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Fortification of *Lassi* with Herbal Extracts – Effects on Quality and Total Phenolic Content

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A B S T R A C T

Lassi, a popular curd based traditional fermented milk beverage of India, was prepared from standardized cow milk *dahi* using 1% commercial yogurt culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Maximum concentration of herbal extracts added during processing of herbal *lassi* were optimized with Ginger, turmeric and carrot extracts @ 2 % (v/v), 1% (v/v) and 15% (v/v) respectively. The antioxidant activity of ginger, carrot, and turmeric extract-based herbal *lassi* was also evaluated. Antioxidant activity of the three varieties of herbal *lassi was* measured by total phenolic content as Gallic Acid Equivalent (GAE) using a double beam UV- visible spectrophotometer at 765 nm. The antioxidant activity of turmeric, ginger, and carrot juice *lassi* was found to be 0.226±0.001, 0.216±0.001, 0.154±0.001 mg of GAE/g respectively and differed significantly ($p<0.05$) with the control *lassi* (without any herbal extract) which showed antioxidant activity of 0.124±.001 mg of GAE/g. Turmeric *lassi* was adjudged best based on antioxidant activity and sensory evaluation and found acceptable up to 9 days when stored at 7±2°C in a glass bottle.

Introduction

The health benefits of different types of foods have been investigated for many years. The growing concern for health and nutrition among consumers has increased the market potential for functional foods throughout the world. Several studies have shown that fermented food products do have a positive effect on health status (Sahlin, 1999). The market for fermented milk products is growing at a faster rate throughout the world. In India, around 9 % of the total milk produced is converted into fermented milk products with an annual growth rate of more than 20% (Singh *et al.*, 2006). Milk-based soft drinks would find its place as a refreshing and nourishment drink and earned universal acceptability all over the world. *Lassi* is one of the most popular fermented dairy beverages in India prepared by the churning of curd. *Lassi* is proven to be an extraordinary nutritional material with all essential and non-essential amino acids. Moreover, these drinks

are light, refreshing, healthful, and nutritious but less acidic than fruit juices and offer a good profit margin. Lassi has been mentioned as one of the best among milk products due to its immense therapeutic, immunostimulatory activity, and nutritional value (Sarkar, 2008; Hussain *et al.,* 2011). Several health benefits associated with the consumption of live probiotic bacteria are in controlling intestinal infections, improved digestion, improved lactose utilization, prevention of colon cancer, lowering of blood pressure, cholesterol, and reduced inflammation, etc. (Dugas *et al.*, 1999). The functionality of *lassi* increases with the addition of probiotic microorganisms and incorporation of antioxidant-rich herbal juices that provides strong antioxidant power endow with value addition to the finished product. Herbs are used to fortify foods throughout history as preservatives, flavour, and therapeutic agents. Although herbs are low-cost commodities, they are nowadays valued as gold or jewels for many centuries (El- Sayed and Youssef, 2019). Consumption of herbs has a significant health-promoting effect and reduces the incidence of cardiovascular disease, cancer, and various degenerative diseases (Singh *et al.,* 2006; Craig, 1999; Shishodia *et al.,* 2005). Nowadays, dairy products are a unique carrier that has been successfully used to deliver phytochemicals and other nutrients for health benefits in our nutrition food system (El-Sayed *et al.,* 2015). Herbs viz. carrot (*Daucus carota* L.), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) have been widely used in our daily foods and were found rich in antioxidant (Molldrem *et al.,* 2004; Bandyopadhyay *et al.*, 2006; He *et al.,* 2015). The antioxidants are the major defensive system that prevents the body from damage by neutralizing the free radicals. Keeping in view the potential of probiotics and antioxidative properties of herbs, the present study was endeavoured to develop antioxidant-rich herbal *lassi* using carrot, ginger, and turmeric extract and assess the antioxidant activity of the developed *lassi*.

