Journal of Dairy Research

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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

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Research Article

Cite this article: 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. https://doi.org/10.1017/S0022029921000868

Received: 28 March 2021
Revised: 5 July 2021
Accepted: 22 September 2021
First published online: 23 December 2021

Keywords:
Dahi; indigenous cattle breed; lactococcal count; Vechur cow milk

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Abstract

Vechur cow is an indigenous cattle breed of Kerala listed as a critical breed by FAO. This research communication is related to the hypothesis that the changes occurring in microbiological quality parameters of Vechur cow milk dahi (VCMD) during storage will be superior to other milk and reflective of the traditional concepts of therapeutic properties attributed to Vechur milk. Microbiological quality of the VCMD stored at room (30 ± 1°C) and refrigerated (4 ± 1°C) temperatures in terms of total viable, coliform, yeast and mold and lactococcal counts is reported in this study, together with titratable acidity and pH. Results are compared with cross-bred cow milk dahi (CCMD) as control. On refrigerated storage, despite the comparable initial microbiological quality, VCMD exhibited significantly lower total viable, lactic acid bacteria, yeast and mold counts than CCMD, from the fifth day onwards for the first two parameters and the tenth day onwards for the last parameter. VCMD exhibited significantly higher pH values than CCMD from the fifth day onwards whereas the titratable acidity was significantly lower from the tenth day onwards. Though this study does not delineate the factors contributing towards the lower microbial population observed in VCMD, it provides an impetus to further researches for scientifically validating its traditionally-reported medicinal properties.

Dahi, a fermented milk product of Indian origin has a very close resemblance to yogurt and is well recognized for its beneficial attributes. The traditional scientific system of Indian medicine Ayurveda, in its well-recognized treatise, Charaka Samhita and Sushruta Samhita, discusses various properties of cow and buffalo milk dahi and emphasizes its therapeutic characteristics (Prajapati and Nair, 2008).This fermented milk product is utilized in a mixture of forms in several Indian cuisines and is consumed either in the main course of meal as a refreshing beverage or as a dessert. In general, it is considered as a healthy refreshing product. Addition of functional ingredients, use of novel starters, utilization of milk from different species or breeds are some of the innovations attempted to harness the high value addition potential of this traditional fermented milk product. Among these options, the use of milk from indigenous cattle breeds presents an attractive option as it potentially exploits the many benefits traditionally attributed to both the milk of indigenous cattle breeds and the fermented milk products. In such an attempt, milk of Vechur cow, an indigenous cattle breed of Kerala was used for the preparation of dahi (Ammiti *et al.*, 2019a) to explore the value addition potential of fermentation technology to address the economic issues associated with the low productivity of these cattle. Vechur cattle are listed under category of Critical Breeds in 'The World Watch List on Domestic Animal Diversity' given by FAO (DAD-IS, 2012). It is well acknowledged that this *Bos indicus* breed needs greater attention for its survival since the breed is facing extinction (Chinnamma *et al.*, 2015). As per the 'Estimated livestock population breed-wise based on breed survey-2013' published by Department of Animal Husbandry, Dairying and Fisheries in 2016 the total number of Vechur cattle is only 2479. Reduction in their population to this very low number warrants special attention and adoption of new measures for their conservation (Sharma and Niranjan, 2016). Value addition of the product of indigenous cattle which ensures financially viable livestock enterprising to farmers is identified as a new possibility for improving the conservation efforts (Srivastava *et al.*, 2019). Taking into consideration that breed specific differences are reported in the processing qualities of milk (Petrera *et al.*, 2016; Yoo *et al.*, 2019) and that the raw milk attributes have a high bearing upon the physicochemical and

microbiological quality of the final product, it was hypothesized that Vechur cow milk product may be behaving differently in terms of microbial quality during storage. In this research communication microbial quality, pH and titratable acidity of Vechur cow milk dahi at room and refrigerated storage are reported and compared with dahi produced from cross-bred cattle.

Materials and methods

Preparation of dahi

Fresh, pooled milk samples of Vechur and cross-bred cows were obtained from the University Cattle Farm, Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India. Vechur cow milk dahi (VCMD) was prepared from two stage homogenized (2500 and 500 psi) standardized (3% fat and 8.5% SNF) Vechur milk in accordance with production parameters (rate of inoculation, incubation temperature and incubation period) optimized through response surface methodology (RSM) keeping cross-bred cow milk as the control (Ammiti *et al.*, 2019b) The heat treated (90°C/5 min) milk was cooled to the incubation temperature, inoculated with *Lactococcus lactis* subsp. *lactis* NCDC 91 (National Collection of Dairy Cultures, Karnal) at the rate of 3% of milk and the incubation was done at 37°C/5 h 30 min. The control dahi was prepared in the same way except that the milk used was cross-bred cow milk, rate of inoculation was 2% and the incubation period was 8 h (Nahar *et al.*, 2007).

