# ASSESSMENT OF ANTIOXIDANT PROPERTIES OF PROBIOTIC (*LACTOBACILLUS ACIDOPHILUS*) ENRICHED FUNCTIONAL KULFI USING BEETROOT (*BETA VULGARIS L.*) AND CARROT (*DAUCUS CAROTA L.*)

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**Abstract:**
The main objectives of this research are to develop probiotic-functional kulfi from beetroot (*Beta vulgaris L.*) and carrot (*Daucus carota L.*) pulp and to assess the kulfi's antioxidant properties, including DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and TPC (Total Phenolic Content). The control kulfi, or Treatment $T_0$ in the current trial, was devoid of probiotic culture as well as beetroot and carrot pulp. Inoculating probiotic culture and use of beetroot and carrot pulp in kulfi had a significant effect on DPPH (%) and TPC (%) content. The control kulfi contain low DPPH and TPC percent and at 1% inoculation levels of probiotic culture (*Lactobacillus acidophilus*), the DPPH and TPC content in kulfi of different treatments was high range. The newly prepared probiotic enriched kulfi were significantly different ($p<0.01$) from control in DPPH (%) content. And the newly prepared probiotic enriched kulfi were both significantly (a $v_s$ b, a $v_s$ c, a $v_s$ d, a $v_s$ e, b $v_s$ c) and insignificantly (b $v_s$ d, c $v_s$ d and c $v_s$ e) different ($p<0.01$) from control in TPC (%) content.

**Keywords:** Antioxidant properties, beetroot, carrot, Probiotics, *Lactobacillus acidophilus*, Indian dairy product, functional foods.

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## Introduction

All age groups like dairy desserts, which can be made with a variety of healthy ingredients. For instance, the popular Indian dairy delicacy Kulfi is made with the same components as ice cream and is typically served cold **(Sharma *et al.*, 2022; Kaur *et al.*, 2021).**

A frozen dessert that serves as an example of the delicate final course is kulfi. It is a product that is made by appropriately mixing and processing other milk products with sugar, flavour, and skim milk powder. Ice cream and other frozen treats like kulfi also attracted people of all ages. Some people do not like these kinds of frozen food, which may include a lot of sugar and fat, due to health issues **(Sobana *et al.*, 2021)**.

Recent innovation in the dairy industry has resulted in novel products with therapeutic and dietary advantages. With the intension for yielding the functional probiotics kulfi, beetroot and carrot was used as a functional food in the experimental kulfi. To increase functional properties of kulfi different percentages of beetroot and carrot pulp have been used in kulfi because recent investigations have produced strong proof that the betalains (betanin) and nitrates found in beetroot, in particular, have recently attracted more attention due to their potent biological action. By scavenging DPPH, avoiding DNA damage, and lowering LDL, betalains have been shown to remove oxidative and nitrative stress **(Chen *et al.*, 2021)**. **Nahla *et al*. (2018)** noted that one of the most widely cultivated vegetables in the world is the beetroot (*Beta vulgaris L.*), and recently, phenolic compound-containing plant extracts have been looked for as potential novel natural food additives. Because of their ability to quench singlet and triplet oxygen, bind free radicals, and breakdown peroxides, phenolic compounds' redox characteristics are primarily responsible for their antioxidant action. On the other hand, one of the more often used vegetables for human nourishment is the carrot (*Dascus carota L.*). It is a vitaminized food that is high in beta carotene, ascorbic acid, and tocopherol **(Susiloningsih *et al*., 2016)**. The human diet does not contain many calories from carrots. However, they are rich in a variety of phytochemicals, including carotenoid compounds (some of which have provitamin A properties), phenolic compounds, ascorbic acid, -tocopherol, vitamins D, K, B1, B6, and biotin, and polyacetylenes many of which have antioxidant and other health-promoting effects **(Livny *et al*., 2003)**.

