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Vechur Cow Milk Dahi-Process Optimization Using Response Surface Methodology

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Abstract This research report optimized production parameters for the preparation of dahi, using milk of Vechur cows – a rare breed of *Bos indicus* cattle named after the village Vechur in Kottayam District, in the state of Kerala, India. The production parameters considered for standardization were starter culture inoculation percent, incubation temperature and period for incubation. The method adopted was Response Surface Methodology (RSM). Twenty experimental runs were conducted with varying levels of independent production variables generated by central composite rotatable experimental design (CCRD). The sensory responses investigated were flavor, body and texture, color and appearance and overall acceptability of dahi. The RSM results showed that the experimental data could be adequately fitted to a second-order polynomial model with correlation coefficients (R^2) of more than 0.90. The study revealed that the effect of all the factors were significant on the responses. The optimum combination parameters of product and their predicted sensory response values were obtained using the desirability function. These sensory scores were validated with experimental scores and no significant differences were found between the two.

Keywords RSM, Vechur cow milk dahi, Sensory attributes.

Introduction

Dahi, a fermented product of Indian origin is supposed to be originated in the period 6,000 –4,000 BC and is utilized in numerous forms in several Indian cuisines. This-well-liked Indian fermented milk product is comparable to plain yoghurt in appearance as well as consistency and has good nutritional and curative value. This product consumed either in the main course of meal as a refreshing beverage or as a dessert is traditionally prepared by fermentation of boiled cow or buffalo milk by lactic cultures. The

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Food Safety and Standards Authority of India, the country's apex food regulator in its recently amended version of regulations (FSSA) 2011) encompasses all the fermented milk products collectively under the same definition as a milk product obtained by fermentation of milk, which may have been manufactured using other permitted raw material, by the action of suitable microorganisms and resulting in lowering of pH with or witrhout coagulation (isoelectric precipitation). The regulations further stipulate that dahi shall have the equal minimum per cent of milk fat along with milk SNF as the milk using which it is made and a minimum titratable acidity of 0.45%. The regulations also speify lactic acid bacteria as the specific starter culture(s) for dahi preparation. The physico-chemical, textural and sensorial attributes of any product is highly dependent on the characteristics of the raw material from which it is prepared. The condition is not different in the case of milk products; their quality being highly reliant on the attributes of the raw milk used. As the composition of milk varies depending on breed, diet and environment, it is quite possible that there could be significant variations in the properties of the final product depending on the type of milk used. Further, the changes occurring during the various stages of processing are also dependent on the milk type and its properties thus resulting in marked differences in the sensorial, textural and physico-chemical properties of the final product. In this regard it is quite likely that optimization of production parameters is an essential step to be carried while standardizing the production process of any product. So the current study aimed at optimizing the production parameters for the production of dahi from the milk of Vechur cows, an indigenous cattle breed named after the village Vechur in Kottayam District of Kerala in India. A recent study based on phylogenetic analysis and genetic distance estimation showed a close relationship between *Bos indicus* cattle including Vechur cow with the reference cattle used, the European dwarf zebu cattle (Zwergzebu) (Pramod et al. 2018).

The Vechur breed is placed in the breed map of cattles published by the National Bureau of Animal Genetic Resources, ICAR, India (NBAGR 2001). Taking into account that Vechur cattles are listed under category of Critical Breeds in The World Watch List on Domestic Animal Diversity given by the Food and Agriculture Organization of the United Nations (FAO) in their Domestic Animal Diversity Information System (DAD-IS 2012), it is essential to find ways to conserve and popularize their rearing among local farmers. Preparation of unique/niche milk products from specific breed may address the economy aspects and boost up the activities towards conservation of that breed. Development of traditional high-quality products from their milk is identified as one of the means for compensating the lower milk yield from the autochthonous/indigenous cattle breeds (Martin-Collado et al. 2010, Petrera et al. 2016). The fact that Vechur cow milk is considered to be of high therapeutic value and was traditionally being widely used in Ayurvedic system gives further boost towards the initiatives of utilizing this milk for the production of healthy milk products like fermented dairy products. In view of these considerations this study aimed to optimize the three independent production parameters i.e., incubation time, incubation temperature and rate of inoculation of starter culture for the production of good quality dahi from Vechur cow milk using response surface methodology (RSM), a collection of mathematical and statistical techniques for empirical model building.

