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Research Article

Effect of incorporation of lemongrass on phenolic content of selected dairy products

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Abstract

Phenolic compounds, known for their antioxidant properties, are rich in milk and milk products. But this can vary depending on various processing parameters and additives. This study focuses on the determination of total phenolic compounds of milk and various milk products like dahi, plain lassi, plain whey and lemongrass flavoured lassi and whey and aqueous lemon grass extract and to have comparative data. Total phenolic content was determined using gallic acid as a standard and using Folin's Ciocalteu method. Absorbance was taken using a UV- Vis Double-beam spectrophotometer at 765 nm. Standardized methods were used to prepare flavoured lassi and whey drink. Results showed that milk had higher phenolic content (482.51±0.7 µg GAE/mL) compared to the dahi (87.63±0.14 µg GAE/mL), plain lassi (64.66±0.36 µg GAE/mL) and plain whey (59.21±0.02 µg GAE/mL). On the other hand, incorporation of 3% (v/v) lemongrass extracts significantly increased the total phenolic content of flavoured lassi (178.32±0.14 µg GAE/mL) and flavoured whey (118.97±0.01 µg GAE/mL). The aqueous extract of lemongrass showed the highest quantity of phenolic content (750.72±0.01 µg GAE/mL) compared to all the products. The findings of the study highlight the impact of processing treatment and the addition of herbs on the amount of phenolic content in the final product.

Keywords: Lassi, Lemon grass, Milk, Total phenolic content, Whey

Highlights

- Total phenolic content was determined using gallic acid as standard and using Folin-Ciocalteu method.
- Significant difference was observed in phenolic content of milk and milk products.
- Addition of lemon grass extract significantly increases the phenolic content in lassi and whey.

INTRODUCTION

Phenolic compounds are natural dietary phytochemicals that are widely spread in the human diet (Tsen et al., 2014). Determining the total phenolic content (TPC) in milk, lassi, whey, and herbal samples is vital because phenolic compounds have antioxidant properties that can provide health benefits. These compounds help in neutralizing harmful free radicals in the body and can reduce the risk of chronic diseases. Additionally, analysing the phenolic content in these samples can provide valuable information about their nutritional value and potential health-promoting effects. Milk is a fluid with immunological, nutritional and anti-inflammatory effects. Milk contains considerable amounts of phenolic compounds including a variety of compounds such as phenol, cresol, thymol and carvacrol and also could increase the antioxidant activity of dairy products by the incorporation of phenolic constituents to these products (Vázquez et al., 2015; Ertan et al., 2017). The functional food has paved the demand for development of food products that not only provide basic nutrition, but also affirm better health and longevity. Dahi is a fermented product obtained after fermentation of milk by addition of starter culture to offer easily digestible nutrients with potential therapeutic effects (Pandhi & Poonia, 2021). Several studies have shown the fermented milk and milk products have some positive effect on health status. Lassi is one of the most popular fermented refreshing dairy beverages in India (Maji et al., 2020). Addition of various herbs and spices are in lassi like carrot, ginger, mango, rose, and lemongrass, makes lassi more nutritious. Whey is the byproduct of cheese and paneer production. Whey contains whey proteins, carbohydrates (mainly lactose), water soluble minerals, vitamins, less amount of lipids (fatty acids and phospholipids) and free amino acids (Önay-Uçar et al., 2014). According to Tlais et al. (2023), the whey production from cheese exceeds 190 million tons per year. Recently, interest has increased considerably in finding naturally occurring antioxidant rich foods or medicinal materials to replace

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or use of synthetic antioxidants, which are being restricted due to their carcinogenicity. Nowadays, herbs are found in 40% of prescription drugs (Zheng & Wang, 2001). Many spices and aromatic herbs are generally considered safe by consumers and used as valuable ingredients for use in food manufacturing and processing, especially in dairy, meat, and bakery products (Kozłowska et al., 2021). Therefore, in the present study, an attempt was made to prepare herbal lassi and herbal whey drink from milk using lemongrass extract and comparing the phenolic content of the lassi and whey drink as well as the raw ingredients like milk, curd and lemongrass extract.