Materials and Methods

Collection of milk and herbs

Raw cow milk was collected from the cattle farm of West Bengal University of Animal and Fishery Sciences, Mohanpur. Cow milk was collected during summer season from the herd of Sahiwal breed mainly fed on paddy straw, hybrid Napier, and nutrifeed seeds. It was subsequently standardized to 3.0% fat and 8.5 % SNF and further used for the preparation of *Dahi*. Ginger, Carrot, and Turmeric were procured from the local market at Mohanpur, Nadia.

Bacterial Culture

Freeze-dried Yoghurt culture (YC-470) containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* of Danisco (Dange-Saint-Romain, France) was purchased from the market.

Preparation of herbal extract

Fresh ginger, carrot, and turmeric were peeled off and then cut into small pieces. After that, each herb pieces were boiled in water for 1 min. Then it is ground in a mixer and the juice is extracted by filtering with a muslin cloth.

Preparation of herbal lassi

Lassi was prepared following the method described by Ashwani *et al.,* (2003) with slight modification (Fig. 1). Milk was first standardized to 3 % fat and 8.5 % SNF. Then heated to 95^{o}C followed by cooling to 45^{o}C. Then milk was inoculated with 1% yogurt culture and incubated at 45±1^{o}C/ 4 to 5 hours. After that, the prepared *dahi* was broken and mixed with 12% sugar and 0.5% stabilizer.

Previously prepared herbal juice was then added and mixed well and stored at refrigeration condition (7±2^{o}C).

Chemical and microbiological analysis

Lassi was analyzed for fat, protein, lactose, sugar following the methods described in AOAC (1990). The standard plate count (SPC), coliform count, and yeast & mold count in *lassi* samples were determined following the methods described by APHA (1995).

Phenolic analysis

Extraction of sample

The extraction of a sample for analysis of phenolic content and antioxidant activity measured was done using the method of Swain and Hills (1959), with some modifications. Three g of the sample was mixed with 25ml methanol followed by homogenized using the Ultra-Turrax homogenizer. The homogenates were kept at 4°C for 12 hrs and then centrifuged at 15000 rpm for 20 min using a vacuum micro-centrifuge. The supernatants were recovered and stored at -20°C until analysis.

Total phenolic estimation

The total phenols of all extract were measured at 765 nm by Folin Ciocalteu reagent (McDonald *et al*, 2001). The dilute methanolic extract (0.5ml of 1:10 g/ml) or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10, diluted with distilled water) and aqueous sodium carbonate (4ml, 1M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using the solutions of Gallic acid prepared in methanol: water (50:50, v/v) with a range of 50 to 250 mg/l. Total phenol values were expressed in terms of Gallic acid equivalent(mg/g of dry mass), which is a common reference compound.

Sensory evaluation

Sensory evaluation of *lassi* was done by using 9 points Hedonic Scale as describe in Amerine *et al.*, (1965) and with a panel of five trained judges of the institution.

Statistical analysis

Observed data were analyzed for one-way ANOVA using the General Linear Model **(**GLM) method of IBM SPSS Statistics, version 21, 2012 software.

Results and Discussion

Chemical composition of *lassi*

Cow milk standardizes to 3% fat and 8.5% SNF was used for preparing *dahi* by using lactic culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus).* This prepared *dahi* was used for the preparation of herbal *lassi* with the addition of sugar syrup @12% and stabilizer @ 0.5% (v/v). *Lassi* samples contained 1.3% fat, 3.5 % protein, 1.2 % lactose, 12% lactose and 0.4 % ash.

Fixation of level of herbal extracts for the preparation of herbal *lassi*

Carrot, ginger, and turmeric are the common herb used at the regular household level. The bioactive compounds from spices and herbs have the potential to decrease or inhibit the risk of degenerative diseases such as diabetes, obesity, cancer, and cardiovascular diseases (Anderson *et al.*, 1999). Phenolic compounds of herbs and spices are good substitutes for the artificial antimicrobial agents used in food manufacturing (Bin *et al.*, 2011). Fortification

of the different herb in dairy products provides natural antioxidant, improve sensory quality, act as a bio preservative, and deliver nutritional as well as medicinal properties (El-Sayad and Youssef, 2019). Therefore, only the highest quality and a fixed level of the herbs can be added to dairy products to improve the appearance, attractiveness, and nutritional value of fortified foods.

