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Impact of climate and traditional practice on quality of homemade dahi

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Research Article

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Abstract

This research communication aimed at assessing the microbial quality and preparation practices of dahi/thayir, a popular traditional fermented milk product, in households from two distinct agroecological zones in Kerala, India, namely the high-range and central midland, We highlighted the significant variations and potential food safety concerns associated with regional differences in production methods. The local climate significantly influenced the incubation temperature of dahi/thayir, thereby influencing the types of microflora involved in the fermentation process. Data on preparation practices and sensory preferences were collected from producers during the sampling process, covering 200 households. There were significant variations in preparation practices between the two regions, particularly concerning the type of container used, inoculation rate and incubation period. Samples from the high-range region exhibited significantly higher acidity and coliform count as well as yeast and mold count than the central midland region. The household fermentation of milk is often uncontrolled, causing increased acidity levels. This uncontrolled fermentation favored the growth of contaminants such as coliforms and yeast, posing a potential threat to food safety. This study underscores the importance of understanding microbial quality variations and preparation practices in dahi/thayir production, emphasizing the need for proper fermentation techniques and hygienic practices to ensure the safety and quality of this traditional fermented milk product.

Dahi, also known as Thayir, is a traditional fermented milk product that holds immense cultural and culinary significance in households across Kerala, India. The preparation of dahi involves the fermentation of milk by beneficial bacteria, resulting in a tangy and creamy product with unique sensory attributes. Kerala is a region with rich cultural diversity and ethnic culinary practices. In Kerala milk is traditionally fermented into 'thayir' (dahi/dahi) by back-slopping of undefined starter culture. The fermented milk 'thayir' is used for the preparation of Kerala cuisines like 'kalan', 'pulissery', 'sambharam' and 'aviyal' based on acidity. However, the preparation practices for dahi can vary significantly from one household to another, leading to a wide variation in the final product's sensory characteristics.

The process of preparing dahi begins with the selection of raw milk, which is typically sourced from local dairy farms or obtained from household-owned cattle. The milk is heated to a certain temperature to eliminate any potential pathogens and to promote desirable fermentation conditions. Once the milk has cooled down, a starter culture, often a spoonful of previously prepared dahi or a commercial starter culture containing specific bacterial strains, is added. This inoculation introduces the beneficial bacteria responsible for the fermentation process.

The incubation period is a crucial step in dahi preparation, as it allows the bacteria to multiply and convert the lactose present in the milk into lactic acid. The incubation temperature plays a pivotal role in determining the type of microflora that will dominate the fermentation process. In households, the incubation of dahi usually takes place at ambient temperatures, which can vary depending on the climate of the region.

Kerala lies along the tropical region of the Indian peninsula with a total coastline of about 560 km and an altitude rising to about 2694 m above mean sea level. The average maximum daily temperature in Kerala is 37°C, while the minimum is 19.8°C (Dili *et al.*, 2010). There is region-wise variation in temperature according to the altitude and rainfall pattern. In hot and humid climates, such as those found in the coastal areas of Kerala, the ambient temperature tends to be higher, resulting in faster fermentation. Conversely, in cooler regions, such as the high ranges of Kerala, the ambient temperature is lower, leading to a slower fermentation process. Based on rainfall pattern and altitude, Kerala has two agroclimatic zones viz Altitude Type I and rainfall pattern (I&II) distributed in the central midland and Altitude Type II and rainfall pattern (I&II) along the high ranges of Kerala (Kerala Agricultural University, 2011).

The variation in incubation temperatures due to different climatic conditions can impact the microbial composition and overall hygiene indicators of dahi (Jaiswal *et al.*, 2021).

Understanding the influence of preparation practices and climatic conditions on the microbial quality of dahi is crucial for ensuring food safety. According to the Food Safety and Standards Authority of India (FSSAI) 2022 guidelines, the process hygiene criteria for fermented milk products include monitoring the coliform count, *Staphylococcus aureus* (coagulase positive) count, and yeast and mold count. By investigating these factors, this study aims to assess the impact of preparation methods and climate on hygiene indicators in dahi prepared in two agroecological zones of Kerala, India. The findings will provide valuable insights for promoting proper fermentation techniques and hygienic practices in dahi preparation, ultimately enhancing the safety and quality of this cherished traditional fermented milk product.