As a probiotic microorganism, *Lactobacillus acidophilus* was inoculated in the experimental kulfi to enhance the therapeutic value. Live bacteria known as probiotics are beneficial to human health, particularly the digestive system **(Tewari, 2019)**. Based on known clinical trials, probiotic supplementation had relatively small impacts on depressive symptoms and had limited short-term effects on gut flora in patients with Major Depressive Disorder (MDD) **(Ng *et al*., 2023)**.

**Verma and Rout (2022)** emphasized that probiotics are safe to consume and promote the growth of good bacteria in our systems. They enhance vitamin synthesis and gut health generally. Utilizing probiotics, especially *Lactobacillus* and *Bifidobacteria*, is one of the most popular approaches to improve the balance of the gut microbiota. Probiotic bacteria also help to improve blood metabolic processes, create healthy microbial (bacterial) balances, and promote immunity **(Chatterjee *et al.*, 2022)**.

The main objective of this study is to manufacture probiotic-functional kulfi using beetroot (*Beta vulgaris*) and carrot (*Daucus carota*)" and to assess the antioxidant properties (DPPH percent and TPC percent) of the control and different newly prepared experimental probiotic functional kulfi.

## Materials and methods

The research study entitled "Assessment of antioxidant properties of probiotic (*Lactobacillus acidophilus*) enriched functional Kulfi using beetroot (*Beta vulgaris L.*) and carrot (*Daucus carota L.*)" was conducted in the lab of the Food Microbiology, Sambalpur University, Orrisa, India.

### Ingredient sourcing and collecting

Buffalo's milk, sugar, and beetroot and carrot pulp, Stabilizers and emulsifier were purchased from a local store in Sambalpur, Orrisa, India to make the probiotic kulfi. *Lactobacillus acidophilus* (14) is freeze-dried cultures that were purchased from The National Collection of Dairy Cultures, NDRI, Karnal (Haryana). It was kept alive and subcultured at the Food Microbiology lab at Sambalpur University, Orissa. Every week, simple skim milk was used to sustain the working cultures.

### Antioxidant analysis of newly prepared probiotic functional kulfi

#### *DPPH free radical-scavenging assay*

The approach was used to calculate the ability to scavenge DPPH free radicals with a few minor adjustments to the test sample concentration and DPPH concentration mixture. A test sample volume of 1 ml was placed in a test tube, forcefully shaken, and incubated for 15 minutes in a dark environment before the absorbance at 517 nm was measured using 0.1 mM of DPPH produced in ethanol. The normal curve was linear between 0 and 30 M Trolox. M Trolox equivalents (TE) per gramme of wet basis are used to express the results **(Sahreen *et al.*, 2010)**.

***Determination of Total Phenolic Content (TPC)***

The conventional spectrophotometric Folin-Ciocalteu method was used to quantify the total polyphenol content (TPC) of the samples that were under analysis, with a few minor adjustments. In a nutshell, 2.5 mL of Folin-Ciocalteu reagent and 30 mL of distilled water were combined with 0.5 mL of defatted sample extract. After waiting for five minutes, 7.5 mL of 7.5% $Na_2CO_3$ was added, and then 50 mL of distilled water was added to the mixture. The absorbance was determined at 760 nm in comparison to the blank solution after an incubation period of one hour in the dark. Gallic acid equivalents per gramme of the sample (mg GAE/g) is the unit of measurement for the results **(Jaćimović *et al*., 2023)**.

**Statistical analysis:**

Graphpad Prism 9 software was used to analyse the data using Analysis of Variance (ANOVA) and Critical Difference (C.D) at 5%.