In the present study, the level of herbal extracts for the preparation of herbal *lassi* was fixed based on sensory evaluation. Herbal *lassi* was prepared by using the different combinations of ginger, turmeric, and carrot extracts individually and evaluated by the sensory test with the control sample where no herbal extract was added.

Effect of different level of carrot extract on the sensory quality of *lassi*

The comparative sensory score of the *lassi* with different levels of carrot extract and control *lassi* samples, stored at 7 ± 2^{o}C are shown in table 1. The mean score of colour, flavour, body, and texture showed a significant difference compared to the control sample. It was also observed that those scores were found higher in the *lassi* having 15% carrot extract compared to the other preparations. The overall acceptability score of control *lassi* was 8.30±0.11 whereas, the carrot extract containing *lassi* scored 7.66 ± 0.12, 8.20 ± 0.11, 7.10 ± 0.12 and 7.0 ± 0.11 for 10%, 15%, 20% and 25% (v/v) carrot juice *lassi* respectively.

The differences between the values of each *lassi* were statistically significant. Also, *lassi* prepared by using 15% (v/v) carrot extract showed significantly higher ($p<0.05$) overall sensory score as compared to other treated *lassi* samples. Therefore using 15 % (v/v) carrot juice was fixed for the preparation of carrot *lassi*. *A s*imilar study was done by Kaur *et al.,* (2019) who said 10 percent carrot pulp improved the overall acceptability in terms of mouthfeel & richness of lassi. The fixation of a higher concentration of carrot extract in the present study could be due to the sensory preference of the panelists who liked the level.

Effect of different levels of ginger extract on the sensory quality of *lassi*

The comparative sensory score of the control *lassi* and *lassi* prepared with different levels of ginger extract, stored at 7 ± 2^{o}C are shown in table 2. The mean score of colour-appearance and body and texture decreased but non-significantly up to 2% concentration and then decreased significantly up to 4%. In the case of flavour, a significant difference was observed in each concentration compared to control lassi. Among those, a comparatively higher flavour score was observed with 2% ginger extract. The overall acceptability score of control *lassi* was 8.30±0.12 whereas, the ginger extract containing *lassi* scored 7.66 ± 0.12, 8.25±0.10, 7.33±0.12, and 6.50±13 for 1%, 2%, 3%, and 4% ginger *lassi* (v/v) respectively.

The differences between the sensory values were statistically significant ($p<0.05$). It was also observed that lassi prepared by using 2% ginger extract (v/v) showed significantly highest overall sensory score as compared to control and other treated *lassi* samples. Therefore, further study was done using 2 % ginger extract (v/v) in *lassi*. The result is in accordance with the findings of Srivastava *et al.,* (2015). They used different ration of ginger extract and finalized 2% ginger extract for the manufacture of herbal yoghurt. Pinto *et al.,* (2009) stated that 4% of ginger juice can be added to ice cream as a flavouring agent.

Table.1 Sensory properties of *lassi* prepared by using different levels carrot extract

Parameters (%)	Levels of Carrot Extract				
	Control	10%	15%	20%	25%
Colour	7.66±0.10^{a}	6.66±0.10^{b}	7.46±0.11^{c}	7.33±0.13^{d}	6.36±0.11^{e}
Flavour	8.33±0.13^{a}	7.40±0.11^{b}	8.13±0.13^{c}	7.20±0.11^{d}	6.8±0.12^{e}
Body and texture	8.33±0.10^{a}	8.25±0.12^{b}	8.23±0.12^{b}	7.66±0.12^{c}	7.33±0.12^{d}
Overall acceptability	8.30±0.11^{a}	7.66±0.12^{b}	8.20±0.11^{a}	7.10±0.12^{c}	7.0±0.11^{d}