Material and methods

Sample and data collection

The dahi samples prepared in households using undefined starter cultures were collected from Central Midland (CM) and High Ranges (HR) of Kerala. Samples from these regions were randomly collected in sterile bottles and transported in icebox to laboratory for analysis. Information on the dahi preparation practices and sensory preferences for dahi were gathered from the producers through a questionnaire-based survey conducted during sample collection. The survey data regarding the type of milk and container used for dahi making, inoculum rate, incubation period, major defects and sensory preference were analyzed statistically using SPSS software package.

Determination of pH and titratable acidity

pH meter (Esico, Microprocessor based pH meter Model 1010) was standardized using standard buffer solutions of known pH (pH 4 and pH 7). The dahi samples were thoroughly mixed using a glass stirring rod and adjusted to a temperature of 27°C by keeping in a water bath. 25 ml of each sample was poured into a glass beaker. pH of the samples were measured by immersing the electrode of pH meter into the samples and kept undisturbed until a steady reading was obtained.

Dahi sample (10 g) was taken in a 100 ml conical flask. To this 10 ml of distilled water was added and mixed thoroughly. Two drops of phenolphthalein indicator was added to the sample and titrated against 0.1 N NaOH until the appearance of faint pink color. Titratable acidity as Lactic acid was calculated using the formula

$$\text{Titratable acidity as lactic acid} = 9(AN)/W$$

Where, A = Volume of standard NaOH required for titration; N = Normality of Standard NaOH solution; W = weight of the sample taken for test

Determination of hygiene indicators in dahi samples

Coliform count of dahi samples were carried out as per BIS (2012). The samples were serially diluted up to 10^{-3} dilution in sterile normal saline (0.85 percent NaCl). One millilitre of appropriate dilution was pour plated using violet red bile agar (Hi Media, Mumbai). Incubation of plates were done at 37°C for 24 h. After incubation characteristic colonies with purplish red color having a diameter of 0.5 mm or greater and sometimes with a reddish zone of precipitated bile were counted and expressed as colony forming units (CFU) per gram of product.

Count of *Staphylococcus aureus* (coagulase positive) in dahi samples were carried out as per BIS (2002). The samples were serially diluted to 10^{-2} dilution and 0.1 ml of this dilution was added to a pre prepared Baird Parker agar plate (Hi Media, Mumbai) and spread carefully using a spreader. The plates were incubated at 37°C for 30 h. After incubation characteristic shiny black colonies with or without narrow gray, white margin were counted and expressed as CFU per gram of product.

Yeast and mold count of dahi samples was carried out as per (BIS, 2018). The samples were serially diluted in normal saline and 10^{-12} dilution was pour plated using yeast extract dextrose chloramphenicol agar (Hi Media, Mumbai). The plates were incubated at 25°C for 5 d. Characteristic smooth, moist and elevated or surface colonies were count and expressed as CFU per gram of product.

Statistical analysis

Data are presented as means ± SEM and statistically evaluated using SPSS software. χ^2 test and t-test were used for comparison of parameters between regions. Natural logarithmic transformation was done before doing independent t-test for bacterial counts.

Results

A total of 100 dahi samples were collected from each of the two states belonging to the two agroecological zones covering the districts of Idukki, Wayanad and Palakkad (High Range: HR), Ernakulam, Thrissur, Palakkad (Central Midland: CM). The data on dahi making practices and consumer preferences were collected through questionnaire during sampling. Survey results indicated that as per the prevailing practices, only cow milk is used for dahi preparation. Irrespective of the agro-ecological zones, most respondents used steel containers (45 percent) followed by glass containers (31.5 percent) for dahi making. Plastic containers were used by 27 percent respondents in HR and 16 percent respondents in CM. Mud pots were reported to be used only in the CM (online Supplementary Fig. S1a). One per cent inoculum was the most commonly followed rate of inoculation in both the zones (online Supplementary Fig. S1b). Significant differences in incubation period were reported by the respondents from both the zones. The average incubation period for fermentation in HR was 12.73 ± 5.2 h and that of CM was 9.89 ± 2.6 h (online Supplementary Fig. S1c).

A significant difference in sensory preferences for dahi based on sourness was reported by producers in this study. Medium low acid dahi was more preferred in both zones (Fig. 1). Major defects reported in these zones were insufficient sourness followed by ropiness, gassiness and wheying off (Table 1). Ropiness was significantly more from HR than from CM. No significant difference between the zones was observed in the occurrence of other defects.

pH and titratable acidity

The average pH and titratable acidity of dahi samples collected from HR and CM are given in the online supplementary Table S1. A significant difference in pH was observed between zones with an average pH of 4.05 ± 0.04 in HR and 3.43 ± 0.02 in CM. A significant difference in acidity was also observed between zones with average titratable acidity values of 1.65 ± 0.06 and 1.49 ± 0.04% lactic acid in HR and CM, respectively.

