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Vechur cow milk yoghurt: response surface methodology-based process optimization and storage studies

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Research Article

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Abstract

In this research article, response surface methodology (RSM) based optimization of three production parameters namely temperature, time and amount of starter culture of Vechur cow milk yoghurt (VCMY) on the basis of sensory evaluation responses comparing cross-bred cow milk yoghurt (CCMY) as the control is reported. The optimized values of production parameters were 2.15 per cent rate of inoculation, 42°C incubation temperature and 4 h incubation period. The optimized product exhibited significantly lower syneresis, a^*, b^* values and higher L^* values than CCMY. Physico-chemical, microbiological, textural and sensory properties of both VCMY and CCMY during room temperature and refrigerated storage were assessed daily until the onset of spoilage (room temperature) or at five day intervals over a period of 15 d (refrigerated). Both room temperature stored products were graded undesirable by the sensory panel upon one day of storage. Significant reduction was observed in the fat, SNF, total solids, protein and pH content and all the tested colour parameters of the optimized product during refrigerated storage. Total viable counts as well as yeast and mould counts and lactic acid bacteria counts of both VCMY and CCMY progressively increased over the 15 d of storage. Significant reductions were observed in the flavour ($P < 0.01$), body and texture, colour and appearance and overall acceptability ($P < 0.05$) scores of both the samples over a period of 15 d. During storage, hardness and adhesiveness values showed an increasing trend whereas the cohesiveness showed a decreasing trend. Storage studies revealed significant differences in the acidity, pH, syneresis, tyrosine value, colour parameters and sensorial attributes of both the yoghurt samples. During the 15 d refrigerated storage period, the VCMY exhibited superior technological attributes to CCMY in terms of lower syneresis %, acidity, microbial population, firmer and less cohesive texture, better flavour, colour and appearance scores. Being the first comprehensive study on the utilization of Vechur cow milk for the preparation of yoghurt, the data generated in the current study would provide a solid base for the exploration of fermentation as a means of value addition of milk of this very rare indigenous cattle breed.

The genetic makeup of the lactating animal has a high bearing upon the milk composition and hence marked breed specific differences are observed in milk composition. Cow milk composition differs among countries due to the use of different breeds, feeding practices and breeding policies (Sharma *et al*., 2018). These compositional differences are directly reflected to the milk's processing qualities as well as the physicochemical and microbiological quality of the products prepared from it. Previous studies have often endorsed superiority in composition and properties of milk of native/indigenous cattle breeds in comparison to mainstream and/or cross-bred cows (Sharma *et al*., 2018; Ammiti *et al*., 2019; Weerasingha *et al*., 2022). Several benefits have been attributed to Indian cow milk in the Indian system of medicine, Ayurveda (Burjor, 2007). However, due to their low productivity and economical limitations, the rearing of indigenous cows is generally not being preferred by farmers despite their low feed requirement, good adaptation potential and high disease resistance. To overcome this low production constraint, cross breeding programmes with high milk producing breeds were adopted by many countries, but these programmes have resulted in the extinction of many indigenous cattle breeds and depletion of good native germplasm with its qualities of disease resistance and heat tolerance (Srivastava *et al*., 2019; Weerasingha *et al*., 2022). Low yield and decreasing utility have led to the decline of most of the indigenous cattle (Niranjan, 2016). It is reported that approximately two breeds of poultry and livestock are lost each week (FAO, 2007). Loss of a defined breed can also be considered as a loss of cultural identity and the heritage of the community to which it belongs (Belew *et al*., 2016), as well as forfeiting of a global insurance policy against future threats to food security (Shah *et al*., 2016).

So, it is essential that new ways are initiated to conserve and popularize these indigenous cattle breeds to prevent the loss of these valuable genetic resources. Development of value-added products from the milk of indigenous cattle is identified as a major possibility to improve the conservation efforts (Srivastava *et al.*, 2019). So, it was hypothesized that adoption of fermentation as a value addition process for Vechur cow milk could result in the development of fermented milk products with superior/different technological attributes. With this background a study was conducted for response surface methodology (RSM) based optimization of production parameters for the preparation of fermented milk products from the milk of Vechur cows, which were very popular in Kerala till 1960s, but became rare when the cross breeding policy was adopted in the state (Iype, 2013). This rare breed of *Bos indicus* cattle named after the village Vechur in Kottayam District, in the state of Kerala of India is placed in the breed map of cattle published by the National Bureau of Animal Genetic resources, ICAR, India (NBAGR, 2001). Food and Agriculture Organisation of the United Nations (FAO) has listed this breed under the category of Critical Breeds in 'The World Watch List of Domestic Animal Diversity' published by FAO (DAD-IS, 2012). We have previously reported the RSM based process optimization of Vechur cow milk *dahi* (Krishna *et al.*, 2019), properties of the optimized product (Ammiti *et al.*, 2019) and microbiological changes occurring during its refrigerated storage (Krishna *et al.*, 2021). In the current study RSM based process optimization and shelf-life studies of Vechur cow milk yoghurt (VCMY) are reported.

