International Journal of Agriculture, Environment and Biotechnology
Citation: *IJAEB*: **17**(02): 131-137, June 2024
DOI: 10.30954/0974-1712.02.2024.6

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

RESEARCH PAPER

Studies on Preparation of Antioxidant Enriched Herbal *Lassi* Incorporated with Lemongrass (*Cymbopogan citratus*) Extract

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Paper No. 1117 **Received:** 11-03-2024 **Revised:** 25-05-2024 **Accepted:** 04-06-2024

ABSTRACT

Herbal *lassi* was prepared by adding concentrated lemongrass (*Cymbopogon citratus*) extract (CLE) at different levels (0.0, 1.0, 1.5, 2.0 and 2.5%). The herbal *lassi* prepared with addition of CLE (2.0%) had significantly (P<0.05) higher scores for the sensory attributes viz. flavour (8.52 ± 0.10), colour and appearance (7.60 ± 0.07), product acidity (8.44 ± 0.11) and overall acceptability (8.47 ± 0.10) as compared to control whereas the score for body and texture (8.28 ± 0.08) resembled to that of control. Therefore, lassi prepared with 2.0 per cent CLE was adjudged as the optimized product. The optimized product had TS (21.22%), fat (3.02%), protein (3.37%), total carbohydrate (14.12%), ash (0.71%), pH (4.71) and titratable acidity (0.68% LA). Further, the herbal *lassi* had higher TPC (50.28 ± 0.04 µM GAE/100 g) and antioxidant capacity i.e. ABTS (19.46 ± 0.03 µM TE/100 g), DPPH (33.53 ± 0.05 µM TE/100 g) and FRAP (24.62 ± 0.04 µM TE/100 g).

HIGHLIGHTS

- *Lassi*, is a popular traditional Indian fermented milk product.
- A technology was developed for preparation of herbal *lassi* incorporated with concentrated lemongrass (*Cymbopogan citratus*) extract (CLE).
- Herbal *lassi* prepared with 2.0 per cent CLE was adjudged as the optimized product and had enhanced antioxidant potential.

Keywords : *Lassi*, Antioxidant, Lemongrass, Sensory, Physico-chemical

The oxidation products from biological reactions or exogenous factors are generated in the body and foodstuffs by the reactive oxygen species (ROS) including free radicals such as superoxide anion radical (O_2^-), hydroxyl radical (OH^-) and singlet oxygen (Phaniendra *et al.* 2015; Chaudhary *et al.* 2023). Free radicals and active oxygen species such as the oxygen molecule behave as double-edged sword. They are important mediators in signal transduction and play a vital role in the production of biologically active and essential compounds. At the same time; they are toxic and known to play a causative role in a variety of diseases (Pham-Huy *et al.* 2008; Juan *et al.* 2021).

There is an increasing evidence for the involvement of ROS in a variety of normal *in-vivo* regulatory systems. When an excess of free radicals is formed, they can overwhelm protective enzymes like superoxide dismutase, catalase and peroxidase,

How to cite this article: Shendurse, A.M., Patel, A.C., Gopikrishna, G., Gawai, K.M. and Prajapati, R.J. (2024). Studies on Preparation of Antioxidant Enriched Herbal *Lassi* Incorporated with Lemongrass (*Cymbopogan citratus*) Extract. *Int. J. Ag. Env. Biotech.*, **17**(02): 131-137.

Source of Support: None; **Conflict of Interest:** None

and cause destructive and lethal cellular effects (apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration. In addition, it has been recognized that oxidative stress plays a significant role in a number of age specific diseases. The factors involved in these diseases are the lipid peroxides and low molecular weight compounds produced during the late stage of the oxidative reaction. For example, many studies have shown increased oxidative damage to all the major classes of biomolecules in the brains of Alzheimer's patients (Halliwell, 2001; Liu *et al.* 2004). Other pathological conditions associated with evidence of significant free radical mediated injury include atherosclerosis, diabetes, rheumatoid arthritis (Abuja and Albertini, 2001; Halliwell, 2001; Halliwell and Whiteman, 2004; Hoelzl *et al.* 2005). Furthermore, cancer is probably a consequence of oxidative DNA-damage (Pham-Huy *et al.* 2008; Chaudhary *et al.* 2023).