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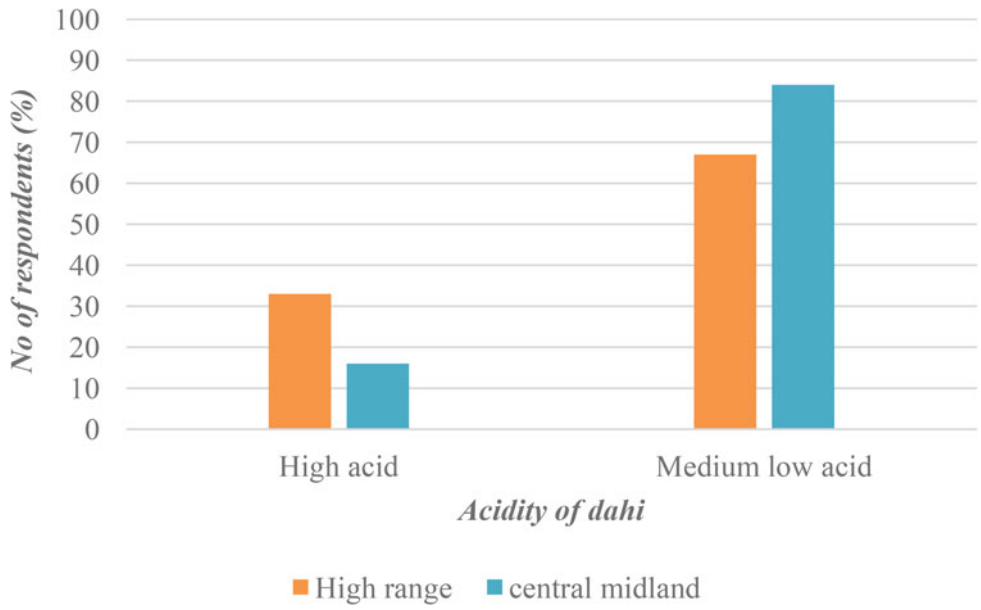

Fig. 1. Consumer preference based on dahi acidity. χ^2 value = 7.812**; *P*-value = 0.005** Significant at 0.01 level.

Hygiene indicators in dahi samples

Coliform count in dahi samples ranged from $<10^1$ cfu/g to 105×10^1 cfu/g in CM and $<10^1$ cfu/g to 80×10^2 cfu/g in HR. No statistical significance in average coliform count was observed (online Supplementary Table S2). The average *Staphylococcus aureus* count in dahi samples collected from CM ranged between $<10^1$ cfu/g and 42×10^1 cfu/g and those from high ranges between $<10^1$ cfu/g and 30×10^1 cfu/g. No significant difference in *S. aureus* count was observed between zones. High yeast and mold count were present in the dahi samples collected from the two agroecological zones of Kerala. In CM, count ranged from $<10^1$ cfu/g to 60×10^6 cfu/g and in HR from $<10^1$ cfu/g to 100×10^7 cfu/g. Once again, there was no significant difference between the regions.

Discussion

Data analysis on dahi making practices in the two agroecological zones of Kerala revealed that cow milk is used mainly for the preparation of dahi in households and small scale units. The reason for this is the higher population of cattle in the state compared to goats and buffaloes (Pulikkamath and Shafeek, 2024). Nayar *et al.* (2010) has also reported the preference of cow milk for direct consumption and preparation of dahi in Kerala. Low cost and better availability of cow milk when compared to milk from other species could be the reason for this (Kebede *et al.*, 2007) made an in-depth study on the influence of type of container on the microflora of spontaneously fermented milk products. They found that the material can have a significant influence on the nonstarter flora of fermented milks. In this study majority (45 percent) of respondents used steel containers. However the practice of using glass, plastic and mud pots also prevailed. Irrespective of the zones most of the respondents followed an inoculation rate of 1 percent. The incubation period, on the other hand, varied. It was significantly higher in HR when compared to CM. Lower ambient temperatures in HR would have necessitated longer incubation period for getting a good coagulum. The fact that 75 percent of the producers preferred low to medium acid dahi irrespective of zones (Fig. 1), highlights the importance of identifying and using medium acid producing starters for improving the marketability of dahi (Deshwal *et al.*, 2021).

