Optimization and development of Misti dahi (sweetened yoghurt) from mixture of cow and goat milk

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Abstract

The present study aims to optimize the relative proportion of cow and goat milk and sugar percent in preparation of misti dahi (sweetened yoghurt). Seventeen experimental runs were conducted with varying levels of independent variables viz. cow milk (60-100v/v), goat milk (20-40v/v) and sugar (8-14%), as generated by Box-Behnken experimental design (BBD). The responses investigated were titratable acidity, syneresis, firmness and overall acceptability of sweetened yoghurt. Response surface methodology (RSM) was used to determine the optimum cow and goat milk proportion and sugar percent. 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 0.94. The study revealed that the effect of all the factors were significant on the responses. The optimum formulation obtained using desirability function was cow and goat milk 73.59 and 18.99 (v/v) respectively and sugar 14%. The values of responses at optimum formulation were, 0.71% titratable acidity, 18.00% syneresis, 3.59N firmness, and 7.97 sensory acceptability. These predicted values were validated with experimental values and found no significant difference.

Keywords

Misti dahi
Goat milk
Optimization

Introduction

Over the years goat milk production has achieved a significant landmark in terms of economic growth in many parts of the world, mainly in arid and semi-arid regions (Haenlein, 2004). India is the leading producer of goat milk (4.0 million MT) in the world that accommodates 30.2 million dairy goats (FAOSTAT, 2008). In spite of many functional benefits of goat milk (Park, 1994; Alferez et al., 2001) the development of fermented goat milk derivatives faces technological hurdles, particularly due to formation of semi-liquid or fragile coagulum (Martin-Diana et al., 2003; Seelee et al., 2009; Tamime et al., 2011). The loose texture is attributed to lower amounts of αs1-casein and higher degree of casein micelle dispersion which also varies with breed, and composition (Storry et al., 1983; Park et al., 2007). Moreover, smaller diameter casein micelles and fat globules and higher non protein nitrogen content in goat milk cause weaker gel in yoghurt (Domagala, 2009). On the other hand, cow milk contains higher amount of all types of proteins which make it highly efficient in making fermented products of better consistency and hardness. Production of yoghurt with mix milk from different animal species have been investigated by many researchers to understand the characteristics inherent to raw milk in the final product (Kavas et al., 2004; Vargas et al., 2008; Bezerra et al., 2012). The mixture of goat milk and cow milk in appropriate proportion could be of highly technological practice that would overcome some rheological and sensory issues of goat milk yoghurt like weak gel and goaty flavor (Martin-Diana et al., 2003; Stelios and Emmanuel, 2004).

Several researches have reported the physicochemical, microbial and sensory properties of yoghurt produced from mixture of cow and goat milk (Vargas et al., 2008; Kucukcetin et al., 2011; Temiz and Kezer 2015). However, to our knowledge, no research has been reported on production of misti dahi (sweetened yoghurt) from mixture of cow and goat milk. In India misti dahi is cherished due to its creamish to light brown colour, firm consistency, smooth texture and pleasant aroma (Raju and Pal, 2011; Obidul Huq et al., 2012). Misti dahi produced from goat and cow milk together could provide a product with enhanced functional attributes which eventually would give more health benefits to consumer.

Materials and Methods

Yoghurt culture maintenance

The starter culture NCDC-263 (National Collection of Dairy Cultures) which is a mixed
thermophilic lactic acid producing bacterial strain comprising *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was procured from National Dairy Research Institute (NDRI), Karnal, India. Freeze dried culture was activated in skim milk medium by incubating it at 42°C for 6h. The mother culture was sub-cultured every fortnight for optimum maintenance of bacterial count. Before use, the working culture was tempered at ambient temperature for 2h. Goat (Assam Hill breed) and cow (Indigenous breed) milk used for preparation of misti dahi were obtained from local firms of Tezpur, Assam, India. The compositional analysis of milk was carried out following standard procedures (AOAC, 2002).