Assessment of microbial quality during room and refrigerated storage

Dahi samples stored at room (30 ± 1°C) and refrigerated (4 ± 1°C) temperatures were assessed for four different microbial parameters; total viable, coliform, yeast and mold counts, three hygiene indicators and the lactococci count. The samples kept at room temperature were assessed daily till it was graded undesirable by the sensory panel whereas the refrigerated samples were analyzed at five day intervals for a period of 15 d. For the enumeration of the microbial population, appropriate dilutions of the samples were pour plated using their respective growth medium and incubated at the particular temperature time combinations. The total viable bacteria of the dahi samples were enumerated by pour plating appropriate dilutions on nutrient agar (HiMedia, Mumbai) and incubation at 37 ± 0.5°C for 48 h (APHA, 1978). Violet red bile agar (VRBA, HiMedia, Mumbai) was used for the enumeration of coliform bacteria, and the plates were incubated at 37 ± 0.5°C for 24 h (IS:5401, 1969). Yeast and mold counts were determined using potato dextrose agar (PDA, HiMedia, Mumbai) incubation at 25 ± 1°C for 5 d and lactococci counts using M17agar (HiMedia, Mumbai) and subsequent incubation at 37 ± 0.5°C for 48 h (Downes and Ito, 2001). All the results were expressed as $\log_{10}$ cfu/g. Considering the well established interrelationship of titratable acidity and pH with microbial population they were also determined as per the standard methods IS: 1166 (1986) and IS: SP 18 (Part XI) (1981) respectively.

Statistical analysis

Repeated measures ANOVA was used for comparing the changes in the parameters between periods within each sample. For comparing changes between the samples in each period paired t-test was used. Data analyses were carried out using the Statistical Package for Social Sciences (SPSS, Version 24) and the results are presented as mean with standard error of six independent batch replications.

Results and discussion

Changes in microbial population, pH and titratable acidity of dahi samples stored at 30 ± 1°C and 4 ± 1°C with progression of storage period are shown in online Supplementary Table S1 and Table 1 respectively. Coliforms were not detected in any of the samples throughout the storage period. As both the cross-bred cow milk dahi (CCMD) and Vechur cow milk dahi (VCMD) samples stored at 30 ± 1°C were graded undesirable by a sensory panel of six trained judges after one day, shelf-life studies of the room temperature stored samples were not carried out beyond day one. The titratable acidity, total viable, yeast and mold and lactococcal counts of both the CCMD and VCMD samples stored at 30 ± 1°C showed significant ($P < 0.01$) increase in one day itself with a concomitant significant ($P < 0.01$) reduction in pH values. No significant ($P > 0.05$) differences were observed between the tested parameters of both the samples under this storage condition except in the case of titratable acidity. Titratable acidity of CCMD was significantly ($P < 0.01$) higher than that of VCMD in 24 h of storage at 30 ± 1°C.

At 4 ± 1°C storage the total viable counts (TVC) of Vechur cow milk and cross-bred cow milk dahi samples significantly increased ($P < 0.01$) from 7.64 to 8.06 $\log_{10}$CFU/g and from 7.65 to 8.12 $\log_{10}$CFU/g respectively over a period of 15 d. Though there were no significant differences between the total viable count of fresh CCMD and VCMD stored at 4 ± 1°C, significant differences ($P < 0.05$) were observed between them on storage with the CCMD exhibiting higher values than VCMD from the fifth day onwards. A similar trend of significantly ($P < 0.01$) higher population in CCMD than VCMD from the fifth day onwards was observed for lactococcal count also. The lactococcal counts obtained for the samples were almost of the same magnitude as that of their total viable counts indicating that the starter culture itself is the major contributor to the TVC. This observation along with the absence of coliforms is indicative of the appropriateness of procedures followed during the product preparation and storage. In the case of yeast and mold counts too, CCMD exhibited higher values than VCMD. However, in this case the difference became significant ($P < 0.01$) only from the 10th day of storage. Though on day 'one' there were no significant differences between the tested microbial populations of both types of dahi, with extending storage period the counts were found to be significantly higher in cross-bred milk dahi. Significant ($P < 0.01$) increase in titratable acidity and decrease in pH values of both the VCMD and CCMD were observed after 5 d of storage at 4 ± 1°C. Statistically relevant ($P < 0.01$) differences in between the titratable acidities of CCMD and VCMD became evident from 10th day with the CCMD showing higher values than the other. Significant ($P < 0.01$) differences in pH were manifested from the fifth day onwards. Overall, it can be summarized that during storage at 4 ± 1°C, VCMD was significantly different from CCMD in that they had significantly lower total viable, lactococcal and yeast and mold counts, titratable acidity and higher pH.