The annual milk production in India is estimated to cross 200 million tons in coming years. About 55 per cent of the milk produced in the country is being utilized for preparation of various dairy products. It is estimated that about 7% of milk produced is converted into fermented milk products. Fermented milk products were developed throughout the world as a means of preserving milk solids against spoilage and have persisted over the centuries in the developing world. The scale of production ranged from house hold production to a large-scale production, wherein use of selected starter cultures, automatic processes and modern equipment are involved. Fermented milk products are popular in view of their organoleptic and other properties such as the characteristic flavour, refreshing taste and improved digestibility. The composition of fermented milk products can be easily tailored to meet various dietary requirements. The dietetic significance of Indian fermented milk products has been established due to its various nutritional and therapeutic properties (Sarkara, 2008).

Lassi, is a popular traditional Indian fermented milk product that is frequently consumed in almost every household in our country and it is produced by fermentation of milk with lactic acid bacteria and considered as a functional food due to its therapeutic and health promoting properties along with well-established nutritional benefits. Empirical evidences suggest that the composition of an average Indian's food basket is gradually shifting towards value added products with additional health benefits (Sarkara, 2008; Singh and Singh, 2014).

Lemongrass (*Cymbopogon citratus*) is a grass variety belonging to Gramineae family. It is a tall, clumped perennial grass growing to a height of 1 m. The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm. It is a tropical grass native to western parts of India and Sri Lanka. It yields aromatic oil used as flavoring, and in perfumery and medicine. It is believed to have a wide range of therapeutic effects, and has been used for centuries in South America and India. There are several species of lemongrass, but few varieties are recommended for food and medicinal use (Francisco *et al*. 2011; Costa *et al*. 2015). Lemongrass extract is obtained from the leaves and shoot of the lemongrass plant and used in several food products including beverages, frozen products etc. The lemongrass extract is reported to possess several health benefits. Only the fresh or dried leaves of lemongrass, and the essential oil derived from them, are used. Lemongrass is widely used as an essential ingredient in Asian cuisines because of its sharp lemon flavor (Adeneye and Agbaje, 2007). It is also reported to be a favorite ingredient in Thai cuisine and dishes. Herbal tea of lemongrass is used as sedative as well as immunostimulant in India. Lemongrass was usually known only for its aromatic properties. However, it also possesses numerous health benefits which make it an invaluable herb. The lemongrass extract is reported to have the following health benefits: antioxidant properties, antibacterial and antifungal properties; detoxify the liver, pancreas, kidney, bladder and the digestive tract and boost the immune system. Lemongrass flavour just like citrus flavours combines well with acidic products (Kumar *et al*. 2017; Ifesan and Olorunsola, 2018). Therefore, an attempt was made in proposed research project to develop the lemongrass incorporated herbal *lassi* with enhanced antioxidants activity.

MATERIALS AND METHODS

Milk

Fresh, chilled, raw cow milk of Kankrej breed was collected from the Livestock Research Station

(LRS), S.D. Agriculture University (SDAU), Sardarkrushinagar (Gujarat).

Lemongrass

Lemongrass (*Cymbopogon citratus*) grown at the Centre for Agro-Forestry, Forage Crops and Green Belt, SDAU was used for the study. Fresh green matured leaves of medium size were selected and cut from the plant.

Lactic cultures

Freeze dried DVS culture (Lyofast MS 059 ET) consisting of specifically selected blend of lactic acid bacteria used for preparing the fermented milk products was obtained from Sacco System, Italy.

Skim milk powder

Spray dried skim milk powder (SMP) was procured from Banas dairy, Palanpur, Gujarat and was used for standardization of solid not fat (SNF) level.

Sugar

Crystalline sugar procured from the local market was used as the sweetening material in the preparation of fermented product.

Preparation of concentrated lemongrass extract (CLE)

Concentrated lemongrass extract (CLE) was prepared by using dried lemongrass leaves powder through refluxing, filtration and centrifugation (10000 rpm/10 min) followed by concentration to 90% using rotary vacuum evaporator.

Preparation of herbal *lassi*

The herbal *lassi* was prepared by adding different levels i.e. T1 (0.0%, control), T2 (1.0%), T3 (1.5%), T4 (2.0%) and T5 (2.5%) of CLE possessing highest antioxidant activity as per the flow diagram mentioned in Fig. 1. The product was optimized on the basis of sensory evaluation. The developed