**Manufacture of sweetened yoghurt**

Fresh cow and goat milk were mixed in different proportion as given by experimental design, preheated to 50°C and standardized to 3% fat and 13% solid not fat. Milk was heated at 90°C for 10min, required amount of sugar was added and the blend was cooled to 42°C. Pasteurized milk was inoculated with 2% working starter culture and incubated at 42°C till the pH reached to 4.6. The prepared misti dahi was stored in refrigerated condition and taken out as and when needed for analysis.

**Box-Behnken experimental design**

The Box-Behnken-Design (BBD) with three numerical factors and three levels is employed to design the experiments. The factors were cow milk ($X_1$=60-100v/v), goat milk ($X_2$=0-40v/v) and sugar ($X_3$=8-14%). The ranges of independent variables were decided from preliminary trials. The BBD generated 17 experimental runs (Table 1). As each factor has been varied at three levels, this response surface design consisted of 12 midpoints of edges and five center points (Seth *et al*., 2015). The values of the factors were converted to coded values (Equation 1) to make the scales of each variable symmetrical before applying them to any statistical tool for analysis.

$$x = \left( \frac{X - X_o}{\partial X} \right)$$

Where, $x$ is the dimensionless coded value, $X$ is the actual value, $X_o$ is the value at center point and $\partial X$ is the step change (Rodriguez-Solana *et al*., 2014).

**Response surface methodology and optimization**

The effect of the independent variables on the yoghurt characteristics like acidity, syneresis, firmness and overall acceptability was studied using response surface methodology (RSM). The response
data were collected based on the experimental runs. The experimental results were fitted with quadratic polynomial equation using the least square method (Equation 2).

\[ Y = \beta_0 + \sum_{i=1}^{p} \beta_i x_i + \sum_{i=1}^{p} \sum_{j=1}^{p} \beta_{ij} x_i x_j + \varepsilon \]  

Where, \( Y \) is the response variable (titratable acidity, syneresis, firmness and overall acceptability), \( x_i \) and \( x_j \) are coded independent variables, \( \varepsilon \) is the residual (error) term, \( \beta_0 \) is the constant, \( \beta_i \) is the linear coefficient, \( \beta_{ij} \) is the quadratic coefficient and \( \beta_{ij} \) is the interaction coefficient (Diamante et al., 2012). The analysis of variance (ANOVA) was carried out on each model to validate the models with a confidence level of 95%. The fitting of the polynomial equation was expressed by the coefficient of determination \( R^2 \) and the adjusted coefficient of determination \( R^2_{adj} \). In order to ensure the quadratic models for response variables are not violated the experimental data were examined through the residual plots to validate that the mathematical models exhibit standard normal distribution. The typical residual plots of all the response variables were plotted against the independent variables as shown in Figure 1. The normal distribution of data in all typical residual plots suggested that the residuals of the responses are distributed normally. The contour and surface plots were generated to see the effect and interaction of independent variables on the response characteristics of sweetened yoghurt. The adequacy and efficiency of predicted response surface models were verified by comparing experimental values and predicted values.

Numerical optimization is a technique applied to obtain exact optimum levels of independent variables which results in the desirable response variables by applying response optimizer. With the numerical optimization, we can modify independent variables to get a final product of good quality. Hence, numerical optimization was adopted to find out the optimum proportion of cow and goat milk and sugar percent. Afterward, three confirmation experiments were carried out to verify validity and accuracy of response surface model.

**Physicochemical analysis of sweetened yoghurt**

Titratable acidity in terms of percent lactic acid of misti dahi was determined according to the method described by AOAC (2002). Syneresis of misti dahi was determined using the drainage method as described by Bansal et al. (2015). A sample of 10 ml of yoghurt was filtered through filter paper for 16 h at 4°C. The syneresis value was computed and expressed in volume percent. Back extrusion was carried out using Universal Texture Analyser TA-HDi (Stable Micro Systems Ltd., Surrey, UK) with settings, viz., 5 kg load cell, probe (A/BE 35), pre test speed 2.0 mm/s, return speed 2 mm/s and 20% compression volume. The samples were tempered to 10°C for 2 h before analysis (Raju and Pal, 2009).