**Treatment combination of control and probiotic functional kulfi**

***Control or normal kulfi ($T_0$)***

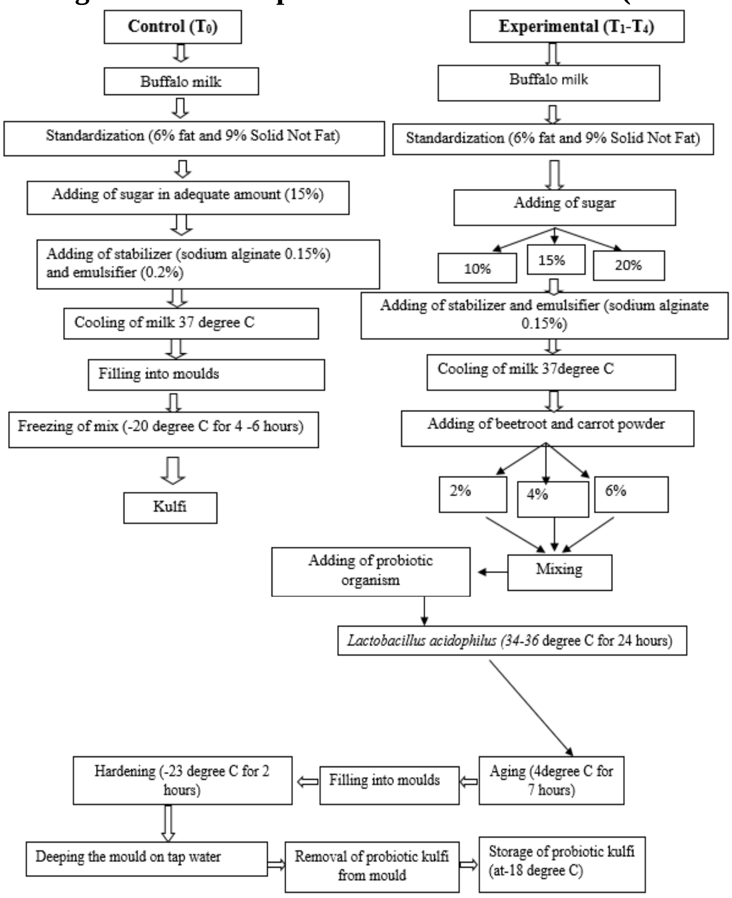
Control or normal kulfi ($T_0$) was prepared by using Buffalo milk with 10 % sugar and without probiotic culture, beetroot and carrot pulp.

***Experimental kulfi ($T_1$ to $T_4$)***

In experimental kulfi, $T_1$ treatment combination was prepared by using Buffalo milk with 10 % sugar, 2 % beetroot pulp and 1% *Lactobacillus acidophilus* as a probiotic culture. $T_2$ treatment combination was prepared by using Buffalo milk with 10 % sugar, 4 % beetroot pulp and 1% *Lactobacillus acidophilus* as a probiotic culture. $T_3$ treatment combination was prepared by using Buffalo milk with 10 % sugar, 2 % carrot pulp and 1% *Lactobacillus acidophilus* as a probiotic culture and $T_4$ treatment combination was prepared by using Buffalo milk with 10 % sugar, 4 % carrot pulp and 1% *Lactobacillus acidophilus* as a probiotic culture

No of treatment – 4+1
No of replications: 5
Total no of trials: 25

**Flow chart for manufacturing control and experimental functional kulfi (Tewari *et al*., 2021)**



**Results and discussion:**

In the current study, which was based on "Assessment of Antioxidant Properties of Probiotic (*Lactobacillus acidophilus*) Enriched Functional Kulfi Using Beetroot (*Beta vulgaris L.*) and Carrot (*Daucus carota L.*)", data were gathered on various aspects and tabulated before being statistically analyzed using Graphpad Prism 9 software. The statistical procedures of analysis of variance and critical difference were used to analyze the data. Critical analysis has been done on the major differences found both within and across treatment combinations.