Materials and methods

Milk samples and yoghurt preparation

Fresh, pooled milk samples of Vechur and cross-bred cows were obtained from University livestock farm, Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India.

To prepare experimental Vechur cow milk yoghurt (VCMY) and control yoghurt (CCMY), standardized (3% fat and 8.5% SNF) and two stage homogenized (2500 psi and 500 psi) milk samples were heat treated at (90°C/5 min), cooled to the incubation temperature, inoculated with the culture NCDC-260 (a mixed culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*, procured from National Collection of Dairy Cultures (NCDC, Karnal)) and incubated at the specified temperatures for specified periods. Full details are in the online Supplementary File.

Optimization of production parameters of yoghurt using response surface methodology

Preliminary trials were conducted to finalize the range (minimum and maximum values) of three production parameters; incubation temperature (IT), rate of inoculation (RI) and incubation period (IP) for deriving the central composite rotatable design (CCRD) for response surface methodology (RSM). The strategy of varying one factor while keeping the other two factors constant (one-factor-at-a-time-method) was followed for this. The minimum and maximum values were determined based on comparison of sensory scores of products prepared as per the details given in Online Supplementary Table S1 according to the procedure given in Supplementary file and flow chart (Online Supplementary Fig. S1).

Experimental design

A five level of three factor CCRD derived by feeding the minimum and maximum values for each production parameter in the RSM software (Design-Expert® software version 8.0.1.0) was employed for optimization of yoghurt formulation using three independent processing variables, Temperature (A: °C), rate of inoculation (B: %) and incubation period (C: h).The sensory attributes viz. flavour (Y_1), body and texture (Y_2), colour and appearance (Y_3) and overall acceptability (Y_4) were recorded as the responses. Each run consisted of 100 ml of standardized, homogenized and pasteurized milk, which was inoculated with respective levels of inoculum and incubated at predetermined temperature for predetermined time given in the CCRD. All the products were subjected to sensory analysis using a nine point hedonic scale (Larmond, 1977) by a panel of six trained judges. The optimized solution was generated as per the details given in the online Supplementary file.

Preparation and analyses of the optimized product

Yoghurt prepared from homogenized standardized Vechur milk as per the optimized combinations derived by RSM were subjected to physico-chemical (fat, SNF, total solids, lactose, protein, titratable acidity, syneresis per cent and colour characteristics) and microbiological (total viable, coliform, yeast and mould, lactic acid bacteria counts) analyses, details of which are provided in the Online Supplementary File.

Analysis of the optimized product during room and refrigerated storage

Yoghurt samples stored at room (30 ± 1°C) and refrigerated (4 ± 1°C) temperatures were assessed for physico-chemical, microbiological, rheological and sensorial attributes as detailed in the online Supplementary File. The samples kept at ambient temperature were assessed daily till the onset of spoilage. In the case of refrigerated samples, they were analysed at five day intervals for a period of 15 d.

Results and discussion

Optimization of production parameters of yoghurt using RSM

Keeping the production parameters of the treatment sample having the highest similarity to the control sample as the optimum value, minimum and maximum values for each production parameters were determined and are given in Online Supplementary Table S2. The coded and actual levels of the three factors (design factors) are also given in the same supplementary table. Design matrices representing different combinations of the three factors suggested by RSM are represented in Table 1. The design consisted of 20 runs (eight factorial points, six axial points in addition to six replicates of central point).

The average values of sensory responses viz. flavour, body and texture, colour and appearance and overall acceptability of the products prepared as per the experimental design are presented in Table 1. For better visualization, the effect of production parameters on the sensorial quality of the VCMY is represented as response surface plots (Online Supplementary Fig. S2). The sensory attributes of yoghurt were subjected to regression analysis in order to assess the effect of levels of rate of inoculation, incubation temperature and incubation period on them and a

Table 1. Experimental design runs for VCMY preparation with corresponding response values

