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RESEARCH ARTICLE

Enhancing antioxidant potential of *Lassi* through fortification with *Paneer* whey partially fermented with *Streptococcus thermophilus*

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Abstract: *Lassi,* a traditional Indian yoghurt-based fermented milk beverage, is known for its functional attributes, including antioxidant activity. The present study aimed to enhance the antioxidant potential of *lassi* by fortifying it with partially fermented paneer (Indian cottage cheese) whey. Paneer whey was fermented using *Streptococcus thermophilus* at 1.5% inoculum for 24 hours at 42/ °C before being incorporated into the yoghurt base. Antioxidant activity of the fortified and control *lassi* samples was evaluated using ABTS [2,22 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. The partially fermented whey fortified *lassi* exhibited significantly higher antioxidant activity (77.22/ ±/ 0.50/ µM TEAC in ABTS assay and 15.90/ ±/ 0.08% DPPH inhibition) compared to the control (70.05/ ±/ 0.67/ µM TEAC and 12.41/ ±/ 0.02%, respectively). These results demonstrate that fortifying *lassi* with partially fermented whey enhances its functional properties while also promoting sustainable utilization of whey, thereby reducing environmental burden and nutrient loss associated with paneer production.

Keywords: ABTS, Antioxidant activity, DPPH, *Lassi*, paneer, whey, yoghurt

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Introduction

Lassi is a traditional Indian dairy product consumed as a refreshing beverage mainly in the Northern parts of India during summer. It is commercially prepared by fermentation of milk (standardized to 4% fat and 8.5% solids not fat) by specific strain of starter culture (Mora, 2019). Good quality *lassi* should have creamy consistency, smooth texture, glossy sheen and white colour with yellowish tinge. The mild acidic flavour and sweetish taste of *lassi* make it a refreshing soft drink. (Ananthakumar and Narayanan, 2021). It is flavoured with either salt or sugar and other condiments or spices like ginger, coriander and mint depending on regional preferences. *Lassi* is an excellent palatable product, full of nutrients, easy to digest, beneficial for gastrointestinal disorder (Padghan et al. 2015).

Streptococcus thermophilus, a proteolytic lactic acid bacterium widely applied in dairy fermentations, has been reported to release bioactive peptides with significant antioxidant potential. Its proteolytic system, comprising extracellular and cell-bound enzymes, enables the hydrolysis of milk and other protein substrates into low-molecular-weight peptides that exhibit radical-scavenging activities such as DPPH and ABTS assays, along with ferric reducing antioxidant power (Guo et al. 2024). Furthermore, when fermenting whey protein concentrates, certain strains of *Streptococcus thermophilus* produce hydrolysates that maintain strong antioxidant activity even after simulated gastrointestinal digestion, demonstrating their potential health relevance and functional stability (Wu et al. 2023; Balthazar et al. 2024).

In recent years, increasing attention has been directed toward the nutritional significance of Indian cottage cheese whey, commonly referred to as "acid whey," which is generated during the acid coagulation of milk proteins. Unlike sweet whey, acid whey possesses a lower pH, which contributes to its comparatively longer storage stability (Macwan et al. 2016). Although traditionally regarded as a major dairy waste contributing to high biochemical oxygen demand (BOD) in the environment, whey is now increasingly recognized as a valuable substrate for the development of functional foods and bioactive compounds. Previous studies have demonstrated the health-

promoting potential of whey-based products; for instance, Anupam et al. (2021) reported that a yogurt-based beverage enriched with whey exhibited antioxidant properties and provided protection against oxidative stress-related conditions. In line with the growing emphasis on sustainable utilization of dairy by-products, the present study investigated the fortification of *lassi* with acid whey derived from paneer production, with the objective of enhancing its antioxidative potential. Two approaches were employed: partial fermentation of whey using *Streptococcus thermophilus* prior to incorporation, and direct fortification with non-fermented whey. The formulated products, along with a control, were systematically evaluated for their physicochemical properties, antioxidant activity, and storage stability in order to assess their functional attributes and potential health benefits.