**Table 2:** Table showing DPPH content (%) and TPC content (%) of newly prepared probiotic functional kulfi.

| Treatment combinations | DPPH (%) ± SD | TPC (%) ± SD |
|---|---|---|
| $T_0$ | 70.13 ± 0.0115[a] | 0.14 ± 0.0231[a] |
| $T_1$ | 75.38 ± 0.0173[b] | 0.84 ± 0.0115[b] |
| $T_2$ | 77.86 ± 0.0115[c] | 0.93 ± 0.0173[c] |
| $T_3$ | 76.46 ± 0.0058[d] | 0.86 ± 0.0115[d] |
| $T_4$ | 79.74 ± 0.0058[e] | 0.95 ± 0.0058[e] |

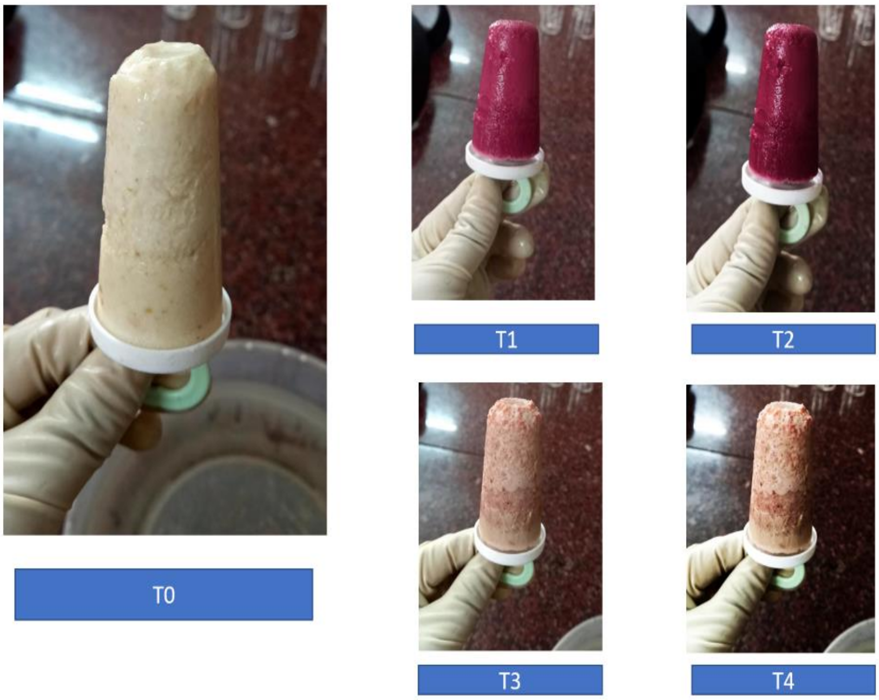



**Figure: 1** Image of control and experimental probiotic functional kulfi

Figure 2 showed the findings of the DPPH (%) analysis. In comparison to the control $T_0$ (70.13 ± 0.0115[a]), the newly prepared experimental probiotic kulfi $T_1$ (75.38 ± 0.0173[b]), $T_2$ (77.86 ± 0.0115[c]), $T_3$ (76.46 ± 0.0058[d]), $T_4$ (79.74 ± 0.0058[e]) contained high DPPH (%). The addition of beetroot (*Beta vulgaris L.*) and carrot (*Daucus carota L.*) pulp with probiotic bacteria (*Lactobacillus acidophilus*) increases the DPPH percentage in experimental kulfi. It was also showed that the mean value of $T_4$ treatment combination was very high compare to the other treatment combinations ($T_1$, $T_2$ and $T_3$).