Standard order	Temperature °C	Inoculation rate (%)	Incubation period (h. min)	Response 1	Response 2	Response 3	Response 4
				Flavour (Y_1)	Body and texture (Y_2)	Colour and appearance (Y_3)	Overall Acceptability (Y_4)
1	37.00	1.00	5.30	7.5	7	7	7
2	47.00	1.00	2.30	6	5.5	6	5
3	47.00	1.00	5.30	7	7.5	7	6.75
4	47.00	3.00	2.30	7.5	7	7	6.5
5	42.00	2.00	4.00	8.6	8.5	8.5	8.6
6	37.00	1.00	2.30	6	5	6	6
7	42.00	2.00	4.00	8.6	8.5	8.5	8.6
8	37.00	3.00	5.30	7	7	7	7.25
9	47.00	3.00	5.30	7	7	7	7.75
10	42.00	2.00	4.00	8.2	8.4	8.3	8.3
11	42.00	2.00	4.00	8.2	8.3	8.3	8.3
12	37.00	3.00	2.30	5.5	6 +	6.5	6
13	33.59	2.00	4.00	6	5	6.5	6
14	42.00	2.00	4.00	8.2	8.5	8.3	8.3
15	42.00	3.68	4.00	7	7	6.5	7
16	42.00	2.00	4.00	8.3	8.2	8.5	8.3
17	42.00	2.00	1.29	5.25	5	5	5
18	42.00	0.32	4.00	6.5	6	6	6.25
19	42.00	2.00	6.31	7.25	6.75	6.25	6.25
20	50.41	2.00	4.00	6.5	6	7	6.75

quadratic regression model for the dependent variables was established to fit the experimental data for each response. The regression analysis obtained after ANOVA resulted in second order equations and are given in the Online Supplementary Table S3.

In the suggested quadratic model, the F-values for all the attributes were more than table F-value ($P < 0.01$) indicating that the developed model was significant (Table 2). The coefficient of determination (R^2) for flavour, body and texture, colour and appearance and overall acceptability were found to be 0.96, 0.97, 0.97 and 0.96 respectively, showing that the fitted quadratic model accounted for over 96 per cent of the difference in experimental data. The closer the R^2 value is to 1.00, the stronger the model is and the better it predicts the response. An R^2 value of >0.75 indicates good fitness of the model (Mandenius and Brundin, 2008). So, the obtained R^2 values of more than 0.75 and very close to 1.00 indicate that the obtained models are strong. A non-significant lack of fit test showed that the model is sufficiently precise for predicting organoleptic characteristics of yoghurt made with any combinations of the variables within the range examined. The adequate precision which measures signal to noise ratio was higher than four for all responses which is highly desirable and hence supporting the suitability of model to navigate the design.

Numerical optimization of the production parameters for preparation of yoghurt provided only one optimized solution (Table 3). The optimized solution exhibited desirability of 0.96 and was selected for the preparation of yoghurt from Vechur cow milk. The predictability of the model for all the responses was determined by comparing the observed values and values predicted by the model (Table 3). As the difference between the predicted and actual values were statistically non-significant ($P > 0.05$), the solution containing 2.15 per cent rate of inoculation, 42°C incubation temperature and 4 h incubation period was adopted as the optimized values of production parameters for the preparation of VCMY. The reproducibility of optimized production parameters of yoghurt was confirmed by the triangle test. The results (Table 3) statistically confirmed the reproducibility of the production procedure ($P < 0.05$).

Analysis of the optimized product

As a direct reflection of the significantly higher protein and lactose contents of the Vechur cow milk than the cross-bred cow milk (data not shown), the protein and lactose contents of VCMY (4.35 and 3.61%, respectively) were found to be significantly ($P < 0.01$) higher than that of CCMY (3.91 and 3.20%, respectively) (Table 4). No significant differences were ascertained between VCMY and CCMYs in the case of fat, SNF, total solids and acidity percentages. FSSAI regulations (2011), stipulate that yoghurt shall have not less than 3.0, 8.5 and 2.9% m/m milk fat, milk solids not fat and milk protein respectively. It is also stipulated that the titratable acidity as lactic acid shall not be less than 0.6 per cent. The optimized products were meeting prescribed standards of fat, solid- not-fat, protein and acidity with VCMY showing values 3.13, 8.54, 4.35 and 0.86 percentages respectively. Syneresis in VCMY (15.93%) was significantly ($P < 0.01$) lower

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Table 2. Analysis of variance (ANOVA) of fitted quadratic model for sensory parameters and responses of VCMY