Material and Methods

Collection of whey fortified *lassi* samples

Three types of *lassi* samples were collected from experiential learning unit (ELU) of Students' Dairy, WBUAFS, Mohanpur campus, Nadia, West Bengal during the month of March, September and December. All samples were collected in PET bottles and kept in a refrigerator (7±2!) for further storage studies. Samples collected from whey-fortified lassi are indicated as T1, T2, T3.

T1= Partially fermented whey fortified *lassi*.

T2= Non-fermented whey fortified *lassi*.

T3 = Control *lassi* without whey.

Chemicals used

Potassium Persulphate solution (2.45 mM), ABTS solution (7 mM) and DPPH solution (0.2 mM) were used to analyse antioxidant activity of the samples. Trichloroacetic Acid, Alkaline Copper Tartarate, Folin-Ciocalteau Reagent, 0.1 N Hydrochloric Acid were used to determine tyrosine value. All the chemicals used during investigation were of analytical grade chemicals and procured from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

Activation and propagation of culture

The particular freeze dried *Streptococcus thermophiles* was activated in the M17 broth at 42°C for 12h and maintained at 7 ± 2 ° C. The active culture was prepared in the sterile skim milk tubes (42°C/12h) and stored at 7 ± 2 ° C for further propagation. Thereafter sub culturing was done for fermenting paneer whey.

Fermentation of whey

The paneer whey was partially fermented with *Streptococcus thermophilus* @1.5% for 24 h at 42ÚC and then added to the Indian yoghurt.

Physico-Chemical analysis of *paneer* whey and *lassi* samples

The pH and acidity, ash, protein and total solid content of the *paneer* whey and *lassi* samples were determined as described in AOAC (1996). The fat and lactose content were determined by the Rose Gottlieb method (AOAC 1990) and Lane-Eynon method given in IS: 1479-PART-II (1961) respectively. The total sugar content of *lassi* samples were determined as per the procedure of Lane Eynon (1923) and modified by Ranganna (1977) while the sugar content (sucrose) was calculated by subtracting reducing sugar (lactose) from the total sugar of *lassi* samples. The extent of protein breakdown was estimated in terms of tyrosine value as delineated by modified method of Juffs (1973).

Determination of antioxidant activity of *lassi* samples

Antioxidant activity of market *lassi* samples and available in ELU were determined by two methods viz; ABTS and DPPH assay.

ABTS [2,2'- Azinobis (3-ethyl benzothiazoline)-6-sulfonic acid] Assay

Total antioxidant activity was measured using the spectrophotometric method of Re et al. (1999). 100 g of *lassi* samples were taken in the centrifuge tubes, centrifuged (3000 rpm for 30 min) and filtered using Whatman no. 40 aseptically in sterile test tubes. ABTS cation radicals were generated by reacting 7/ mM ABTS with 2.45/ mM potassium persulfate (1:1) and incubating in the dark for 12–16 hours. The working solution was prepared by diluting the stock with methanol to an absorbance of 0.70/ ±/ 0.02 at 734/ nm. For the assay, 3.9/ mL of ABTS working solution was mixed with 0.1/ mL of the diluted sample, incubated for 5 minutes and absorbance was measured at 734/ nm. Methanol served as blank and antioxidant activity was calculated based on a Trolox standard curve by following equation (Fig 1).