Materials and Methods

Vechur cow and cross bred cow milk

Fresh, pooled milk samples of Vechur and cross bred cow's were obtained from University cattle farm, Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India.

Dahi culture maintenance

Mesophilic dahi starter culture NCDC-91 (National Collection of Dairy Cultures, *Lactococcus lactis* spp. *lactis*) procured from National Dairy Research Institute, Karnal was aseptically transferred into sterile skim milk (15 Ibs pressure at 121^{o}C for 15 minutes) and incubated till its coagulation at 37^{o}C. These cultures were subjected to four consecutive transfers to get them optimally activated. Routine maintenance of the culture was done by fortnight transfer in sterilized skim milk with refrigerated storage at 4^{o}C in between

Table 1. Design of study –preliminary trials.

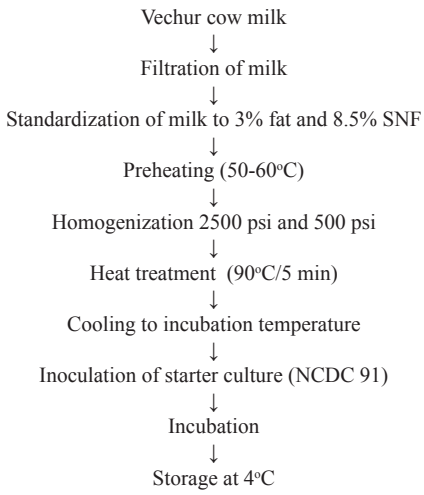
Sl. No.	Product	Variable parameter and value	Constant parameters and values
1.		Rate of inoculation 2 per cent (T_1) 2.5 per cent (T_2) 3.5 per cent (T_3) 4.5 per cent (T_4) 5 per cent (T_5)	Incubation temperature 37°C
	Dahi from vechur cow milk	Incubation temperature 32°C (T_1) 35°C (T_2) 38°C (T_3) 41°C (T_4) 44°C (T_5)	Rate of inoculation 2% Incubation period-8h
		Incubation period 1h (T_1) 3h (T_2) 5h (T_3) 7h (T_4)	Rate of inoculation-2% Incubation Temperature-37°C
	Dahi from cross bred cow milk-control sample(T_6)	Rate of inoculation-2% incubation period -8h incubation temperature-37°C	

the transfers.

Preliminary trials

Preliminary trials were conducted to finalize the range (minimum and maximum values) of the three production parameters, incubation temperature (IT), rate of inoculation (RI) and incubation period (IP) to be adopted for deriving the Central Composite Rotatable Design (CCRD). 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 Table 1 according to the flow chart given in Fig.1. In brief, standardized (3% fat and 8.5% SNF) and two stage homogenized (2500 psi and 500 psi) Vechur cow milk was heat treated at (90°C/5min), cooled to the incubation temperature, inoculated with the dahi culture NCDC 91 (NCDC, Karnal) and incubated at the representative temperatures. The products obtained were stored under refrigeration and subjected to sensory analysis (color and appearance, flavor, body and texture and overall acceptability) using a nine-point hedonic scale where 9 points indicate extremely like and 1 point indicates extremely dislike (Larmond 1977). Control samples were prepared from cross bred cow milk sample standardized to 3.0% fat and 8.5% SNF. The minimum and maximum values for the three production parameters were decided by comparing the sensory scores of the treatment samples with the corresponding control sample.

Fig. 1. Flowchart adopted for the standardization of procedure for the preparation of dahi.



Optimization of production parameters of dahi and using Response Surface Methodology (RSM)

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 8.01.0) was employed for optimization of dahi formulation using 3 independent variables, temperature (A-°C), rate of inoculation (B-%) and incubation period (C-h). The sensory attributes viz.flavor, boby and texture, color and appearance and overall acceptability 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. Data obtained from sensory analysis were fed to RSM software to generate the regression coefficients

Table 2. Minimum and maximum values selected for optimization of production parameters of dahi.