	Partial coefficients of sensory parameters			
Partial coefficient terms	Flavour	Body and texture	Colour and appearance	Overall acceptability
Intercept	8.30	8.31	8.32	8.34
A-Temperature A-Temperature	0.17*	0.27**	0.09	0.074
B-Inoculation	0.09	0.27**	0.17*	0.29**
C-Incubation	0.50**	0.49**	0.34**	0.54**
A^2	−0.66**	−0.87**	−0.45**	−0.61**
B^2	−0.48**	−0.52**	−0.62**	−0.53**
C^2	−0.66**	−0.74**	−0.85**	−0.88**
AB	0.31**	0.00	0.063	0.28*
AC	−0.31**	−0.12	−0.062	0.094
BC	−0.19	−0.37**	0.19*	0.031
Lack of fit	0.21 ns	0.059 ns	0.06 ns	0.052 ns
Model *F* value	2.14*	5.61*	5.41*	6.05*
R^2	0.96	0.97	0.97	0.96
Press	4.87	3.48	2.27	6.85
Adequate precision	15.69	19.89	20.05	17.27

*- Significant at five per cent level ($P < 0.05$), **- Significant at one per cent level ($P < 0.01$), ns- non significant

than CCMY (19.01%). This could be attributed to the significantly higher protein content of VCMY than CCMY as an increase in protein content is found to decrease syneresis (Rani *et al.*, 2012). Composition of the milk used for yoghurt preparation is considered to be one of the major factors affecting whey separation of the yoghurt gels (Magdaleno, 2016). No significant differences were noted between microbial counts of control and treatment yoghurt samples. Coliforms were absent in both the samples. The logarithmic value of lactic acid bacteria count (which was calculated by summing up the individual values of lactobacilli and lactococcal

Table 3. Results of RSM-based optimization: A. Optimized solution obtained, B. Comparison of predicted, observed values and C. Sensory scores for triangle test

A. Optimized solution obtained after response surface analysis			
Inoculation rate (%)	Incubation temperature °C	Incubation period (h)	Desirability
2.15	42.5	4	0.96
B. $^{\$}$Comparison of Predicted and Observed values			
Attributes	Predicted value	Observed value	*t*-value
Flavour	8.38	8.34 ± 0.02	0.74 ns
Body and texture	8.45	8.35 ± 0.31	0.79 ns
Colour and appearance	8.35	8.32 ± 0.88	0.92 ns
Overall acceptability	8.46	8.31 ± 0.91	0.81 ns
C. $^{\#}$Sensory scores for triangle test			
Sensory attributes	Control Yogurt	A	B
Flavour	8.41 ± 0.01^{a}	8.22 ± 0.05^{b}	8.25 ± 0.08^{b}
Body and texture	8.33 ± 0.01^{a}	8.23 ± 0.98^{a}	8.33 ± 0.06^{a}
Colour and appearance	8.22 ± 0.06^{b}	8.32 ± 0.54^{a}	8.32 ± 0.02a
Overall Acceptability	8.4 ± 0.08^{a}	8.38 ± 0.67^{a}	8.35 ± 0.09a

$^{\$}$Values are the mean ± standard error of six replications.

*-Significant at five per cent level ($P < 0.05$) **-Significant at one per cent level ($P < 0.01$), ^{ns}- non-significant,a–bMeans with different superscripts within the row are significantly different ($P <$ 0.05).

$^{\#}$Values are the mean ± standard error of three replications, A and B are coded samples of yogurt prepared with Vechur cow milk.

Table 4. Characteristics of optimized product

Parameter	VCMY	CCMY	*t*-value
Physico-chemical attributes			
Fat (%)	3.13 ± .021	3.15 ± .022	0.542 ns
SNF (%)	8.54 ± .004	8.54 ± .004	0.000 ns
Total solids (%)	11.67 ± .023	11. 69 ± .021	0.523 ns
Protein (%)	4.35 ± .096	3.91 ± .0307	5.754**
Lactose (%)	3.61 ± .024	3.20 ± .025	8.364**
Acidity (% lactic acid)	0.86 ± .004	0.86 ± .005	0.255 ns
Syneresis (%)	15.93 ± .162	19.01 ± 0.16	15.406**
Microbiological parameters			
Total viable count (log$_{10}$CFU/g)	7.65 ± 0.022^a	7.65 ± 0.016^a	0.165 ns
Yeast and mould count (log$_{10}$CFU/g)	0.426 ± 0.140^a	0.596 ± 0.085^a	1.032 ns
Lactic acid bacteria (log$_{10}$CFU/g) count	7.91 ± 0.009^a	7.91 ± 0.004^a	0.043 ns
Colour characteristics			
L^*	88.83 ± 0.008^a	88.52 ± 0.014^b	31.687**
a^*	−3.88 ± 0.047^a	−3.61 ± 0.003^b	46.667**
b^*	11.48 ± 0 0.003^a	12.41 ± 0.003^b	212.449**

Values are the mean ± standard error of six replications
*-Significant at five per cent level ($P < 0.05$) **-Significant at one per cent level ($P < 0.01$), ^{ns}- non-significant,a–bMeans with different superscripts within the row are significantly different ($P < 0.05$)

counts) was 7.91 CFU/ml for both the control and treatment samples indicating no significant difference between them. Yeast and mould counts of VCMY and CCMY were 0.43 ± 0.14 and 0.60 ± 0.09 log$_{10}$CFU/ml. Microbiological parameters of the yoghurt samples were within the limits specified by FSSAI indicating their conformity to existing legal standards.