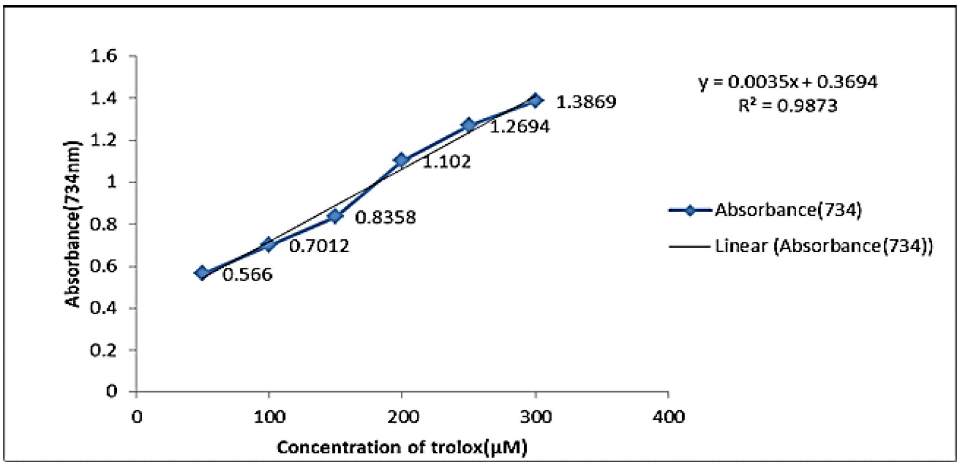
$$\%\ ABTS\ free\ radical\ inhibition\ activity = (Blank\ abs - Sample\ abs) \times 100 / Blank\ abs$$

Where blank $_{abs}$ is the absorbance of the blank sample and sample $_{abs}$ is the absorbance of the sample. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), i.e., $\mu M\ Trolox/100\ g\ dry\ weight\ (DW)$.

DPPH (2,2-Diphenyl-1-picryl hydrazyl) Assay

The antioxidant activity of *lassi* samples were measured using the DPPH assay as described by Mbunde et al. (2018) with slight modifications. 5g of *lassi* sample was extracted in 50 ml methanol for 48 hours at 37°C. It was then filtered through Whatman No. 42 filter paper and 1 ml filtrate was taken for analysis. A 0.2/ mM DPPH solution (7.88/ mg in 100/ ml methanol) was prepared in an

Fig. 1 Standard curve of Trolox (ABTS).



amber flask. For analysis, 4/ ml of DPPH solution, 1/ ml of extract and 2/ ml methanol were mixed and incubated in the dark for 30/ minutes. Absorbance was read at 517/ nm. The control contained DPPH solution and methanol only, with methanol as the blank. Free radical scavenging activity expressed in terms of per cent inhibition was calculated using the formula:

$$Inhibition\ (\%) = (Ac - A) \times 100 / Ac$$

Where, Ac is the absorbance of control and A is the absorbance of test. The antioxidant activity of whey was assessed following Liu et al. (2005) with slight modifications. Whey (5/ ml) was diluted to 25/ ml with methanol, centrifuged (6000/ rpm for 15/ minutes) and filtered. Equal volumes (2/ ml) of filtrate and 0.2/ mM DPPH solution were mixed, incubated in the dark for 30/ minutes and absorbance was taken at 517/ nm. The control was prepared in the same manner while methanol was used as blank. Free radical scavenging activity (% inhibition) was calculated using the same formula as mentioned above.

Statistical analysis

The statistical analysis of the data obtained during the course of investigation was done for meaningful interpretation of the results and statistical software IBM SPSS statistics 20 was used. Least significant difference at ($p < 0.05$) was used to determine significant differences between means from all the treatments.

Results and Discussion

Physico-Chemical properties

The paneer whey used for fortification exhibited an average composition of fat 0.33 ± 0.016%, protein 0.87 ± 0.015%, lactose 4.76 ± 0.16%, and ash 0.54 ± 0.015%, with a pH of 5.8 ± 0.1. The pH and titratable acidity of lassi samples T1, T2, and T3 varied significantly ($p < 0.05$), with pH values ranging from 3.83 to 4.34, consistent with previous reports (Ghule et al. 2016; De, 1980; Jadhav, 1991; Rathaur & Solankey, 2002).