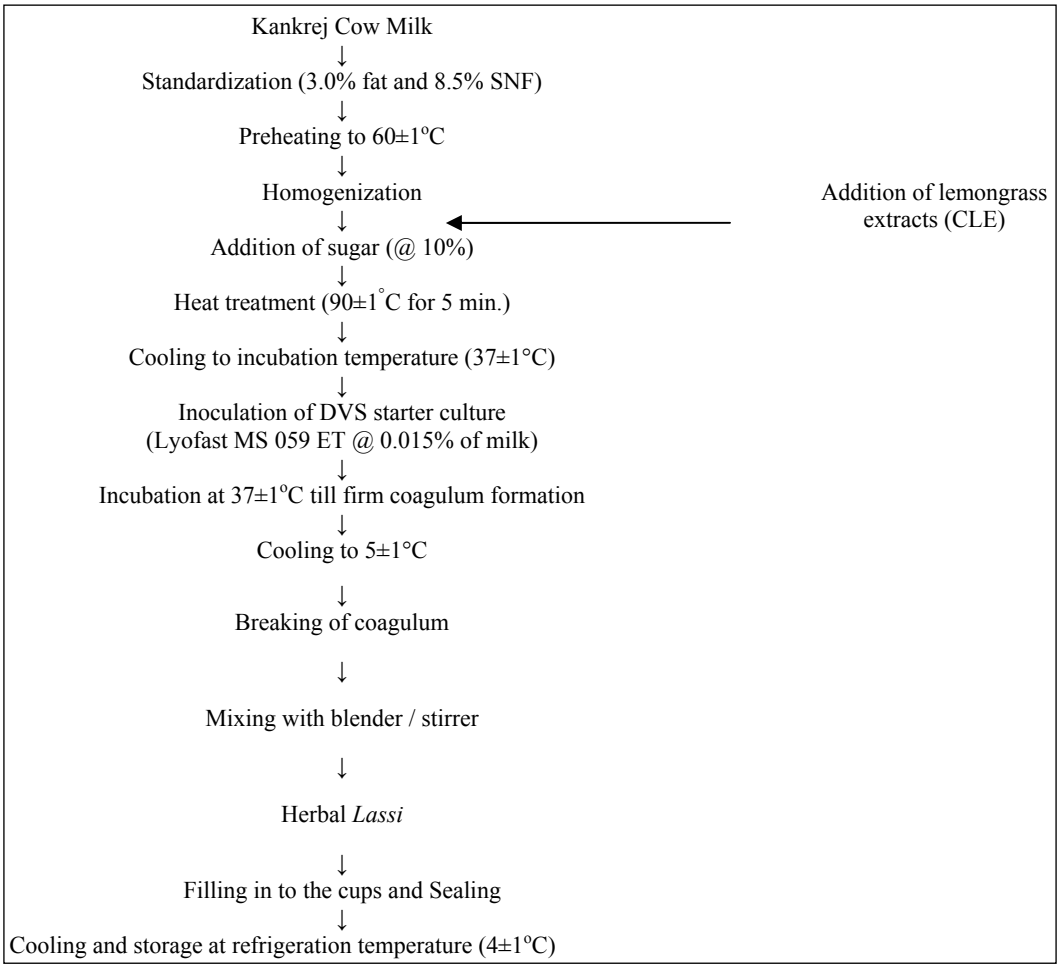

Fig. 1: Flow diagram for preparation of herbal *lassi*

product was analyzed for physico-chemical analysis (total solids, fat, protein, total carbohydrate, ash, titratable acidity and pH) as well as for the total phenolic content (Kahkonen *et al.* 1999) and antioxidant potential by ABTS assay (Re *et al.* 1999; Guo and Jauregi, 2018), DPPH assay (Brand-Williams *et al.* 1995) and FRAP assay (Benzie and Strain, 1996).

STATISTICAL ANALYSIS

Statistical analysis was carried out using completely randomized design (CRD) with 3 repetitions. The data are presented as means ± standard error of mean (SEm) or means ± standard deviation (STD). Analysis of variance (ANOVA) was used to determine the main effects of treatments (Gacula and Singh, 1984; Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Optimization of levels of concentrated lemongrass extract (CLE) in *lassi*

The sensory scores of *lassi* prepared by addition of CLE at different levels are presented in Table 1. The results depicted that addition of different levels of CLE in *lassi* had a marked influence on all sensory attributes except body and texture. *Lassi* prepared with addition of CLE at different levels i.e. 0.0% (T1, control), 1.0% (T2), 1.5% (T3), 2.0% (T4) and 2.5% (T5) had flavour scores of 6.51 ± 0.12, 7.67 ± 0.09, 8.11 ± 0.08, 8.52 ± 0.10 and 8.09 ± 0.11, respectively, while the scores for overall acceptability were 6.56 ± 0.11, 7.49 ± 0.10, 7.97 ± 0.11, 8.47 ± 0.10 and 7.95 ± 0.08, respectively. It was observed that with the increase in level of addition of CLE in *lassi* from 1.0 to 2.0 per cent, the sensory scores for flavour and overall acceptability also increased significantly (P<0.05). Further increase in the level of CLE to 2.5 per cent, resulted in significant (P<0.05) decrease in the scores of both i.e. flavour and overall acceptability. The scores of colour and appearance as well as product acidity of lemongrass incorporated *lassi* samples had significantly (P<0.05) increased with the increase in the level of CLE (1.0 to 2.5 per cent) as compared to control because judges preferred coloured *lassi* over the uncoloured control *lassi*. The *lassi* prepared with CLE (2.0%) had significantly (P<0.05) higher scores for the sensory attributes viz. flavour (8.52 ± 0.10), colour and appearance (7.60 ± 0.07), product acidity (8.44 ± 0.11) and overall acceptability (8.47 ± 0.10) as compared to control whereas the score for body and texture (8.28 ± 0.08) resembled to that of control. Therefore, *lassi* prepared with 2.0 per cent CLE was adjudged as the optimized product.