Parameters	Determined from preliminary trials		
	Optimum Value	Mini-mum	Maxi-mum
Temperature °C	38	35	41
Inoculation (%)	3.5	2	4.5
Incubation period (h)	5	3	7

and ANOVA of fitted polynomial models for sensory parameters and responses to get the optimized combinations of production parameters. The optimized solution was generated by numerical optimization using response surface methodology software. For this the desired goals for each production parameter and responses were chosen and different weights were assigned to each goal. The responses were kept maximum and the production parameters in range, during the course of optimization. The optimized combinations of the products were validated by comparing observed values of sensory data with the predicted values generated by RSM software. To ensure that there is no significant lot to lot variation in sensory attributes of optimized products the triangle test was carried out using two coded samples (A and B) of dahi prepared separately from the same lot of Vechur cow milk adopting the optimized solution.

Statistical analysis

Data obtained from sensory analysis were fed to RSM software (Design-Expert® Software Version 8.0.1.0) to generate the regression coefficients and ANOVA of fitted polynomial models for sensory parameters and responses.

Results and Discussion

Preliminary trials

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 represented in Table 2.

Table 3. Coded and actual levels of the three factors (design factors) used in Central Composite Design.

Parameters	Lower limit *-a*	Factorial point -1	Center coordi-nate 0	Factorial point +1	Upper limit *+a*
A : Temperature °C	32.95	35	38	41	43.05
B : Inoculation (%)	1.15	2	3.25	4.5	5.35
C : Incubation (h : min)	1.38	3	5	7	8.21

Optimization of production parameters of dahi and using Response Surface Methodology (RSM)

Minimum and maximum levels of incubation temperature, rate of inoculation of starter culture, and incubation period of dahi selected based on preliminary trials were fed to a five level of three factor Central Composite Rotatable Design (CCRD) for optimization of levels. The coded and actual levels of the three factors (design factors) are given in Table 3. Design matrices representing different combinations of the three factors suggested by RSM are given in Table 4. 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. flavor, body and texture, color and appearance and overall acceptability of the products prepared as per experimental design are given in Table 5. For all the tested responses, Minimum values were obtained for the combination of 38°C incubation temperature, 3.25% rate of inoculation and 1h 38 minutes incubation period while the maximum value was obtained for the combination of 38°C incubation temperature, 3.25% rate of inoculation of starter culture and 5 h incubation period.

The sensory scores of dahi 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 quadratic regression model for the dependent variables was established to fit the experimental data for each response. In the

Table 4. The CCRD for three factors : Temperature, rate of starter inoculation and incubation period for dahi.

Standard order	Temperature °C	Inoculation rate (%)	Incubation period (h : min)
1	38.00	3.25	5.00
2	32.95	3.25	5.00
3	38.00	3.25	8.21
4	38.00	3.25	5.00
5	35.00	2.00	3.00
6	35.00	4.50	7.00
7	41.00	2.00	7.00
8	41.00	4.50	7.00
9	35.00	2.00	7.00
10	41.00	2.00	3.00
11	43.05	3.25	5.00
12	35.00	4.50	3.00
13	38.00	3.25	5.00
14	38.00	3.25	5.00
15	38.00	3.25	1.38
16	38.00	3.25	5.00
17	38.00	5.35	5.00
18	38.00	1.15	5.00
19	38.00	3.25	5.00
20	41.00	4.50	3.00

suggested quadratic model, the F-values for all the responses, (i.e. 4.51, 4.04, 2.56 and 3.87 for flavor, body and texture, color and appearance and overall acceptability respectively), were more than table F-value ($p<0.05$) indicating the developed model was significant (Table 6). The fit of the model was

Table 6. Analysis of variance (ANOVA) of fitted quadratic model for sensory parameters and resposes of dahi.