The fat content of whey-fortified samples T1 and T2 did not differ significantly from the control (T3) ($p > 0.05$), while values were slightly lower than those reported by Bhoir et al. (2012) for traditional lassi (2.88–3.26%). In contrast, protein and total solids content differed significantly among the treatments ($p < 0.05$). Protein content in the fortified lassi was comparable to earlier findings in skim milk lassi (Chaudhari, 1959; Laxminarayana & Shankar, 1980), whereas variations in total solids likely reflect the incorporation of additional components, which influence texture, taste, and mouthfeel, as noted by Pagote and Balachandran (1993), Jadhav (1991), and Kalokhe (1991).

The sucrose content of the control lassi (11.91 ± 0.03%) differed significantly from fortified samples ($p < 0.05$), exceeding the range reported by Bhoir et al. (2012) (8.51–9.46%). Overall, whey fortification had a significant impact on protein, total solids, and sucrose content, while maintaining fat and acidity within typical ranges for lassi.

An increase in the tyrosine content (from 2.85 ± 0.02 to 4.12 ± 0.03 mg tyrosine/100 mL) was observed in the fermented whey-fortified lassi samples, indicating enhanced protein hydrolysis. This elevated proteolytic activity may have contributed to the release of antioxidative peptides in the fortified lassi. These results are in agreement with Wu et al. (2023), who reported that fermentation of whey protein concentrates with specific strains of *Streptococcus thermophilus* generates hydrolysates exhibiting substantial antioxidant activity. A similar trend was observed throughout the storage period of the lassi samples.

Changes in antioxidant activity of *lassi* during storage at 7±2°C

Antioxidant activity of whey fortified *lassi* along with control *lassi* samples varied significantly ($p<0.05$) during storage period and increased gradually with storage days (Fig. 2 and 3). From

the Table 2 and 3, it is clearly evident that increase in antioxidant activity (determined by both ABTS and DPPH method) in partially fermented whey fortified *lassi* samples were higher as compared to non-fermented whey fortified and control lassi samples. The initial antioxidant activity of the T1, T2 and T3 samples were 76.77±0.168, 72.26±0.058 and 70.17±0.066 µM TEAC respectively determined by ABTS and 16.03±0.097, 13.36±0.073 and 11.16±0.100 % DPPH inhibition respectively which was increased to an average antioxidant activity of 82.44±1.218,74.74±0.081,71.16±0.026 µM TEAC by ABTS and 17.34±0.064, 14.57±0.108 and 11.87±0.017 % DPPH inhibition respectively on the 7th day of storage.

The increase in the antioxidant activity might be due to the increase in the antioxidative peptides in the partially fermented whey fortified *lassi* during storage. Garay et al. (2021) reported that whey protein fortified drink based on goat whey has high antioxidant capacity because of the presence of all essential amino acids and branched chain amino acids in high amount. Sadighbathi et al. (2023) reported high levels of antioxidant activity in postbiotic-enriched cheese whey and skim milk supplemented yoghurt showed during 21 days of storage at 4°C which is in close agreement with the present findings. Maleki et al. (2015) also reported that DPPH radical scavenging activities for fermented hazelnut milk increased from 50.47±1.81 to 81.65±2.28%. However no previous work has been found on the antioxidant activity of fermented or non-fermented whey fortified *lassi* samples.

Antioxidant activity of whey

Table 1: Physico-chemical quality of whey fortified *lassi*

Parameters	T1	T2	T3
pH	4.29±0.04^{c}	4.41±0.01^{b}	4.56±0.03^{a}
Titratable acidity (% LA)	0.59±0.01^{a}	0.50±0.02^{b}	0.51±0.02^{b}
Fat (%)	1.85±0.03^{a}	1.78±0.01^{a}	1.49±0.10^{b}
Protein(%)	2.25±0.01^{a}	2.13±0.01^{b}	1.78±0.02^{c}
Sucrose (%)	11.28±0.07^{b}	11.27±0.03^{b}	11.91±0.03^{a}
Total solids (%)	20.50±0.02^{a}	19.42±0.07^{b}	19.03±0.20^{c}
Ash (%)	0.35±0.01^{b}	0.35±0.02^{b}	0.45±0.01^{a}
Tyrosine Value (mg tyrosine/100ml)	4.12±0.03^{a}	3.08±0.02^{b}	2.85±0.02^{c}