MATERIALS AND METHODS

Collection of milk and herb: Fresh Cow milk was collected from the Mini dairy unit of the livestock department, and tender lemongrass leaves were collected from the organic farm of the Centurion University of Technology and Management, Paralakhemundi, Odisha.

Bacterial culture: Lactic acid culture was collected from the mini dairy unit of Centurion University of Technology and Management, Paralakhemundi, Odisha.

Artificial sweetener and natural sugar: The sugar-free Natura and natural sugar were collected from the Dairy Technology laboratory of Centurion University of Technology and Management, Odisha.

Chemicals: Gallic acid (Merck), Folin-Ciocalteu reagent (Pallav), Sodium Carbonate (SRL), Methanol (NICE), Citric Acid (SRL). All the chemicals used for the analysis purpose was analytical grade.

Methods of preparation

Preparation of artificial sweetener solution- Sugar-free Natura at 0.7% was prepared using distilled water.

Preparation of lemongrass extract- Lemongrass extract was prepared according to the method of Maji et al. (2025). Fresh tender lemongrass was cut into small pieces. After that, they were dipped in boiled water for 10 s followed by filtration using a strainer. It was then ground in a mixer grinder. The juice was filtered with the help of a muslin cloth by pressing it properly without the addition of excess water.

Preparation of Dahi- Dahi was prepared according to the method of Ashwani et al. (2003). Cow milk collected from mini dairy unit was heated to 90°C and cooled to 37°C. Inoculation was done using 1.5% bacterial culture followed by incubation at 37ºC for 6-7 h.

Preparation of lemongrass flavoured lassi- Lassi was prepared according to the methods standardized by Maji et al. (2025). For the preparation of lassi, the prepared dahi was mixed in a mixer grinder at low speed and added with 0.7% artificial sweetener. For the preparation of lemongrass-flavour lassi, 3% lemongrass extract was incorporated in plain lassi and blended using a mixer grinder. After that it was stored at refrigeration conditions (7±2°C).

Preparation of lemongrass flavoured whey- Lemongrass flavoured whey was prepared according to the standardized method of Gantayat et al. (2023). Cow milk was heated to 80ºC and cooled to 70ºC. Coagulation was done by using 1% citric acid, followed by the separation of whey using a muslin cloth. Plain whey drink was prepared by the addition of 10% sugar, and flavoured whey drink was prepared using 3% lemongrass extract in plain whey. After that, it was stored at refrigerated temperature (7±2ºC).

Analysis of phenolic content: The phenolic content of milk, curd, plain lassi, flavoured lassi, plain whey, flavoured whey and lemongrass extract was analysed using gallic acid as a standard. To prepare the gallic acid standard curve, the method of Maji et al. (2018) was followed.

Preparation of gallic acid standard curve: Different concentrations of gallic acid (250 µg/mL, 200 µg/mL, 150 µg/mL, 100 µg/mL, 50 µg/mL) were prepared in methanol: water (1:1 v/v). The prepared concentrations of gallic acid were then mixed with 5 mL of Folin-Ciocalteu reagent (10% v/v) and 4 mL of sodium carbonate solution (7% w/v) to make a volume of 10 mL. The solution was vortexed for 10 min and then centrifuged at 10,000 RPM for 5 min. After that, absorbance was measured by using a UV-Vis spectrophotometer at 760 nm against blank (Fig. 1).