Composition and physico-chemical characteristics of herbal *lassi*

The control and CLE incorporated herbal *lassi* samples were analyzed for compositional and physico-chemical characteristics. The average values of total solids (TS), fat, protein, total carbohydrate, ash, pH and titratable acidity are depicted in Table 2. The values of TS, fat, protein, total carbohydrate and ash were recorded highest for control *lassi* i.e. 21.61 ± 0.15, 3.08 ± 0.02, 3.32 ± 0.02, 14.47 ± 0.05 and 0.74 ± 0.01 per cent, respectively, whereas slightly lower values were observed for *lassi* prepared with CLE i.e. 21.22 ± 0.14, 3.02 ± 0.02, 3.37 ± 0.13, 14.12 ± 0.07 and 0.71 ± 0.02 per cent, respectively. Similarly, the control and CLE added *lassi* had pH values of 4.65 ± 0.01 and 4.71 ± 0.01 and titratable acidity of 0.70 ± 0.002 and 0.68 ± 0.002% LA, respectively.

Table 1: Sensory scores of herbal *lassi* prepared with incorporation of CLE at different levels

Sensory Attributes	Level of addition of CLE (%)					CD (p<0.05)
	Control (T1)	1.0 (T2)	1.5 (T3)	2.0 (T4)	2.5 (T5)	
Flavour	6.51 ± 0.12^{d}	7.67 ± 0.09^{c}	8.11 ± 0.08^{b}	8.52 ± 0.10^{a}	8.09 ± 0.11^{b}	0.14
Body and texture	8.30 ± 0.09^{a}	8.30 ± 0.07^{a}	8.27 ± 0.10^{a}	8.28 ± 0.08^{a}	8.26 ± 0.09^{a}	0.11
Colour and appearance	6.48 ± 0.11^{a}	7.01 ± 0.11^{b}	7.58 ± 0.08^{c}	7.60 ± 0.07^{c}	8.23 ± 0.10^{d}	0.17
Product acidity	7.42 ± 0.10^{a}	8.01 ± 0.09^{b}	8.41 ± 0.12^{c}	8.44 ± 0.11^{c}	8.39 ± 0.11^{c}	0.15
Overall acceptability	6.56 ± 0.11^{a}	7.49 ± 0.10^{b}	7.97 ± 0.11^{c}	8.47 ± 0.10^{d}	7.95 ± 0.08^{c}	0.19

Means in each row with different superscripts (a, b, c, d) were significantly different (LSD test, P < 0.05) from each other. Data are presented as means ± SEm (n = 21).

Table 2: Composition and physico-chemical characteristics of control and CLE incorporated herbal *lassi*

Parameters	Control *lassi*	CLE incorporated herbal *lassi*
Proximate composition (%)		
Total solids	21.61 ± 0.15	21.22 ± 0.14
Fat	3.08 ± 0.02	3.02 ± 0.02
Protein	3.32 ± 0.02	3.37 ± 0.13
Total carbohydrate	14.47 ± 0.05	14.12 ± 0.07
Ash	0.74 ± 0.01	0.71 ± 0.02
Physico-chemical characteristics		
pH	4.65 ± 0.01	4.71 ± 0.01
Titratable acidity (% lactic acid)	0.70 ± 0.002	0.68 ± 0.002

Values represent average of three trials i.e. Mean ± STD (n=3).

Table 3: Antioxidant capacity and total phenolic content of control and CLE incorporated herbal *lassi*

Product	Antioxidant capacity (µM TE*/100 g)			Total phenolic content (µM GAE†/100 g)
	ABTS	DPPH	FRAP	TPC
Control *lassi*	9.67 ± 0.02	13.93 ± 0.04	10.82 ± 0.03	27.45 ± 0.03
CLE incorporated herbal *lassi*	19.46 ± 0.03	33.53 ± 0.05	24.62 ± 0.04	50.28 ± 0.04

Data are presented as mean ± STD (n=3).