Values are mean of three replicates along with standard error. a, b, c different superscripts within a row denotes significant differences ($p<0.05$) among samples.

Table 2: Changes in antioxidant activity of whey fortified *lassi* and control *lassi* by ABTS method during storage at 7±2°C

Days	(µM) TEAC by ABTS		
	T1	T2	T3
0	76.77±0.168^{cA}	72.26±0.058^{dB}	70.17±0.066^{dC}
3	78.76±0.138^{bA}	73.25±0.169^{cB}	70.54±0.058^{cC}
5	79.76±0.115^{bA}	74.35±0.130^{bB}	71.01±0.026^{bC}
7	82.44±1.218^{aA}	74.74±0.081^{aB}	71.16±0.026^{aC}

Values are mean of three replicates along with standard error. A, B, C different superscript within a row denotes significant ($p<0.05$) difference among sample types; a, b, c different superscripts within a column denotes significant ($p< 0.05$) differences among day

Table 3. Changes in antioxidant activity of whey fortified *lassi* and control *lassi* by DPPH method during storage at 7±2°C

Day	% DPPH inhibition		
	T1	T2	T3
0	16.03±0.097^{dA}	13.36±0.073^{dB}	11.16±0.100^{cC}
3	16.73±0.046^{cA}	13.75±0.038^{cB}	11.43±0.055^{bC}
5	16.97±0.034^{bA}	14.07±0.032^{bB}	11.73±0.038^{aC}
7	17.34±0.064^{aA}	14.57±0.108^{aB}	11.87±0.017^{aC}

Values are mean of three replicates along with standard error. A, B, C different superscript within a row denotes significant ($p<0.05$) difference among sample types; a, b, c different superscripts within a column denotes significant ($p< 0.05$) differences among day.

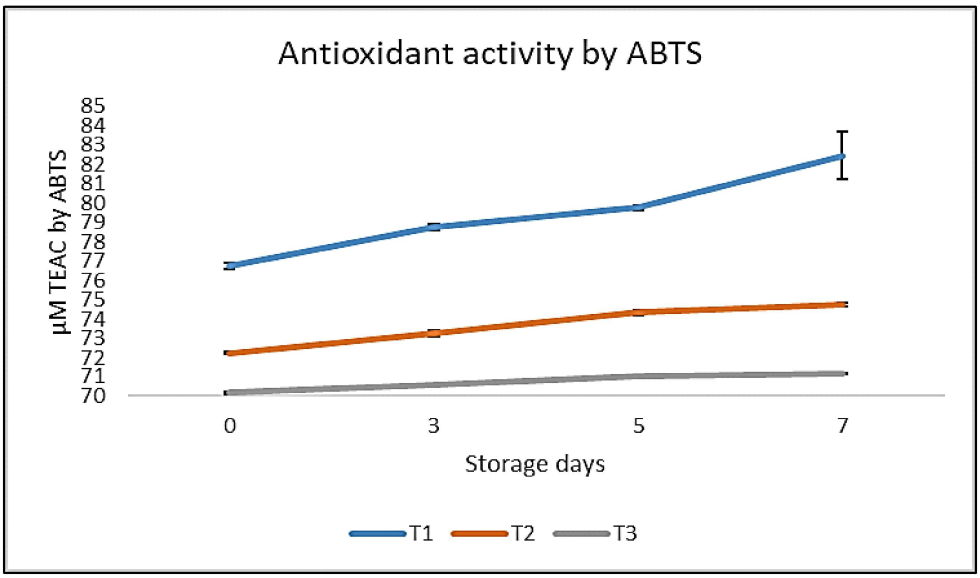


Fig. 2 Antioxidant activity of *lassi* by ABTS. Values are the mean of the three replicates (n=3) with standard error.