Microbial quality of dahi prepared from different types and sources of milk have been reported in many studies. However,

https://doi.org/10.1017/S0022029921000868 Published online by Cambridge University Press

Table 1. Microbial quality of dahi prepared from milk of Vechur and cross-bred cattle and stored at 4 ± 1°C

Parameters	Days	Vechur cow milk dahi	Cross-bred cow milk	Significance
Total viable count (log_{10}cfu/g)	Day 0	7.64 ± 0.048^{ax}	7.65 ± 0.056^{ax}	ns
	Day 5	7.86 ± 0.050^{bx}	7.90 ± 0.072^{by}	$P < 0.05$
	Day 10	7.96 ± 0.051^{cx}	8.03 ± 0.089^{cy}	$P < 0.05$
	Day 15	8.06 ± 0.017^{dx}	8.12 ± 0.078^{dy}	$P < 0.01$
Coliform count (log_{10}cfu/g)	Day 0	Nil	Nil	–
	Day 5	Nil	Nil	–
	Day 10	Nil	Nil	–
	Day 15	Nil	Nil	–
Yeast and mold (log_{10}cfu/g)	Day 0	0.547 ± 0.021^{ax}	0.59 ± 0.062^{ax}	ns
	Day 5	1.00 ± 0.016^{bx}	1.12 ± 0.019^{bx}	ns
	Day 10	1.17 ± 0.078^{bcx}	1.35 ± 0.029^{cy}	$P < 0.01$
	Day 15	1.37 ± 0.098^{cx}	1.50 ± 0.027^{dy}	$P < 0.01$
Lactococcal count (log_{10}cfu/g)	Day 0	7.63 ± 0.031^{ax}	7.62 ± 0.010^{ax}	ns
	Day 5	7.81 ± 0.014^{bx}	7.85 ± 0.024^{by}	$P < 0.01$
	Day 10	7.92 ± 0.033^{cx}	7.98 ± 0.070^{cy}	$P < 0.01$
	Day 15	8.04 ± 0.058^{dx}	8.10 ± 0.078^{dy}	$P < 0.01$
Titratable acidity (% lactic acid)	Day 0	0.72 ± 0.033^{ax}	0.72 ± 0.033^{ax}	ns
	Day 5	0.79 ± 0.083^{bx}	0.82 ± 0.017^{bx}	ns
	Day 10	0.84 ± 0.083^{cx}	0.89 ± 0.033^{cy}	$P < 0.01$
	Day 15	0.92 ± 0.050^{dx}	0.98 ± 0.017^{dy}	$P < 0.01$
PH	Day 0	$4.02 \pm .027^{ax}$	3.87 ± 0.004^{ax}	ns
	Day 5	3.90 ± 0.010^{bx}	3.66 ± 0.083^{by}	$P < 0.01$
	Day 10	3.81 ± 0.067^{cx}	3.51 ± 0.083^{cy}	$P < 0.01$
	Day 15	3.69 ± 0.067dx	3.43 ± 0.083dy	$P < 0.01$

ns, not significant.
Figures are the mean ± standard error of six replications, a-d: means with different superscript vary significantly ($P < 0.01$) within a column, x-y: means with different superscripts vary significantly within a row as shown.

to our knowledge this is the first study reporting the microbial quality of Vechur cow milk dahi during storage. As expected, room temperature storage favored microbial growth making the product unacceptable within one day of storage. The observation of lower microbial counts, titratable acidity and higher pH in VCMD than CCMD needs to be further investigated in the light of its significantly higher content of lactose, a readily fermentable sugar (Ammiti *et al.*, 2019*a*) and the higher antimicrobial properties reported for Vechur cow milk lactoferrin (Anisha *et al.*, 2012). The breed-wise differences observed in microbiological attributes is in agreement with the earlier reports of substantial differences among cattle breeds for product quality (Malacarne *et al.*, 2006; Yoo *et al.*, 2019).

In conclusion, and as hypothesized, Vechur cow milk dahi behaved differently in terms of microbial quality with its count being significantly lower than the cross-bred cow milk dahi for all the tested parameters except coliforms, which were absent in all the samples throughout the storage. This observation is of relevance as it can provide a great impetus to the ongoing efforts to market quality products from milk of indigenous cattle breeds in an attempt to assure premium price to the product. It also provides scientific substantiation of traditional concepts of therapeutic properties of Vechur cow milk.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029921000868.

Acknowledgement. The authors wish to acknowledge the financial support by the Kerala Veterinary and Animal Sciences University to carry out this post graduate research work.

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https://doi.org/10.1017/S0022029921000868 Published online by Cambridge University Press