Data represented as MEAN±SE; a- e Different superscripts within a row differ significantly ($p < 0.05$); n=5

Table.2 Sensory properties of *lassi* prepared by using different levels of ginger extract

Parameters (%)	Level of Ginger extract				
	Control	1%	2%	3%	4%
Colour and Appearance	7.66±0.11^{a}	7.55±0.11^{a}	7.50±0.10^{a}	7.20±0.10^{b}	7.0±0.11^{c}
Flavour	8.33±0.12^{a}	8.0±0.12^{b}	8.55±0.11^{c}	6.66±0.12^{d}	6.0±0.12^{d}
Body and texture	8.33±0.11^{a}	8.3±0.12^{a}	8.1±0.11^{a}	7.66±0.12^{b}	7.26±0.12^{c}
Overall acceptability	8.30±0.12^{a}	7.66±0.12^{b}	8.25±0.10^{a}	7.33±0.12^{d}	6.5±0.13^{d}

Data represented as MEAN±SE; a-d Different superscripts within a row differ significantly ($p<0.05$); n=5

Table.3 Sensory properties of *lassi* prepared by using different levels of turmeric extract

Parameters (%)	Levels of Turmeric extract				
	Control	1%	2%	3%	4%
Colour and Appearance	7.66 ±0.11^{a}	7.50±0.13^{a}	7.45±0.13^{a}	7.33±0.12^{b}	6.66±0.12^{c}
Flavour	8.33±0.10^{a}	8.2±0.11^{b}	7.33±0.13^{c}	6.0±0.11^{d}	4.33±0.11^{d}
Body and texture	8.33±0.11^{a}	8.25±0.13^{b}	7.7±0.11^{c}	7.0±0.12^{d}	7.0±0.12^{d}
Overall acceptability	8.30±0.11^{a}	8.23±0.12^{a}	7.33±0.10^{b}	6.66±0.11^{c}	5.66±0.11^{d}

Data represented as MEAN±SE; a-d Different superscripts within a row differ significantly ($p<0.05$); n=5

Table.4 Total phenolic content of herbal *lassi* prepared by using ginger, carrot and turmeric extracts

Parameters	**Types of Lassi**			
	Control lassi	**Ginger lassi**	**Turmeric lassi**	**Carrot lassi**
Total phenolic content (mg GAE/gm)	**0.124±.001^{a}**	**0.216±0.001^{b}**	**0.226±0.001^{c}**	**0.154±0.001^{d}**

Data represented as MEAN±SE; a-d Different superscripts within a row differ significantly ($p<0.05$). Average of five trials (n=5)

Table.5 Effect of storage on titratable acidity and microbial quality of turmeric *lassi* at refrigeration temperature (7±2°C)

Parameters	Days				
	0	3	5	7	9
Acidity (% LA)	0.312±0.01^{a}	0.402±0.01^{a}	0.531±0.02^{a}	0.672±0.02^{b}	0.690±0.03^{c}
SPC (log_{10}cfu/ml)	4.78±0.02^{a}	5.90±0.02^{b}	6.50±0.04^{c}	7.66±0.04^{d}	8.60±0.05^{e}
Coliform (log_{10}cfu/ml)	Nil	Nil	Nil	Nil	Nil
Yeast and mold (log_{10}cfu/ml)	Nil	Nil	Nil	Nil	Nil

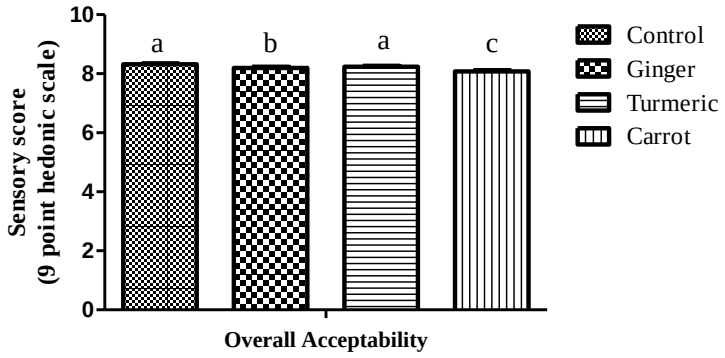
Data represented as MEAN±SE; a- e Different superscripts within a row differ significantly ($p<0.05$); n=5