The physical structure of milk which can modify the L^* value is affected by milk composition parameters including fat, protein, Ca, P and processing conditions (Dufossé and Galaup, 2021). While the L^* value of VCMY (88.83) was significantly ($P < 0.01$) higher than that of CCMY (88.52) it's a^* and b^* values (−3.88, 11.48) were significantly lower ($P < 0.01$) than the other (−3.61, 12.41). This is in agreement with the breed wise colour variation of fermented milk products reported by Yoo *et al.* (2019) and Weerasingha *et al.* (2022). The negative a^* values indicate the dominance of green-tone over the red in both the yoghurt samples. As all the b^* values recorded in the present study were above zero it can be inferred that yellowness is dominating over the blue in all samples. β-Carotene pigment in the milk fat component is responsible for the yellowness of milk. Based on the exhibited colour parameters the prepared yoghurt samples could be regarded as relatively dark greenish yellow in colour. Similar to the current study Vechur cow milk dahi also exhibited significantly lower a^* and b^* values than cross-bred cow milk yoghurt (Ammiti *et al.*, 2019).

Storage studies of the optimized product

The yoghurt samples were assessed for the changes in their physico-chemical, microbiological, sensorial and rheological attributes during their storage at 30 ± 1°C (Online Supplementary Table S4) and 4 ± 1°C (Table 5 and Online Supplementary Table S5). Due to the high acidic flavour and bitterness, the yoghurt samples stored at 30 ± 1°C were graded undesirable by the sensory panel upon one day of storage. Hence the storage studies of the ambient temperature stored samples were not carried out beyond day one.

No significant differences were observed between the fat, SNF and total solids contents of VCMY and CCMY on day zero or after one day of storage at 30 ± 1°C. Statistically significant ($P < 0.01$) reduction was observed in the protein content of both the samples after one day of storage at 30 ± 1°C. This was very much in agreement with the significant ($P < 0.01$) increase observed in the tyrosine value of both the yoghurt samples.

During storage at 4 ± 1°C, no significant differences were observed between the fat, SNF, total solids and protein contents of VCMY and CCMY (Online Supplementary Table S4) while significant differences were observed in the case of acidity, pH, syneresis, tyrosine value and the colour parameters (L^*, a^* and b^*: Table 5). Acidity of VCMY was found to be significantly lower than that of CCMY from the 5th day of storage onwards. Agreeing with this, pH values of VCMY were higher than that of CCMY throughout the storage period. The higher acid-buffering capacity observed for Vechur cow milk than cross-bred cow milk (Krishna *et al.*, 2018) could be one of the reasons for the lower acidity exhibited by VCMY. Occurrence of syneresis during storage is attributed to aggregation of protein particles and their deposition under gravity (Kesenkas *et al.*, 2011). VCMY exhibited significantly ($P < 0.05$) lower syneresis percentage than CCMY throughout the storage period. This observation is very much in agreement with the significantly lower syneresis reported for yoghurt prepared from indigenous cattle types (Thamankaduwa white and Lankan cattle compared with Jersey and Friesian; Weerasingha *et al.*, 2021). As excessive acidification below pH 4 may promote whey separation as well as gel defects in the finished product (Jaros and Rohm, 2003), the increasing trend of syneresis observed in the current study could also be attributed to the fact that all the

Table 5. Properties which showed significant difference in between Vechur and cross-bred cow milk yoghurt on storage at 4 ± 1°C