Partial coefficient terms	Partial coefficients of sensory parametrs			
	Flavor	Body and texture	Color and appearance	Overall accept-ability
Intercept	8.32	8.14	8.31	8.22
A-Temperature	0.23	0.32	0.32	0.32
B-Inoculation	0.34	0.34	0.34	0.34
C-Incubation	0.49*	0.49*	0.49*	0.49*
A^2	-0.99**	-0.95**	-1.02**	-0.98**
B^2	-0.90**	-0.77**	-0.83**	-0.80**
C^2	-1.25**	-1.12**	-1.18**	-1.15**
AB	-0.12	-0.12	-0.12	-0.12
AC	-062*	-0.62*	-0.62*	-0.62*
BC	-0.12	-0.13	-0.13	0.13
Lack of fit	0.06^{ns}	0.075^{ns}	0.16^{ns}	0.081^{ns}
Model F value	4.51*	4.04*	2.56*	3.87*
R^2	0.91	0.91	0.90	0.91
Press	29.94	26.84	27.97	27.10
Adequate precision	9.10	8.73	8.54	8.83

Table 5. Sensory responses of dahi prepared with Vechur cow milk at different levels of inoculation rate, incubation temperature and incubation period.

Standard order	Response 1 Flavor	Response 2 Body and texture	Response 3 Color and appear-ance	Respose 4 Overall accept-bility
1	8	8	9	8.5
2	6.5	6	6	6
3	5	5	5	5
4	8.5	8	8	8
5	4	5	5	5
6	6	7	7	7
7	6	6	6	6
8	5	5	5	5
9	7	7	7	7
10	6	6	6	6
11	4	4	4	4
12	4	4	4	4
13	8.5	9	8.5	9
14	8	8	8	8
15	4	4	4	4
16	9	8	9	8
17	5	5	5	5
18	6	6	6	6
19	8	8	8	8
20	5	5	5	5

estimated by the coefficient of determination (R^2). 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 >0.75 is reported to be indicative of the good fitness of the model (Mandenius and Brundin 2008). The coefficient of determination (R^2) values for flavor, body and texture, color and appearance in addition to overall acceptability of dahi were found to be 0.91,0.91, 0.90 and 0.91 respectively (Table 6) indicating the model as significant. The R^2 values also indicated that fitted quadratic models accounted for 91% of the variation in the data for flavor, body and texture and overall acceptability whereas in the case of color and appearance it was 90%. Adequate precision is a statistical index that indicates the signal to noise ratio and values higher than 4 is considered desirable (Kalita et al. 2017). The respective 'adequate precision' values were found to be 9.10, 8.73,8.54 and 9.10 which were much higher than the minimum desirable value of 4 . So these results indicated that the models could be used to navigate the design. Lack of fit test is a measurement of the failure of a model to

Table 7. Criteria adopted for optimization of production parameters–Dahi.

Constraint	Goal	Lower limit	Upper limit
Inoculation rate (%)	In range	1.15	5.35
Incubation temperature °C	In range	32.95	43.05
Incubation period (h : min)	In range	1.38	8.21
Flavor	Maximize	4	9
Body and texture	Maximize	4	9
Color and appearance	Maximize	4	9
Overall acceptability	Maximize	4	9

represent the experimental data at the point excluded in the regression (Gan et al. 2007). In the present study the lack of fit test was found to be non-significant indicating that the model is sufficiently precise for predicting organoleptic characteristics of dahi made with any combinations of the variables within the evaluated range.

The regression analysis data (Table 6) also shows the partial coefficients of all the sensory responses in terms of linear, second orders and interaction levels for all the three independent parameters. At the linear level incubation period was found to have significant effect ($p<0.05$) on all the sensory responses assessed whereas the effect of rate of starter inoculation and incubation temperature were found to be non-significant ($p<0.05$) for all the sensory responses. At the interaction level, incubation temperature and incubation period were found to be significant ($p<0.05$) for all the responses. At quadratic level all variables had highly significant ($p<0.01$) effect on all the sensory responses tested.

The regression analysis obtained after ANOVA resulted in the following response surface equation for all the responses depicting them as a function of independent variables (A-Temperature, B-Inoculation, C-Incubation) and their linear and quadratic interactions :

Flavor = -186.01740 + 8.88538 × A+4.98052 × B+7.49581 × C-0.033333 × A × B-0.10417 × A × C-0.050000 × B × C-0.10966 × A^2-0.57509 × B^2-0.31303 × C^2.

