibles were then transferred to muffle furnace, which was ignited at 600 °C for 3.5 h to make ash. The crucibles were then cooled in desiccators and weighed.

The percentage ash was calculated by the formula:

$$\text{Ash (\%)} = (W2 - W1) / W$$

Where,

W1= Weight of silica crucible

W2= Weight of silica crucible + ash

W= Weight in grams of lassi

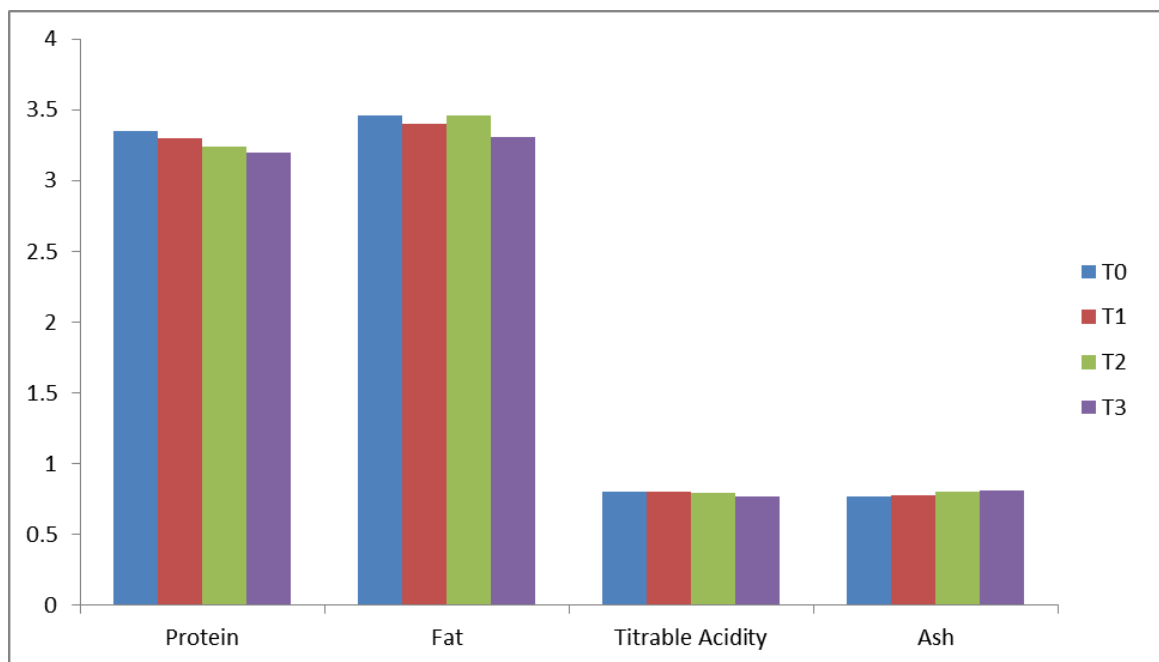
Results and Discussion

Chemical parameters

The protein (%), fat (%), carbohydrate (%), ash (%), acidity (%) and total solids (%) of different types of herbal honey lassi have been shown in Table 2. A significantly change in trend was observed in the fat, protein, carbohydrate and ash content of herbal honey lassi in various levels of the Tulsi and honey combination (Figure 1 and Figure 2).

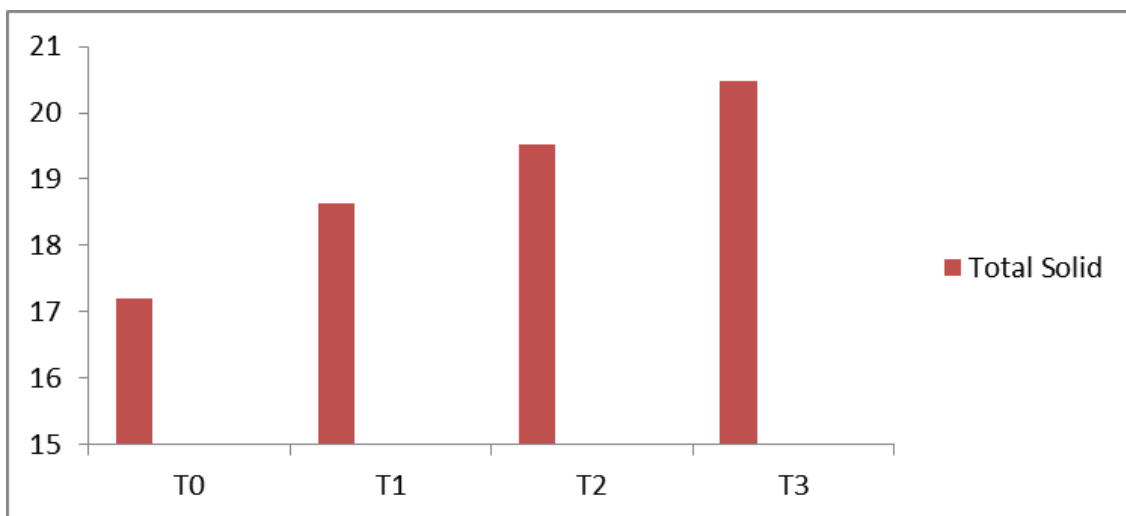
Table 2: Physicochemical and biochemical parameters of herbal honey lassi (in percent).