The sweetened yoghurt was submitted for sensory evaluation by ten panelists (7 male, 3 female, aged 20-35 years) of the Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam, India. Selection of the panel members was done based on their capacity to discriminate samples with reproducibility and repeatability of the results (Stone and Sidel, 1993). A multi-comparison test was performed by the selected panelist. The panelists had to give acceptable score of sensory attributes using a 9-point hedonic scale where 9 point indicates ‘extremely like’ and 1 point indicates ‘extremely dislike’. The panelists were asked to taste the sample (30 mL/panelist) and drink water as neutralizer. The yoghurt sample contained in a white cup covered with lid and coded randomly with two digit numbers were given to panelists for scoring in a room with proper light luminosity. The sensory attributes were flavour, whiteness, presence of whey, consistency, presence of lumps after breaking the gel, taste, acidity and overall acceptability.
Results and Discussion

Chemical composition of raw milk

The average composition of cow and goat milk used in the preparation of yoghurt is shown in Table 2. Values of fat, lactose, ash and total solid contents of goat milk significantly varied from that of cow milk. However, titratable acidity, pH values and protein content of cow and goat milk had no variation. It was observed that goat milk contained higher amount of all the components compared to that of cow milk. A similar finding was reported by Vargas et al. (2008). It is worth mentioning that, the compositional variation could be due to variation in the breed, season of milking, lactation period etc.

Model fitting and statistical analysis

RSM was applied to see the effect of cow milk, goat milk and sugar proportion in yoghurt formulation on the response values- titratable acidity, syneresis, firmness and sensory overall acceptability of sweetened yoghurt. Variance and regression analysis was carried out to fit the suggested quadratic models and investigate the statistical significance of model factors. The adequacy of the model was investigated by the F-values and corresponding p values of the regression models. It was observed that, the predicted models for all the response variables were adequately fitted to the observed experimental data (p≤0.001). The sequential sum of squares, F-values and the corresponding $R^2$, $R^2_{adj}$, CV, PRESS and adequate precision values are presented in Table 3. The linear, quadratic and interaction effect of each response variables are also presented in Table 3. The $R^2$ values for titratable acidity, syneresis, firmness and overall acceptability of sweetened yoghurt were 94.86, 95.90, 95.94 and 95.80% respectively. In this study, empirical models explained a high percentage ($R^2>0.94$) of response variations. Therefore, we interpret that the variation in the response values are well explained by the predicted polynomial model. The accuracy of the fitness of the models was also judged by the lack of fit values for each response and it was observed that there were no lack of fits (p>0.05) in any response model. The difference between $R^2$ and $R^2_{adj}$ is less than 2% implying there are no insignificant terms added to the models (Homayoonfal et al., 2014). The precision and reliability of the response data in the model fitting was confirmed by the CV values that varied between 2.42 to 5.37. Adequate precision is a statistical index that indicates the signal to noise ratio and the values higher than 4 is desirable. The response models yielded adequate precision values between 12.34 and 16.28. The results obtained from experimental observation were compared with the predicted values of the polynomial equations. The results showed that the models can establish optimum condition for preparation of sweetened yoghurt.

Titratable acidity

The predicted model for titratable acidity is given in equation 3.

$$Titratable\ acidity = 0.78 + 0.045X_1 + 0.069X_2 + 0.079X_1^2 + 0.093X_2^2 - 0.032X_1X_2 + 0.053X_1^2X_2 + 0.065X_2^3$$

The analysis of variance showed that the linear, interaction and quadratic effects of cow milk on titratable acidity of sweetened yoghurt were significant (Table 3). The perfect curvature of surface plot suggests that the effect of cow milk on acidity could be well explained by the pattern of variation with goat milk and sugar (Figure 2-a). Sugar demonstrated significant role on the acidity of yoghurt with a positive linear effect (P≤0.001). The interaction of cow milk and sugar significantly increased the titratable acidity. This could be explained by the tolerance of Streptococcus thermophilus and Lactobacillus bulgaricus to osmotic pressure. Ray et al. (1972) and Tramer (1973) stated that S. thermophilus has a greater tolerance to sucrose than L. bulgaricus which could explain our current findings indirectly. Ghosh and Rajorhia (1990) have reported that Streptococcus thermophilus has the ability to produce lactic acid from sucrose and was found to be highest when sugar level was 6-8%. Moreover, sugar as a bulking agent had a significant effect (P≤0.01) on the acidity of sweetened yoghurt (Raju and Pal, 2011). In another research it was found that, mean percentage of acidity of yoghurt made from goat
milk was highest followed by yoghurt from cow milk and buffalo milk (Nahar et al., 2007). However, goat milk had least effect except in interaction with cow milk (P≤0.05) in the current study. Increased acidity in goat milk yoghurt was explained by enhanced microbial activity and peptidase activity of *L. bulgaricus* (Tamime and Robinson, 1999). Since, maximum volume fraction of goat milk in yoghurt formulation was 40% the effect might have not been so significant in the current research.