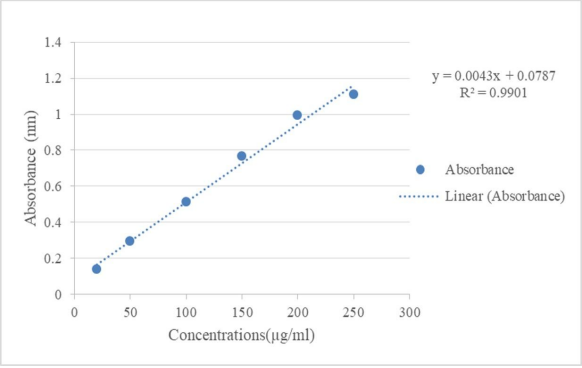


Fig. 1. Standard curve of Gallic Acid

Table 1. Different sample and ratio of dilution with solvent mixture

Sl. no	Name of Sample	Sample dilution ratio in solvent mixture (Methanol: Water)	Reference
1	Milk	1:5	Sreeramulu & Raghunath (2011) (with some modifications)
2	Dahi	3:25	Maji et al. (2018)
3	Plain lassi	3:25	Maji et al. (2018)
4	Lemongrass flavoured lassi	3:25	Maji et al. (2018)
5	Plain Whey	1:1	Thongzai et al. (2022) (with some modification)
6	Lemongrass flavoured whey	1:1	Thongzai et al. (2022) (with some modification)
7	Lemongrass extract	1:50	Sah et al. (2012) (with some modification)

Extraction of samples for determining total phenolic content: To get a clear extract from the sample, different proportions of sample and solvent mixture were tried. The different sample preparations of all the samples are described in Table 1. Samples in different proportions were dissolved in methanol: water (7:3), mixed using an incubator shaker for 2 h at room temperature. After that, the samples were kept for 12 h in refrigeration condition. The samples were then centrifuged at 10,000 RPM for 25 min, and the upper methanolic layer was used for the analysis of phenolic content. In case of milk sample, the Upper layer was again filtered using Whatman no. 1 filter paper and the clear filtrate obtained was used for further analysis using UV-Vis spectrophotometer at 765 nm against blank.

Statistical analysis: All measurements were performed in triplicate and reported as mean ± standard deviation. One-way analysis of variance (ANOVA) with Tukey's test was performed to see the significant difference between the methods during the analysis process. All statistical analysis was performed at 95% confidence interval with GraphPad Prism 5 software.

Table 2. Phenolic content of milk and milk products and Lemongrass extract

Sample Name	Total Phenolic Content (µg GAE/mL)
Milk	482.51±0.7[a]
Dahi	87.63±0.14[b]
Plain Lassi	64.66±0.36[c]
Lemongrass Flavoured Lassi	178.32±0.14[d]
Plain Whey	59.21±0.02[e]
Lemongrass Flavoured Whey	118.97±0.01[f]
Lemongrass Extract (aqueous)	750.72±0.01[g]

Values are represented as Mean±SE; n=3; [a-g] - each superscript column wise differ significantly ($p<0.05$).

RESULTS

The total phenolic content of milk, dahi, plain lassi, flavoured lassi, plain whey, flavoured whey and lemongrass was analyzed and expressed as µg GAE/mL. It was observed that the lemongrass extract has a very high quantity of phenolic content (750.77±0.00 µg GAE/mL), indicating as a rich source of phenolic compounds (Table 2). The TPC of milk and curd was 482.51±0.7 µg GAE/mL and 87.63±0.14 µg GAE/mL, respectively (Table 2).

From the results, it was observed that the average concentration of TPC of lemongrass lassi were found to be 178.32±0.14 µg GAE/mL whereas the plain lassi showed 64.66±0.36 µg GAE/mL. The findings clearly showed the positive correlation between the addition of lemongrass and TPC of the lassi. There was a significant increase in the TPC observed in the lemongrass flavoured lassi compared to plain lassi.

The TPC of lemongrass flavoured whey, along with plain whey was determined. The average concentration of TPC of lemongrass whey was found to be 118.74±0.01 µg GAE/mL, whereas the plain whey showed final concentrations of 59.21±0.02 µg GAE/mL (Table 2). From the result, it was revealed that 3% (v/v) lemongrass flavoured whey showed significantly higher TPC than plain whey drink. The difference in TPC among the whey samples was found to be statistically significant.