A. Physicochemical				
Parameters	Days	VCMY		
Parameters	Days	Milk	CCMY	*Z*-value
Acidity (% lactic acid)	Day 0	0.86 ± 0.003^{ax}	0.85 ± 0.004^{ax}	1.265^{ns}
	Day 5	0.98 ± 0.003^{bx}	1.04 ± 0.004^{by}	12.45**
	Day 10	1.13 ± 0.006^{cx}	1.23 ± 0.007^{cy}	10.17**
	Day 15	1.37 ± 0.018^{dx}	1.44 ± 0.015^{dy}	2.719^{*}
pH	Day 0	3.82 ± 0.004^{ax}	3.67 ± 0.004^{ay}	22.98**
	Day 5	3.66 ± 0.007^{bx}	3.53 ± 0.006^{by}	12.90**
	Day 10	3.52 ± 0.010^{cx}	3.40 ± 0.010^{cy}	7.85**
	Day 15	3.42 ± 0.031^{dx}	3.32 ± 0.031^{dy}	7.36**
Syneresis per cent	Day 0	15.93 ± 0.162^{ax}	19.01 ± 0.116^{ay}	15.406**
	Day 5	19.65 ± 0.152^{bx}	21.68 ± 0.079^{by}	11.850**
	Day 10	25.75 ± 0.152^{cx}	26.50 ± 0.063^{cy}	4.550**
	Day 15	30.55 ± 0.226^{dx}	32.48 ± 0.116^{dy}	7.596^{**}
Tyrosine (mg.5m^{L-1})	Day 0	0.240 ± 0.00^{ax}	0.242 ± 0.00^{ax}	2.125^{ns}
	Day 5	0.248 ± 0.00^{bx}	0.245 ± 0.00^{by}	7.862*
	Day 10	0.269 ± 0.00^{cx}	0.251 ± 0.00^{cy}	38.334**
	Day 15	0.290 ± 0.00^{dx}	0.258 ± 0.00^{dy}	94.077**
L*- Lightness axis	Day 0	88.83 ± 0.008^{ax}	88.52 ± 0.005^{ay}	31.687**
	Day 5	88.64 ± 0.007^{bx}	88.21 ± 0.004^{aby}	47.352**
	Day 10	88.49 ± 0.003^{cx}	87.98 ± 0.005^{by}	80.638**
	Day 15	88.34 ± 0.040^{dx}	88.13 ± 0.225^{by}	25.77 **
a*- Red-green axis	Day 0	-3.88 ± 0.004^{ax}	-3.61 ± 0.003^{ay}	46.667**
	Day 5	-3.92 ± 0.002^{bx}	-3.65 ± 0.002^{by}	89.443**
	Day 10	-3.96 ± 0.002^{cx}	-3.69 ± 0.003^{cy}	69.303**
	Day 15	-4.07 ± 0.003^{dx}	-3.72 ± 0.002^{dy}	78.262**
b*- Blue-yellow axis	Day 0	11.48 ± 0.003^{ax}	12.41 ± 0.003^{ay}	212.449**
	Day 5	11.41 ± 0.003^{ax}	12.24 ± 0.003^{by}	183.436**
	Day 10	11.35 ± 0.002^{ax}	12.13 ± 0.002^{cy}	253.266**
	Day 15	11.13 ± 0.003^{bx}	11.08 ± 0.043^{dx}	1.148^{ns}
B. Microbiological				
Parameters	Days	VCMY	CCMY	*t*-value
Total viable count (log10cfu/g)	Day 0	7.64 ± 0.022^{ax}	7.68 ± 0.016^{ax}	0.165^{ns}
	Day 5	7.76 ± 0.008^{bx}	7.90 ± 0.011^{by}	8.969**
	Day 10	7.95 ± 0.011^{cx}	8.02 ± 0.027^{cy}	2.365*
	Day 15	8.05 ± 0.014^{dx}	8.09 ± 0.021^{dx}	1.602^{ns}
Yeast and mould count (log10cfu/g)	Day 0	0.426 ± 0.14^{ax}	0.59 ± 0.085^{ax}	1.032^{ns}
	Day 5	1.01 ± 0.043^{bx}	1.12 ± 0.037^{bx}	1.828^{ns}
	Day 10	1.22 ± 0.032^{bcx}	1.37 ± 0.024^{cy}	3.695^{**}
	Day 15	1.43 ± 0.031^{cx}	1.54 ± 0.017^{dy}	3.154**
Lactic acid bacteria (log10cfu/g)	Day 0	7.91 ± 0.010^{ax}	7.91 ± 0.004^{ax}	0.043^{ns}

(*Continued*)

Table 5. (*Continued.*)