Table 8. Optimized solution obtained after response surface analysis for dahi.

Solution no.	Inoculation rate %	Incubation temperture °C	Incubation period (h:min)	Desirability
1	3.02	37.3	5.30	0.875

Body and texture =-175.43913 +8.52090 × A + 3.94721 × B + 6.85031 × C-0.033333 × A × B-0.10417 × A × C -0.050000 × B × C-0.10524 × A^2-0.49305 × B^2-0.28098 × C^2.

Color and appearance = -185.12363 + 9.00048 × A + 4.18346 × B + 6.99229 × C-0.033333 × A × B-0.10417 × A × C-0.050000 × B × C-0.11155 × A^2-0.52939 × B^2-0.29518 × C^2.

Overall acceptability = -180.28138 +8.76069 × A+4.06534 × B+6.92130 × C-0.033333 × A × B-0.10417 × A × C-0.050000 × B × C-0.10840 × A^2 -0.51122 × B^2 -0.28808 × C^2. (A-Temperature, B-Inoculation, C-Incubation).

Optimized solution and its validation

The criteria set for the numerical optimization of the production parameters are given in Table 7. For optimization to the desired variables, the goal was set to in range for incubation temperature, rate of inoculation and incubation period. For the sensory attributes, flavor, body and texture, color and appearance in addition to overall acceptability, the goal was set to maximum. Based on the criteria only one optimized solution (desirability 0.875) was obtained and was selected for the preparation of dahi from Vechur cow milk (Table 8).

Verification of the optimized solution was done statistically (t test with equal variances) comparing the actual sensory scores obtained with the predicted values (Table 9). Since no statistically significant ($p<0.05$) differences were observed between the predicted and actual values, the optimized solution of 3.02% rate of inoculation, 37.3°C incubation tempera-

Table 9. Comparison of predicted and observed values.

Attributes	Predicted value	Observed value	t-value
Flavor	8.43	8.41 ± 0.02	0.88^{ns}
Body and texture	8.27	8.2 ± .31	0.81^{ns}
Color and appearance	8.43	8.39 ± 0.88	0.69^{ns}
Overall acceptability	8.35	8.31 ± 0.91	0.71^{ns}

ture and 5 : 30 hours incubation period was adopted as the optimized values of production parameters for the preparation of dahi from Vehur cow milk. The optimized production conditions for Vechur cow milk dahi were different from those adopted for control dahi especially in terms of rate of inoculation, which was higher and the incubation period, a shorter one. Decrease in incubation period with increase in rate of inoculation is quite expected as the introduction of more number of organisms as starter cultures obviously will result in higher acid production and thus a faster coagulation.

On assessing the reproducibility of the optimized production parameter for dahi statistically (t test with equal variances) by comparing the sensory scores obtained for the coded samples (A and B), no significant ($p<0.05$) differences were observed between the samples confirming the reproducibility of the procedure adopted (Table 10).

Table 10. Sensory scores for triangle test-Dahi.

Sensory attributes	A	B
Flavor	8.22 ± 0.05^{a}	8.25 ± 0.08^{a}
Body and texture	8.23 ± 0.98^{a}	8.33 ± 0.01^{a}
Color and appearance	8.5 ± 0.54^{a}	8.51 ± 0.02^{a}
Overall acceptability	8.47 ± 0.67^{a}	8.5 ± 0.09^{a}

Conclusion

Vechur cow milk, though considered to have high medicinal value and was extensiverly used in the Ayurvedic system of medicine, to our knowledge there are no scientific reports about its utilization for the production of any products till now. As far as we know this is the first report in this direction. This study optimized the production parameters for the preparation of dahi from Vechur cow milk by RSM as an incubation temperature of 37°C, starter culture inoculation rate of 3% and incubation period of 5h 30 minutes. The successful development of good quality dahi with Vechur cow milk proves its suitability as the raw material for the production of dahi, a fermented milk product and thus can boost up the on-going efforts towards conservation of this rare breed by shedding light to the possibilities of exploration of value addition potential of this milk for addressing the economic aspects associated with their rearing of fermented milk products.

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