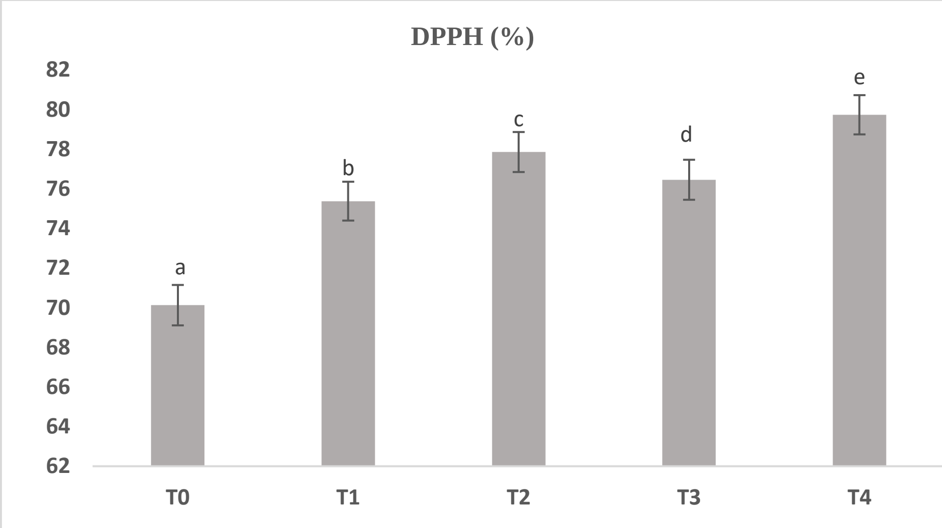



**Figure: 2** Graphical representation of DPPH percentage of different treatment combinations.

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed significant difference).

Figure 3 showed the findings of the TPC (%) analysis. In comparison to the control $T_0$ (0.14 ± 0.0231[a]), the newly prepared experimental probiotic kulfi $T_1$ (0.84 ± 0.0115[b]), $T_2$ (0.93 ± 0.0173[c]), $T_3$ (0.86 ± 0.0115[d]), $T_4$ (0.95 ± 0.0058[e]) contained high TPC (%). The addition of beetroot (*Beta vulgaris L.*) and carrot (*Daucus carota L.*) pulp with probiotic bacteria (*Lactobacillus acidophilus*) increases the TPC percentage in experimental kulfi. It was also showed that the mean value of $T_4$ treatment combination was very high compare to the other treatment combinations ($T_1$, $T_2$ and $T_3$).

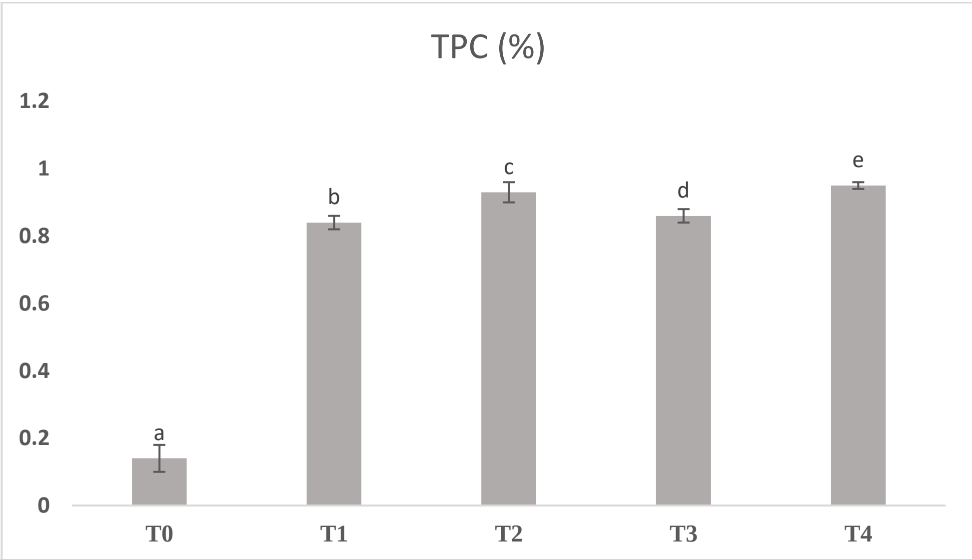



**Figure: 3** Graphical representation of TPC percentage of different treatment combinations.

(All the samples were evaluated in triplicate. In the similar column, different superscript alphabet showed significant difference).