B. Microbiological				
Parameters	Days	VCMY	CCMY	*t*-value
	Day 5	8.07 ± 0.008^{bx}	8.11 ± 0.014^{by}	4.141**
	Day 10	8.25 ± 0.010^{cx}	8.27 ± 0.007^{cx}	1.376 ^{ns}
	Day 15	8.34 ± 0.007^{dx}	8.33 ± 0.015^{dx}	0.697 ^{ns}
C. Rheological				
Parameters	Days	VCMY	CCMY	*t*-value
Hardness(g)	Day 0	203.9 ± 0.88^{ax}	180.8 ± 0.48^{ay}	7.11**
	Day 5	213.7 ± 0.71^{bx}	192.6 ± 0.56^{by}	9.06**
	Day 10	222.1 ± 0.40^{cx}	212.6 ± 0.31^{cy}	10.63**
	Day 15	246.1 ± 0.93^{dx}	225.4 ± 0.14^{dy}	6.54**
Cohesiveness	Day 0	0.40 ± 0.006^{ax}	0.41 ± 0.002^{ay}	7.82**
	Day 5	0.39 ± 0.002^{bx}	0.41 ± 0.001^{ay}	9.11**
	Day 10	0.38 ± 0.026^{cx}	0.39 ± 0.001^{by}	9.86**
	Day 15	0.37 ± 0.001^{bx}	0.40 ± 0.003^{cy}	10.694**
Adhesiveness	Day 0	119.9 ± 0.91^{ax}	108.5 ± 0.59^{ay}	14.93**
	Day 5	129.5 ± 0.61^{bx}	115.1 ± 0.82^{by}	8.87**
	Day 10	136.3 ± 0.75^{ax}	126.2 ± 0.83^{cy}	16.27**
	Day 15	150.7 ± 0.26^{dx}	141.1 ± 0.61^{ay}	10.40**
D. Sensory				
Parameters	Days	VCMY	CCMY	*Z*-value
Flavour	Day 0	8.3 ± 0.21^{ax}	8.0 ± 0.0^{ay}	31.0**
	Day 5	7.4 ± 0.20^{abx}	7.0 ± 0.0^{aby}	40.0**
	Day 10	6.7 ± 0.16^{abx}	6.9 ± 0.08^{aby}	4.375*
	Day 15	6.1 ± 0.15^{bx}	6.4 ± 0.8^{bx}	1.712 ^{ns}
	χ^2 value	17.746**	17.727*	
Body and texture	Day 0	8.0 ± 0.0^{ax}	7.8 ± 0.16^{ay}	62.5**
	Day 5	7.8 ± 0.16^{ax}	7.4 ± 0.20^{aby}	40.0**
	Day 10	7.2 ± 0.10^{abx}	7.2 ± 0.10^{aby}	4.375*
	Day 15	6.8 ± 0.66^{bx}	6.6 ± 0.20^{bx}	1.078 ^{ns}
	χ^2 value	16.412*	15.420*	
Colour and appearance	Day 0	8.5 ± 0.22^{ax}	8.0 ± 0.05^{ay}	21.5**
	Day 5	8.0 ± 0.00^{abx}	7.9 ± 0.08^{ax}	2.25 ^{ns}
	Day 10	7.7 ± 0.21^{abx}	7.0 ± 0.00^{aby}	40.0**
	Day 15	6.1 ± .023^{bx}	6.5 ± 0.12^{bx}	1.875 ^{ns}
	χ^2 value	17.586*	17.089*	

Values are the mean ± standard error of six replications.
*-Significant at five per cent level ($P < 0.05$) **-Significant at one per cent level ($P < 0.01$),^{ns}- non-significant,a–bMeans with different superscripts within the row are significantly different ($P < 0.05$).

stored samples had pH below this value. Though there was no significant difference in tyrosine value of fresh samples, after 5 d of storage at 4 ± 1°C VCMY exhibited significantly higher ($P < 0.01$) tyrosine values than CCMY. The direct relationship observed between the increase in tyrosine values and reduction in protein contents of both the yoghurt samples stored at 4 ± 1°C manifests that the products were undergoing marked proteolysis. A significant increase in the extent of proteolysis during

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refrigerated storage of yogurt was also reported by Ramachandran and Shah (2010) and Amani *et al.* (2016). Increase in tyrosine value, a measure of degree of proteolysis with storage was reported by Gursel *et al.* (2016) while studying the quality attributes of goat milk yoghurt. He also observed that tyrosine level may be considered as an indicator of bitter taste development in yogurt. Throughout the storage period L^* value of VCMY was found to be significantly ($P < 0.05$) higher than that of CCMY while a^* and b^* values were found to be significantly ($P < 0.05$) lower. This is in agreement with the significant breed specific differences in the L^*, a^* and b^* values of set yoghurt during 21 d of refrigerated storage reported by Weerasingha *et al.* (2022).