Table.6 Effect of storage in sensory quality of turmeric *lassi* at refrigeration temperature (7±2°C)

Parameters (%)	No of Days				
	0	3	5	7	9
Colour	7.66±0.12^{a}	7.66±0.10^{a}	7.66±0.10^{a}	7.33±0.11^{a}	7.0±0.13^{a}
Flavour	8.66±0.10^{a}	8.56±0.10^{a}	8.33±0.11^{a}	7.66±0.11^{a}	7.33±0.13^{b}
Body and texture	8.66±0.12^{a}	8.66±0.12^{a}	8.54±0.11^{a}	8.33±0.13^{a}	7.66±0.12^{b}
Overall acceptability	9.0±0.12^{a}	8.83±0.12^{a}	8.83±0.13^{a}	8.66±0.12^{a}	7.33±0.02^{b}

Data represented as MEAN±SE; a – b Different superscripts within a row differ significantly ($p<0.05$); n=5

Fig.1 Effect of different herbal extract on the sensory properties of herbal *lassi* prepared by ginger (2%), turmeric (1%) and carrot (15%) extract. Each bar represents Mean ±SE; a-c Different superscript within each bar differ significantly ($p<0.05$); n= 5



Effect of different levels of turmeric extract on the sensory quality of *lassi*

The comparative sensory score of *lassi* prepared by using different levels of turmeric extract and control *lassi* sample, stored at 7 ± 2°C are shown in table 3.

The mean score of colour and appearance decreases but not significantly after increasing the concentration up to 2% and then decreased significantly up to 4%. This could be due to the high yellow colour in lassi which affects the appearance. In the case of flavour and body and texture, scores were decreased significantly with increasing the concentration of turmeric extract. The overall acceptability score of control *lassi* and the lassi containing 1% turmeric extract was comparable but the scores started to fall

significantly with higher concentrations. The overall acceptability score of control lassi was 8.30±0.11 whereas the turmeric extract containing *lassi* scored 8.23±0.12, 7.33±0.10, 6.66±0.10, and 5.66±0.11 for 1%, 2%, 3%, and 4% turmeric *lassi* (v/v) respectively. It was also observed that lassi containing 1% (v/v) turmeric extract showed a significantly higher overall sensory score as compared to other treated *lassi* samples. Therefore the further study was done using 1 % turmeric extract (v/v) in herbal *lassi.* These results are in line with those obtained by Foda *et al.*, (2008) who reported that turmeric in different concentrations had a significant effect on the sensory quality and acceptability of fresh and stored yoghurt. Also, Manoharan *et al.*, (2012) studied the effect of different levels of curcumin addition in ice cream as a colouring agent and based on sensory evaluation a rate of 0.5% was adjudged the best suitable level for addition in ice cream.

Total phenolic content of herbal *lassi* prepared by using ginger, carrot, and turmeric extract in *lassi*

On the basis of sensory evaluation 15 % (v/v) carrot juice, 2 % (v/v) ginger and 1% (v/v) turmeric incorporated *lassi* were selected for preparation of herbal *lassi*. The total phenolic content of these three herbal lassi samples was determined and it was measured in terms of mg GAE/gm. The total phenolic content of ginger *lassi*, turmeric *lassi*, and carrot *lassi* was found to be 0.216±0.001, 0.226±0.001 is 0.154±0.001 mg GAE/gm respectively whereas, the control sample showed 0.124±0.001 mg GAE/gm (Table 4).