**Conclusion:**

It was concluded that the inoculating probiotic culture and use of beetroot and carrot pulp in kulfi had a significant effect on DPPH (%) and TPC (%) content, as seen in Table 1. The control kulfi contain low DPPH and TPC percent and at 1% inoculation levels of probiotic culture (*Lactobacillus acidophilus*), the DPPH and TPC content in kulfi of different treatments was high range. The newly prepared probiotic enriched kulfi were significantly different ($p<0.01$) from control in DPPH (%) content. And the newly prepared probiotic enriched kulfi were both significantly (a $v_s$ b, a $v_s$ c, a $v_s$ d, a $v_s$ e, b $v_s$ c) and insignificantly (b $v_s$ d, c $v_s$ d and c $v_s$ e) different ($p<0.01$) from control in TPC (%) content.

**References:**


1. Chatterjee, G., Negi, S., Basu, S., Faintuch, J., O'Donovan, A., & Shukla, P. (2022). Microbiome systems biology advancements for natural well-being. Science of The Total Environment, 155915.
2. Chen, L., Zhu, Y., Hu, Z., Wu, S., & Jin, C. (2021). Beetroot as a functional food with huge health benefits: Antioxidant, antitumor, physical function, and chronic metabolomics properties. Food Science & Nutrition, 9(11), 6406-6420.
3. Jaćimović, S., Popović-Djordjević, J., Sarić, B., Krstić, A., Mickovski-Stefanović, V., & Pantelić, N. Đ. (2022). Antioxidant properties and multi-elemental analysis of dark chocolate. Foods, 11(10), 1445.
4. Kaur, N., Kaur, A., Sridhar, K., Sharma, M., Singh, T. P., & Kumar, S. (2021). Development and quality characteristics of functional Kulfi fortified with microencapsulated betalains. International Journal of Food Science & Technology, 56(10), 5362-5370.
5. Livny, O., Reifen, R., Levy, I., Madar, Z., Faulks, R., Southon, S., & Schwartz, B. (2003). β-carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. European journal of nutrition, 42, 338-345.
6. Nahla, T. K., Wisam, S. U., & Tariq, N. M. (2018). Antioxidant activities of beetroot (Beta vulgaris L.) extracts. Pakistan Journal of Nutrition, 17(10), 500-505.
7. Ng, Q. X., Lim, Y. L., Yaow, C. Y. L., Ng, W. K., Thumboo, J., & Liew, T. M. (2023). Effect of Probiotic Supplementation on Gut Microbiota in Patients with Major Depressive Disorders: A Systematic Review. Nutrients, 15(6), 1351.
8. Sahreen, S., Khan, M. R., & Khan, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food chemistry, 122(4), 1205-1211.
9. Sharma, M., Inbaraj, B. S., Dikkala, P. K., Sridhar, K., Mude, A. N., & Narsaiah, K. (2022). Preparation of Curcumin Hydrogel Beads for the Development of Functional Kulfi: A Tailoring Delivery System. Foods, 11(2), 182.
10. Susiloningsih, E. K. B., Sarofa, U., & Sholihah, F. I. (2016). Antioxidant properties and sensory properties carrot (Daucus carrota) soyghurt. In MATEC Web of Conferences (Vol. 58, p. 01002). EDP Sciences.
11. Tewari S, David J, Gautam A. (2019). A review on probiotic dairy products and digestive health. J Pharmacogn Phytochem 8:368–372.
12. Tewari, S., David, J., & Gautam, A. (2021). Physicochemical analysis of probiotic functional Kulfi by using Indian blackberry (Syzygium cumini L.). Journal of Pharmacognosy and Phytochemistry, 10(5), 236-246.
13. Verma, M., & Rout, P. K. (2022). Probiotics: Promising Opportunity for Future Functional Foods. In Recent Advances in Food Biotechnology (pp. 75-96). Springer, Singapore.