DISCUSSION

Comparison between the TPC in milk, milk products and lemongrass extract: The total phenolic content in milk was higher than other milk products like dahi, lassi and whey (Fig. 2). The lower TPC content may be due to processing techniques like fermentation and coagulation, which may reduce or

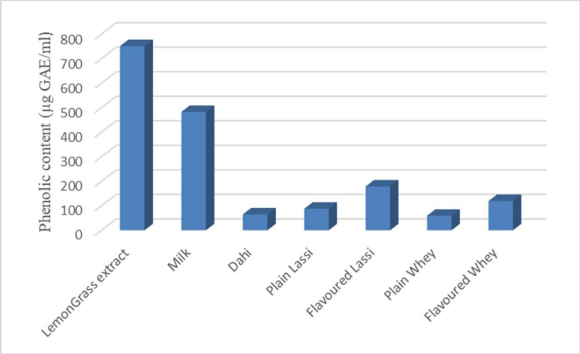


Fig. 2. Phenolic content of lemongrass extract, milk and milk products

redistribute the phenolic compounds. Loss of phenolic compounds during fermentation was studied by Othman et al. (2009). Additionally, exposure to light, heat, and oxygen during processing and storage can lead to the degradation of phenolic compounds. Sik et al. (2023), found a lower amount of polyphenol in the cheese and the whey prepared from the same milk. In the present study, dahi, lassi, and whey demonstrated comparatively lower phenolic contents, likely due to processing and dilution effects that may reduce phenolic compounds.

The TPC in plain lassi was found comparatively less than the lemongrass flavoured lassi. That is due to the addition of the lemongrass extract, which relatively increases the TPC in it as lemongrass has high total phenolic content (Table 2). Similar results were also observed by Maji et al. (2020), where the addition of different herbs increased the TPC in lassi. Then phenolic content in plain whey was found less because many phenolic compounds are associated with the casein and fat portion of the milk. On addition of lemongrass extract in plain whey to make it flavoured whey, its phenolic content increases relatively. Similar result was observed by Naik et al. (2023) and Zaman et al. (2023). The addition of carrot extract increases the phenolic content in whey.

In another research by Han et al. (2019), the interaction between the phenolic content with casein and whey protein was studied. They concluded that the addition of herbal extract increases the interaction between casein and polyphenol content. Whereas in case of whey negligible interaction was observed between them. The findings are completely aligned with the results obtained in this study.

The evaluation of phenolic content in milk and milk products is crucial for understanding their potential health benefits and nutritional value. Phenolic compounds, which are naturally present in milk, contribute to antioxidant, and antimicrobial properties, making them important for overall health. The variation in phenolic content among different milk products was observed, and that depends on various processing factors such as processing methods (fermentation, heating, and coagulation), milk source, and animal diet. Addition of herbal extract can increase the overall phenolic content in milk and milk products. In this study incorporation of lemongrass extract significantly increased the phenolic content of different milk products and the data obtained gave a clear descriptive idea about it. Regular assessment of phenolic content can provide valuable insights into the functional properties of dairy products, encouraging the development of functional foods that offer added health benefits. Addition of lemongrass extract into milk and milk products not only boosts the phenolic content in them significantly but also gives a great and appealing taste to it, which can further help to make the products more suitable for consumers of all age groups.

Conflict of interest: The authors declare that they have no conflict of interest.

Author's contribution: GG, GP, SS: Writing- original draft, methodology, investigation, analysis; SM: Writing – review & editing, supervision, validation, project administration, methodology, conceptualization; MKB:Writing- review editing, conceptualization.

Data availability statement: Data set generated during research will be available from the corresponding author upon reasonable request.

Use of artificial intelligence tools: During the preparation of this work, the authors used google scholar and Gemini to review literature. After using the service, the authors edited the content as needed and takes full responsibility for the content of the publication.

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