Physicochemical and biochemical parameters of herbal honey lassi (in percent)					
Treatments	Protein	Fat	Total Solid	Titration acidity	Ash
Control (T ₀)	3.35	3.46	17.19	0.80	0.77
T ₁	3.30	3.40	18.64	0.80	0.78
T ₂	3.24	3.46	19.52	0.79	0.80
T ₃	3.20	3.31	20.48	0.77	0.81



*T represents various treatments of lassi and Tulsi with different levels of honey.

Fig 1: Biochemical parameters of herbal honey lassi (in percent)



*T represents various treatments of lassi and Tulsi with different levels of honey.

Fig 2: Total solids in herbal honey lassi (in percent)

Protein

The protein percentage was significantly highest (3.35%) in the lassi prepared without addition of honey, while protein content was the lowest (3.18%) in lassi prepared with addition of 10 percent honey. It was observed from the present study that as the level of honey increased, there was decrease in the level of protein content in lassi.

Fat

The fat percent was significantly highest in Normal lassi prepared without honey (Control sample) i.e. with addition of sugar, while fat content was lowest in treatment having 10 percent honey (3.28). The fat percentage in honey with 10 percent honey is not significantly higher than that of lassi with 12 percent honey. The results indicate that with the increase in level of honey, there was significant reduction in fat percent of herbal honey lassi. This may be due to the fact that fat content of honey was considerably less as compared to the fat content of dahi. Shuwu *et al.* (2011) ^[11] observed that with the increase in the level of honey, there was proportionate decrease in level of fat content in lassi. Thus, the present result agrees with their revelation.

Total solid

The total solid percentage was significantly highest in lassi prepared with addition of 10 percent honey while total solids content was the lowest in lassi prepared without addition of honey. The statistical analysis showed that total solids content of lassi was significantly increased with the addition of honey. It was seen that as the level of honey increases, there was an increase in level of total solids in lassi. This might be due to higher solids content of honey. The result obtained in present study are in agreement with the result reported by Shuwu *et al.* (2011) ^[12], who noted that with the increase in the level of honey, there was proportionate increase in the level of total solids content in lassi.

Titration acidity

The acidity content was the highest (0.80 percent) in plain lassi prepared without addition of honey i.e. with sugar, while acidity content was the lowest (0.77 percent) in lassi prepared with addition of 10 percent honey. It was observed that addition of different level of honey did not significantly affect the level of acidity of lassi, but there was a declining trend in the acidity level with increase in honey content. Shuwu *et al.* (2011) ^[12] reported that with the increase in levels of honey, there was proportionate decrease in the level of acidity of the lassi.

Ash content

The ash percent was the lowest (0.77 %) in lassi prepared without addition of honey i.e. plain lassi (with addition of 15% sugar), while ash content was the highest (0.81 %) in lassi prepared with addition of 10 percent honey and 2 percent Tulsi. As the level of addition of Tulsi extract is same in all treatment so this cant brought significant change in ash content of lassi. It was observed that with the increase in the level of honey in lassi, there was decrease in the ash content. This may be due to the low content of minerals in honey. The result of present study agrees with the result of Shuwu *et al.* (2011) ^[12]. They have reported that with increase in the level of honey there was proportionate decrease in the level of ash content in lassi.

Conclusion

Studying the quality of products i.e. herbal honey lassi based on incorporation of honey in the product at different levels i.e.

0, 8, 10 and 12% during optimisation process. It was revealed that addition of honey at 10% level showed relatively high score for chemical acceptability as compared to control, 8% and 12% honey addition. Hence, honey concentration of 10% was selected for manufacturing of herbal honey lassi and for further shelf-life study. A significantly change in trend was observed in the fat, protein, carbohydrate and ash content of herbal honey lassi in various levels of the Tulsi and honey combination. The fat percent was significantly highest in normal lassi prepared without honey (Control sample) i.e. with addition of sugar, while fat content was lowest in treatment having 10 percent honey (3.28 %). The protein percentage was significantly highest (3.35%) in the lassi prepared without addition of honey, while protein content was the lowest (3.18%) in lassi prepared with addition of 10 % honey. The titrable acidity content was the highest (0.80%) in plain lassi (prepared without addition of honey i.e. with sugar) while acidity content was the lowest (0.77 %) in lassi prepared with addition of 10 percent honey. The total solid percentage was significantly highest in lassi prepared with addition of 10 percent honey while total solids content was the lowest in lassi prepared without addition of honey. The ash percent was the lowest (0.77 %) in lassi prepared without addition of honey i.e. plain lassi (with addition of sugar), while ash content was the highest (0.81 %) in lassi prepared with addition of 10 percent honey and 2 percent Tulsi.

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