Table 1. Defects reported during dahi making in two agroecological zones of Kerala

Defects	HR (*n* = 100)	CM (*n* = 100)	*Z*-value (*P*-value)
Ropiness	43	23	3.708** (0.002)
Insufficient sourness	45	36	1.302^{ns} (0.193)
Gassiness	17	20	0.547^{ns} (0.585)
Wheying off	6	9	0.807^{ns} (0.420)

n denotes number of dahi samples. Each defect was compared between regions by using *z*-test for independent proportions; ** Significant at 0.01 level; ^{ns} non-significant

Insufficient sourness was the most reported defect in fermented milk. Slow starters are majorly responsible for this defect. Type of starter culture, their optimum temperature, environmental conditions and practices followed during product preparation are critical factors that decide the sourness of final product. The ropiness defect was significantly greater in HR than in CM. Increase in viscosity is generally more appreciable at low temperatures, and can also arise from exopolysaccharide-producing starters. Gassiness arising from yeast and coliforms are well reported spoilage in fermented milks. Wheying off is usually associated with excessive acidity due to the reduction in water-holding capacity of proteins. Excessive acidity often arises due to lack of arrest in fermentation and due to excessive agitation after fermentation (Mistry, 2001)

The average pH of the dahi samples collected from the two agro ecological zones were found to be 4.05 ± 0.04 and 3.43 ± 0.02 in HR and CM, respectively. The average acidity of samples collected from both zones were comparatively higher than the reported acidity of 0.73 ± 0.01 percent lactic acid in dahi samples

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made with cow milk using defined dahi starter cultures (Krishna *et al*., 2021) Autochthonous lactose fermenters would have contributed to the higher acidity in the dahi samples as most of these samples were prepared by back slopping.

The maximum permissible limit as per Food safety and Standards Authority of India (FSSAI, 2022) is 100 cfu/g for coliforms coagulase positive *S. aureus*, and yeast and mold. In this work, average counts for all the three exceeded the permissible limits. Similarly, high counts have been reported in traditional dahi samples in Assam (Deb and Seth, 2014). High coliform counts are suggestive of poor hygienic practices. Even though milk used for dahi making is pasteurized or boiled before fermentation, post processing contaminants including coliforms can enter the product through water and utensils. The high titratable acidity of dahi samples can be related to the presence of lactose fermenters like coliforms in the sample.

The presence of *Staphylococcus aureus* (coagulase positive) was detected in samples, with an average count of 0.76 ± 1.87 ln cfu/g in HR and 1.29 ± 1.84 ln cfu/g in CM. Food handlers are the major contributors of *S. aureus* in manually processed foods. Bhattarai and Das (2016) reported the presence of toxin-producing strains of *S. aureus* in dahi samples. Acid tolerance of *S. aureus* was studied by Zhou and Fey (2020), who confirmed robust acid tolerance mechanisms in this bacterium. They also found that *S. aureus* is less sensitive to lactic acid compared to other organic acids like acetic acid and hydrochloric acid. Thus, staphylococci entering the product due to poor handling practices have the potential to withstand the acidic environment of dahi. Education and training for household producers and small-scale units on proper handling, pasteurization and sterilization of utensils can significantly reduce contamination risks from coliforms and *S. aureus*. Introducing defined starter cultures, specifically those producing desired acidity, could address issues of insufficient sourness and prevent defects like ropiness and excessive acidity.

During household fermentation of milk, the incidence of uncontrolled fermentation is higher, leading to increased acidity that favors the growth of yeast and molds. Gassiness defect is also associated with yeast (Bhattarai and Das, 2016). Akabanda *et al*. (2010) successfully isolated lactose-fermenting yeast *C kefyr* from fermented milk. The presence of yeast at higher levels in the microflora of fermented products can impact the overall quality of the product both positively and negatively. Hence, identifying yeast is essential to categorize it as a contaminant or a beneficial component. Chandran *et al*. (2022) reported the prevalence of lactose fermenting yeast in dahi, suggesting that not all yeast presence should be considered contamination. Further research and characterization are necessary to understand the role of these yeasts fully and determine whether they align with or extend the traditional definition of dahi.

To improve the quality of dahi, potential interventions and extension service like field level trainings, inputs focusing on enhancing hygienic practices, optimizing fermentation conditions and utilizing appropriate starter cultures are needed. Additionally, promoting the commercial production of dahi, in small scale and large scale, can standardize production practices, thereby improving safety and marketability.