** - TE- trolox equivalent, † - GAE- gallic acid equivalent.*

Table 4: Cost of production of Herbal *Lassi*

	Raw material cost for preparing 100 kg of Herbal *Lassi*				
		Herbal *Lassi*		Control *Lassi*	
Ingredients	**Cost (₹/kg)**	**Quantity (kg)**	**Cost (₹)**	**Quantity (kg)**	**Cost (₹)**
Milk	₹ 50.00	100.00	₹ 5000.00	100.00	₹ 5000.00
Sugar	₹ 55.00	10.00	₹ 550.00	10.00	₹ 550.00
DVS culture	450.00/5 units	4 units	₹ 360.00	4 units	360.00
LGE Concentrate	₹ 200.00	02.00	₹ 400.00	—	—
Total		112.00	₹ 6310.00	110.00	₹ 5910.00
Cost (₹/100 kg *Lassi*)		100.00	₹ 5633.93	100.00	₹ 5372.73
Cost (₹/kg *Lassi*)			₹ 56.34		₹ 53.73
Manufacturing cost for *Lassi*					
Cost type	**Assumption made**		**Cost of *Herbal Lassi* (₹/ Kg)**		**Cost of control *Lassi* (₹/ Kg)**
Raw material cost	—		₹ 56.34		₹ 53.73
Processing and allied cost	15% of raw material cost		₹ 08.45		₹ 08.06
Packaging cost	₹ 3.00 per cup/glass		₹ 15.0		₹ 15.0
Total cost (₹/kg *Lassi*)			**₹ 79.79**		**₹ 76.79**

Antioxidant capacity and total phenolic content of herbal *lassi*

The total phenolic content (TPC) as well as antioxidant activity by ABTS, DPPH and FRAP assays were measured for the control and CLE incorporated *lassi* and the results are presented in Table 3. The data of antioxidant capacity by ABTS, DPPH and FRAP assays showed lowest values for control *lassi* i.e. 9.67 ± 0.02, 13.93 ± 0.04 and 10.82 ± 0.03 µM TE/100 g, respectively, whereas higher antioxidant capacity was exhibited by the *lassi* prepared with CLE which possessed the values of 19.46 ± 0.03, 33.53 ± 0.05 and 24.62 ± 0.04 µM TE/100 g, respectively. Similarly, the trend of TPC (µM GAE/100 g) was observed as control *lassi* (27.45 ± 0.03) < CLE *lassi* (50.28 ± 0.04). The results

indicated higher TPC and antioxidant potential of CLE incorporated herbal *lassi* as compared to control *lassi*.

Computing the cost of herbal *Lassi*

The raw material cost involved in preparing herbal *lassi* is shown in Table 4. Other associated costs such as cost of processing, works overhead, publicity and advertising have been assumed to be 15.0 per cent of the raw material cost and packaging cost was considered separately. The manufacturing cost involved in preparing herbal *lassi* is also depicted in the Table 4.

The current price of a popular brand of rose and mango flavour of *lassi* (1000 ml) is ₹ 60.0, while that of strawberry *lassi* (1000 g) is ₹ 100.0. In the current experiment, the cost of 1000 g serving size of herbal *lassi* added with 2.0 % CLE was computed at ₹ 79.79.

The cost of production of 200 g of CLE incorporated herbal *lassi* and control *lassi* was ₹ 15.96/- and 15.36/, respectively.

CONCLUSION

The results of present investigation revealed that the herbal *lassi* with enhanced antioxidant potential could be prepared by incorporating 2.0 per cent of CLE (concentrated lemongrass extract). The developed product had higher overall acceptability as compared to control. The TS, fat, protein, total carbohydrate and ash for herbal *lassi* prepared with CLE was 21.22 ± 0.14, 3.02 ± 0.02, 3.37 ± 0.13, 14.12 ± 0.07 and 0.71 ± 0.02 per cent, respectively, while it had pH of 4.71 ± 0.01 and titratable acidity of 0.68 ± 0.002% LA. The cost of production of 200 g of CLE incorporated herbal *lassi* was ₹ 15.96/-. Further, the TPC and antioxidant potential of CLE incorporated herbal *lassi* was higher as compared to control.

ACKNOWLEDGMENTS

Centre for Agro-Forestry, Forage Crops and Green Belt, S.D. Agri. University, Sardarkrushinagar for providing lemongrass.

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