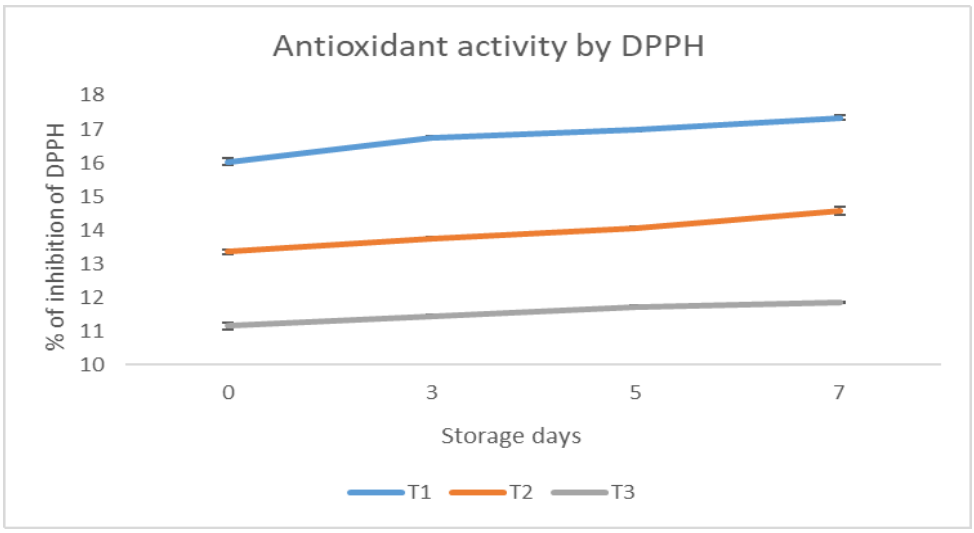


Fig. 3 Antioxidant activity of *Lassi* by DPPH. Values are the mean of the three replicates (n=3) with standard error

Table 4: Antioxidant activity of whey

Type	ABTS	DPPH
Non-fermented whey	47.16±3.834[b]	11.26±0.531[b]
Fermented whey	64.26±2.887[a]	14.15±0.449[a]

Values are mean of three replicates along with standard error. a, b are different superscript within a row denotes significant ($p<0.05$) difference among sample types

Antioxidant activity of both non-fermented and fermented whey was determined by ABTS and DPPH method (Table 4), which depicted that partially fermented whey samples has higher antioxidant activity as compared to non-fermented whey. The higher antioxidant activities in fermented whey may be due to the result of fermentation of whey proteins and production of antioxidative peptides by *Streptococcus thermophilus*. The results found support from Dinkçi et al. (2023) which revealed that goat Whey Protein Concentrate based probiotic beverages especially indicated the highest antioxidant activity. Dalaka et al. (2023) reported that antioxidant activities of the sweet whey digestates were significantly higher than those of sweet whey which further strengthen the findings in the present study.

Conclusion

The present study demonstrated that the physico-chemical properties, sensory characteristics, and storage stability of

fermented paneer whey-fortified *lassi* vary significantly compared to non-fermented paneer whey-fortified and control *lassi*. Fortification of the *lassi* resulted in marked improvements in the antioxidant potential, with the fortified samples showing statistically significant differences compared to non-fortified and control counterparts. These differences can be attributed to the factors such as the source and composition of whey, incubation time, starter cultures, and storage conditions. Overall, the results suggest that *lassi* fortified with paneer whey not only retains its nutritional value but also exhibits enhanced antioxidant properties, indicating its potential as a functional food that may contribute positively to human health.

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