In conclusion, the analysis of dahi-making practices in Kerala's agroecological zones revealed that cow milk is predominantly used for dahi preparation due to its availability and affordability, with steel containers being the most common, though glass, plastic, and mud pots are also utilized. The high range zone requires a longer incubation period due to lower temperatures. Producers prefer low to medium acid dahi, highlighting the need for medium acid-producing starters. Common defects include insufficient acidity and ropiness, while high microbial counts of coliforms, *S. aureus*, and yeast and mold indicate poor hygiene. Notably, the presence of yeast can affect product quality both positively and negatively, necessitating proper identification to determine its role. Overall, ensuring hygienic practices and proper starter culture selection, including the characterization of yeast, are essential for high-quality dahi production.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S002202992400061X.

References

Akabanda F, Owusu-Kwarteng J, Glover R and Tano-Debrah K (2010) Microbiological characteristics of Ghanaian traditional fermented milk product, Nunu. *Nature and Science* **8**, 178–187.

Bhattarai RR and Das SKL (2016) Evaluation of microbiological quality of indigenous Dahi from Eastern Nepal. *Sunsari Technical College Journal* **2**, 23–26.

BIS (2002) IS 5887-8-2. 2002 Methods for Detection of Bacteria Responsible for Food Poisoning, Part 8: Horizontal Method for Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and other species). Bureau of Indian Standards 3p.

BIS (2012) IS, 5401 Part-1 2012 Microbiology of Food and Animal Feeding Stuffs – horizontal Method for the Detection and Enumeration of Coliforms. Part-1: colony count technique (2nd Revision). . Bureau of Indian Standards 3p.

BIS (2018) IS 5403 1999 Method for Yeast and Mould Count of Foodstuffs. Bureau of Indian Standards 3p.

Chandran A, Linsha CK and Beena AK (2022) Prevalence of *Candida* species in 'thairu' a traditional fermented milk of Kerala, India. *European Journal of Nutrition & Food Safety* **14**(11), 15–19.

Deb M and Seth D (2014) Studies on the physico-chemical and microbilogical analysis of plain Dahi of Assam (India). *Journal of Bioresources and Engineering Technology* **1**, 27–32.

Deshwal GK, Tiwari S, Kumar A, Raman RK and Kadyan S (2021) Review on factors affecting and control of post-acidification in yoghurt and related products. *Trends in Food Science and Technology* **109**, 499–512.

Dili A, Naseer M and Varghese TZ (2010) Passive environment control system of Kerala vernacular residential architecture for a comfortable indoor environment: a qualitative and quantitative analyses. *Energy and Buildings* **42**, 917–927.

FSSAI (2022) Food Safety and Standards (Food products standards and food additives) Regulations, 2011. Ministry of Health and Family Welfare, India December, 20, 2022 38-40.

Jaiswal P, Minz PS, Vairat AD, Kumari K and Raju PN (2021) Quality Dahi preparation in automated controlled ambient conditions. *International Journal of Bio-resource and Stress Management* **12**, 82–88.

Kebede A, Viljoen B, Gadaga T, Narvhus J and Lourens-Hattingh A (2007) The effect of container type on the growth of yeast and lactic acid bacteria during production of Sethemi, South African spontaneously fermented milk. *Food Research International* **40**, 33–38.

Kerala Agricultural University (2011) *Package of Practices Recommendations Crops*, 12th Edn. Thrissur: Kerala Agricultural University.

Krishna AM, James L, Beena AK, Rajakumar SNR and Mercey KA (2021) Changes in microbiological parameters, pH and titratable acidity during the refrigerated and room temperature storage of dahi prepared from the milk of Vechur cows: an indigenous cattle breed of Kerala. *Journal of Dairy Research* **88**, 436–439.

Mistry VV (2001) Fermented milks and cream. Food Science and Technology-New York-Marcel Dekker 301–26.

Nayar R, Sathian CT, Mercey KA, Surendran D and Jenifer P (2010) Influence of religion on consumption pattern of livestock products in Kalpetta, Wayanad District, Kerala. *Journal of Dairying, Foods and Home Sciences* **29**, 60–63.

Pulikkamath A and Shafeek A (2024) A preliminary analysis and estimation of the status of feed and fodder in Kerala. *Heliyon* **10**(10), e31200.

Zhou C and Fey PD (2020) The acid response network of *Staphylococcus aureus*. *Current Opinions in Microbiology* **55**, 67–73.

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