**Syneresis**

The analysis of variance for syneresis depicts that the goat milk demonstrated a positive effect on syneresis of misti dahi in linear and quadratic terms (P≤0.001) (Table 3). The developed polynomial model for syneresis is given in equation 4.

\[
\text{Syneresis} = 21.46 + 1.50X_1 - 0.22X_2 - 1.52X_1^2 + 0.75X_2^2 - 1.44X_1X_2 + 0.50X_1X_2^2 + 0.63X_3^2 + 0.03 \tag{4}
\]

Domagala (2012) has reported similar findings in his research. Even the interaction of goat milk with cow milk exhibited a negative effect on syneresis (P≤0.001). This could be explained by the higher proportion of cow milk than goat milk which might have overcome loose texture in misti dahi, thus less syneresis. The pattern of variation of syneresis as an effect of independent variables can be observed from Figure 2-b. Domagala (2009) reported that the yoghurt gel depends on the type of milk and total protein content and he interpreted that yoghurt made from cow milk showed lower syneresis than yoghurt made from goat milk irrespective of the total solids milk contain. Moreover, increasing syneresis trend with addition of goat milk could be explained by the level aggregation and compaction of casein micelles at lower volume fraction of goat milk (Vargas *et al*., 2008). Positive effect of sugar on misti dahi syneresis can be explained by the water binding capacity in yoghurt which increases the serum phase viscosity (Schkoda *et al*., 1997).

**Firmness**

Firmness describes the resistance of yoghurt gel to external forces. The firmness value of yoghurt in all the experimental runs varied between 1.469 to 4.134 N. Cow milk and sugar demonstrated a positive effect on the firmness of misti dahi whereas, a negative effect was observed in case of goat milk (Table 3). The developed polynomial model for firmness of yoghurt is presented in equation 5.

\[
\text{Firmness} = 3.69 + 0.13X_1 - 0.34X_2 + 0.39X_3 - 0.42X_1^2 - 0.35X_2^2 + 0.60X_3^2 + 0.22X_1X_2 + 0.30X_1X_3 + 0.03 \tag{5}
\]

The pattern of variation of firmness as an effect of independent variables can be observed from Figure 2-c. Firmness of yoghurt is dominantly attributed by the cow milk casein aggregation and forming gel network. Casein forms network by making phosphate bond with β-lactoglobulin with the help of κ-casein in the micellar surface. The aggregation depends on the amount of casein and whey protein present in milk, the quantity and type of inoculum added and the time of incubation (Ghosh and Rajorhia, 1990; Vargas *et al*., 2008; Kucukceketin *et al*., 2011). Raju and Pal (2009) demonstrated the effect of sugar on the texture of sweetened yoghurt and concluded that, sugar acts as a bulking agent strengthening the gel

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### Table 3. Analysis of variance (ANOVA) for response surface quadratic models

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structure of yoghurt. Further, it was observed that goat milk had a significant negative effect on the firmness (p≤0.01). This trend was observed in many research findings, which is attributed to the lack of αs1-casein in goat milk, which otherwise would give firmness to the yoghurt (Michalski et al., 2002; Vargas et al., 2008; Kucukcetin et al. 2011). Moreover, weaker gel structure is explained by the characteristic casein micelle dispersion which doesn’t allow the process of aggregation.