Significant reductions were observed in the fat, SNF, total solids, protein, pH content and all the tested colour parameters of both the samples during the storage period. The tested parameters which showed significant increase over the storage period were acidity, tyrosine value and syneresis. This observation is in agreement with the previous reports of similar trends in pH and titratable acidity of yoghurt during storage (Sahan *et al.*, 2008; Weerasingha *et al.*, 2021). The reduction of lightness in dairy products could have resulted from non-enzymatic browning reactions including lipid peroxidation, degradation of ascorbic acid and Maillard reactions (Chudy *et al.*, 2020). As observed in the current study, previous studies (Cais-Sokolińska *et al.,* 2016; Weerasingha *et al.*, 2022) also reported decreases in the L^* values during storage of yoghurt. It is reported that degree of photo-oxidation and the level of antioxidants present in milk may affect the a^* value of yoghurt. So, the reduction observed in the a^* values of both VCMY and CCMY could be due to oxidation reactions during storage (Popov-Raljić *et al.*, 2008). Observations of the current study are very much in agreement with previous reports (Jakubowska and Karamucki, 2020; Weerasingha *et al.*, 2022) of significant reduction in a^* values of yoghurt during storage. Significant reduction observed in the b^* values of VCMY and CCMY is in agreement with the decrease in b^* values of set-yoghurts made from Lankan cattle, Jersey and Friesian milk with the storage reported by Weerasingha *et al.* (2022).

The total viable, yeast and mould and lactic acid bacteria counts of both the CCMY and VCMY samples stored at 30 ± 1°C showed significant ($P < 0.05$) increase within one day (Online Supplementary Table S4). No significant differences were observed between the tested microbiological parameters of both the samples under this storage condition. On storage at 4 ± 1°C total viable counts as well as yeast and mould and lactic acid bacteria counts of VCMY and CCMY increased progressively over the 15 d period (Table 5). Initially there were no significant differences between the tested microbial populations of VCMY and CCMY, but as the storage period progressed all the tested microbiological parameters except the lactic bacteria count were found to be significantly higher in CCMY. The higher antimicrobial properties reported for Vechur cow milk lactoferrin (Anisha *et al.*, 2012) could have contributed towards the lower microbial counts exhibited by VCMY.

During storage, hardness and adhesiveness values showed an increasing trend whereas the cohesiveness showed a decreasing trend (Table 5). Similar changes were reported by Amani *et al.*, 2016. While the hardness and adhesiveness of VCMY was significantly higher than CCMY throughout the storage period its cohesiveness was significantly lower. Hardness is viewed as the most important parameter for evaluation of texture. As per Brennan and Tudorica (2008), higher firmness makes fermented milk less prone to structural rearrangements and so less susceptible to syneresis. This observation was found true in the current study as the Vechur cow milk yoghurt, which exhibited lower syneresis throughout the study, also exhibited higher hardness values. These could be attributed to its higher protein content, which increases the extent of cross-linkage in gel network and thereby results in a denser structure (Paseephol *et al.*, 2008). Our results are in agreement with Shahbandari *et al.* (2016), who reported a gradual increase in hardness of yoghurt when stored at 4°C. Texture parameters, such as cohesiveness, adhesiveness along with gumminess are reported to be important for set-type yoghurts (Domagala, 2006), as they should be spoonable, firm and free of slimy or undesirable textures (Tamime and Robinson, 1999). As cohesiveness is related to the force of internal bonds in yogurt structure (Yilmaz-Ersan *et al.*, 2017) the lower cohesiveness value observed for VCMY indicates its smoother texture. Kumar and Mishra (2003) reported that cohesiveness and adhesiveness can influence the gel strength and the force that would be needed to take away the yoghurt attached to spoon or mouth while eating the product. Significant differences were observed between the VCMY and CCMY for all the textural attributes tested, with the VCMY always showing higher values for hardness, adhesiveness and lower values for cohesiveness.

In the case of all the tested sensory parameter scores except colour and appearance, statistically significant ($P < 0.01$) reductions were observed after one day of storage at 30 ± 1°C of both VCMY and CCMY (Online Supplementary Table S4). Both yoghurt samples developed high acidity and bitterness resulting in drastic reduction of all the sensory scores. This observation is substantiated by the significant ($P < 0.01$) increase observed in the tyrosine values and acidity of both the yoghurt samples. In the case of yoghurt samples stored at 4 ± 1°C significant reductions were observed in their flavour ($P < 0.01$), body and texture, colour and appearance as well as overall acceptability ($P < 0.05$) scores over a period of 15 d (Table 5 and Online Supplementary Table S5). Though there were significant ($P < 0.05$) differences between VCMY and CCMY samples in their other scores, no significant differences were observed in their overall acceptability scores.

In conclusion, as hypothesized, Vechur cow milk yoghurt was found to be technologically superior than cross-bred cow milk yoghurt in terms of lower syneresis %, acidity, microbial population, firmer and less cohesive texture, better flavour, colour and appearance scores. Putative health benefits attributed to the milk of indigenous cattle breeds (Sana *et al.*, 2022), together with the superior technological attributes we have described open up the possibility of adopting fermentation as a means for value addition of Vechur cow milk. Such an initiative would be a noteworthy stride in resolving the economic and endangerment issues associated with this indigenous cattle breed of Kerala.

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