The results showed that the differences in Total phenolic content among all the *lassi* samples were found statistically significant. Moreover, it was revealed that 1% (v/v) turmeric *lassi* showed significantly higher ($p<0.05$) Total phenolic content compared to the other samples. The results are in accordance with the findings of Sharma *et al.*, (2011), who found that the maximum polyphenols were present in turmeric extract (166.7mg/100g) followed by ginger (23.9 mg/100 g).

Effect of the different herb on the sensory quality of herbal lassi

Three types of herbal *lassi* prepared by the fixed level of different herb (ginger, carrot, and turmeric) were evaluated for the sensory property. The difference was made based on the overall acceptability of the three herbal lassies. The overall sensory scores of the three herbal lassies are depicted in Figure 1. The sensory score of ginger *lassi*, carrot *lassi*, and turmeric *lassi* was observed 8.20±0.11, 8.15±0.11, and 8.25±0.12 respectively. The difference in the sensory score between all the treated *lassi* samples was statistically significant ($p<0.05$). Moreover, it was revealed that turmeric *lassi* had the highest overall sensory score compared to the other herbal *lassi.*

On the basis of antioxidant activity and sensory score, 1% (v/v) turmeric *lassi* was selected for further analysis of storage stability.

Effect of storage on the chemical, microbiological, and sensory properties of turmeric *lassi* prepared with 1% turmeric extract stored at refrigeration temperature (7±2°C)

The storage stability of turmeric *lassi* was studied at refrigeration temperature (7±2°C). The products were kept in a glass bottle and were analyzed for change in acidity, standard plate count (SPC), yeast and mold, coliform, and sensory characteristics at 2 days interval i.e. 0^{th}, 3^{rd}, 5^{th}, 7^{th}, and 9^{th} days of storage at refrigeration temperature (7±2°C). The

change in different parameters of turmeric *lassi* is shown in tables 5 and 6. The result revealed that up to the 5th day of storage, there was no significant difference in acidity was observed whereas, after the 7th day acidity starts to increase significantly. From the microbiological analysis, it was observed that the standard plate count (SPC) of turmeric *lassi* increased from the initial day to the 9th day of storage whereas, in the case of coliform, yeast, and mold count, it was observed nil from the initial day to 9th day. A similar result was observed by Mourya, (2012), where he reported the absence of coliform in the curcumin fortified *lassi* sample during the storage period. The presence of coliforms in milk and milk products is indicative of unhygienic conditions or practices followed during production, processing, handling, and storage. Coliforms were not detected throughout the storage, which is indicative that the lassi sample was safe for consumption.

The effect of storage at 7±2^{o}C, on the sensory score of turmeric *lassi*, was done and represented in table 6. It was revealed that up to the 7th day of storage, among colour, flavour, body, and texture, there was no significant difference was observed whereas from 9th day onwards the difference starts to decrease at a significant level. From the overall acceptability of turmeric *lassi*, it was observed that up to the 7th-day a non-significant difference was observed. So it was finalized that the turmeric lassi can be stored to the acceptable condition up to the 9th day in a glass bottle at 7±2^{0}C temperature.

The result is in agreement with those obtained by Mourya, (2012), where he found that the curcumin fortified *lassi* had a shelf life of 20 days at 4 ± 1°C and 90-95% RH when packed in low-density polyethylene (LDPE) pouches or in polyethylene terephthalate (PET) bottles.

In conclusion *lassi* is a fermented milk product having good nutritional and therapeutic value. It can be made more nutritious and antioxidant-rich by the addition of different types of herbs viz. Ginger, turmeric, and carrot. Based on sensory evaluation, 2% (v/v) ginger extract, 1% (v/v) turmeric extract and 15% (v/v) carrot extract was finally added to the *lassi*. On the basis of antioxidant activity and sensory evaluation *lassi* prepared by the addition of turmeric juice @ 1% (V/V) was adjudged best. The final products showed a shelf life of 9 days on the basis of chemical, microbiological, and sensory tests when kept in a glass bottle and stored at 7±2°C.

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