Sensory evaluation

Sensory attributes of the sweetened yoghurt were judged and scaled to 9 point multi comparison test. The flavour, whiteness, whey separation, consistency, lumpiness, taste and acidity of sweetened yoghurt were compared with the control sample. The sensory scores of all the attributes significantly decreased (p<0.05) with the addition of goat milk except whiteness. The yoghurt taste and flavour was evaluated with low scores, because with the addition of goat milk the acetaldehyde production decreases (Abrahamsen and Rysstad, 1991). The lower consistency of yoghurt was not preferred by the panelists. The whiteness of yoghurt increased with the increase of goat milk which could be due to the increased reflectance of light by large number of smaller size fat globules present in goat milk. The whey separation from yoghurt was least preferred which increased with increase of goat milk. The visible lumps in yoghurt decreased with the increased goat milk with a reverse trend of consistency (Vargas et al., 2008).

Overall acceptability depicted the preference of sweetened yoghurt sample over a standard sample (sweetened yoghurt from cow milk only). Figure 2-d shows the effect of sugar and cow milk on overall acceptability of yoghurt. It was observed from the analysis of variance for overall acceptability that goat milk had negative effect in linear terms (P≤0.001) (Table 3). The predicted polynomial model for overall sensory acceptability is shown in equation 6.

\[
\text{Overall acceptability} = 7.88 + 0.17X_1 + 0.76X_2 + 0.19X_3 - 0.38X_1^2 - 0.37X_2^2 - 0.16X_3^2 - 0.22X_1X_2
\]

Flavour attribute of yoghurt made from goat milk drastically reduces the likeness of the product by the sensory panelist. This might be due to the goaty flavor caused by the short chain fatty acids (Karademir et al., 2002; Slacanac et al., 2010, Costa et al., 2014). The typical yoghurt flavor is attributed by the development of acetaldehyde in yoghurt. The development of acetaldehyde in goat milk yoghurt is very low compared to cow milk yoghurt. It was illustrated that with the increase of goat milk proportion, the sensory scores reduced (Vargas et al., 2008). However, the degree of un-liking of sweetened yoghurt was less compared to plain yoghurt. This might be due to the relative dominance of the sugar over the goaty flavor. Other sensory attributes as mentioned earlier are not reported with thinking that, they all contribute to the overall acceptability of the yoghurt.
Optimization and model verification

The independent variables were optimized numerically using statistical software Design Expert, trial version- 8.0.4.1 (Stat-Ease Inc.). The variables were kept in range during optimization. The goals were assigned to each response parameters. The acidity was kept in range; overall acceptability and firmness were at maximum and syneresis was at minimum. From the numerical analysis, it was observed that 66.75 v/v cow milk, 12.65 v/v goat milk and 14% sugar gave an optimized product of desirability 0.91. The corresponding optimized response values found were 0.71% lactic acid for acidity, 18.00% syneresis, 3.59N firmness, 40.37Ns consistency, 78.66% DPPH activity and 7.97 overall acceptability. Validation of optimized yoghurt formulation was done by preparing yoghurt in triplicate and comparing the observed result with the optimum predicted data. For checking the variability of predicted responses, two-tailed, one sample t-test was carried out. The results of the t-test demonstrated no significant difference between the values of experimental responses and the predicted responses. Thus, suitability of the models to predict various responses was ascertained.

Conclusion

Designed experiments using Box-Behnken successfully exhibited the effect of independent variables (cow milk, goat milk and sugar) on the response variables (acidity, syneresis, whiteness index, firmness and overall acceptability) of sweetened yoghurt developed from mixture of cow and goat milk. The developed models found to be statistically valid and demonstrated adequate information regarding the behaviour of sweetened yoghurt characteristics upon variation of process variables. Addition of goat milk decreased gel firmness and increased syneresis. The preferred sweetened yoghurt was obtained by mixing goat milk and cow milk and sugar in an optimized way keeping in view on the overall acceptability and gel firmness. The optimized combinations of independent variables found were cow milk (73.59 v/v), goat milk (18.99 v/v) and sugar (14%). The one sample, 2-tailed t -test demonstrated no significant differences between the predicted and original values (P ≤0.10). It is concluded that goat milk can be used with cow milk for preparation of sweetened yoghurt with acceptable sensory attributes.

References


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