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Study of the variation in viscosity during addition of stabilizers to obtain an optimised reconstituted Indian yoghurt (dahi) powder based drink

[1*]Routray, W., [2]Mishra, H. N. and [1]Jusoh, Y. M. M.

[1]*Bioresource Engineering Department, Macdonald Campus, McGill University, Quebec, Canada, H9X 3V9*
[2]*Department of Agricultural and Food Engineering, Indian Institute of Technology Kharagpur, Kharagpur, India, 721302*

Abstract: Central composite design with two factors (guar gum and locust bean gum) was applied to study the variation of viscosity (dependent variable) with respect to variation of amount of guar gum and locust bean gum (independent variables) where the ratio of dahi powder to water (1:10) was kept constant. Optimisation of the composition was carried out, using SAS programming with response surface methodology. All the terms in the final regression equation were found to be significant at α=0.05, except the term presenting the individual effect of locust bean gum. Guar gum was observed to have stronger effect on viscosity of the drink as compared to locust bean gum. The optimum lowest viscosity of 2.003 Pa-s was obtained by incorporating 0.445 g guar gum and 0.905 g of locust bean gum with 20 g of dahi powder and 200 g of water, to prepare the Indian yoghurt drink.

Keywords: Dahi, dahi powder, dahi drink, viscosity, stabilizer

Introduction

Dahi is a fermented food product similar to yoghurt, popular in Indian subcontinent, for its characteristic (sweet-tart) taste. It is produced using mixed mesophilic cultures of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis* along with *Leuconostoc* species. Along with taste dahi has many health benefits (Lin and Yen, 1999; Agarwal and Bhasin, 2002) and in the tropical climate, it has higher storability as compared to the milk. In India, dahi is not specifically classified as a health food but is consumed as a part of basic food, although the manner in which it is consumed varies according to the season, region and individual food habits. It is served plain or with salt or cane sugar or sometimes with other flavour additives during hot summers in India. Dahi based drink, generally known as lassi, is one of the popular drinks consumed to quench thirst during hot summers in India.

Storage parameters are important criteria which determine the profit of the food industry. As mentioned before dahi has higher shelf life than milk, but it is still susceptible to microbial and chemical spoilage. Decrease in the water content of any food product leads to retardation of the microbial activity and chemical degradation. Therefore drying is one of the best methods of preservation of food products. Powder produced by drying of dahi can be mild or extra sour and can be consumed as drink by mixing with water. The conventional methods to produce yoghurt powder are freeze-drying and spray drying (Kim and Bhowmik, 1990; Bielecka and Majkowska, 2000; Kumar and Mishra, 2004). When packaging is done hermetically, shelf-life of yoghurt powder is expected to be high under normal room temperature conditions (Schur, 1978).

One of the major quality attributes of the dahi is texture. Fermentation of the milk sugar (lactose) produces lactic acid, which destabilizes the casein micelles and produces gel like structure in yoghurt. Stabilizers or emulsifiers are used during the manufacture of some of the dairy products including yoghurt, where the main purpose is to enhance and maintain the desirable characteristics of yoghurt. Some of the important properties of consideration in case of yoghurt are body and texture, viscosity or consistency, appearance and mouth feel. Their application in most countries is governed by legislative regulation. Drying of dahi leads to loss of textural properties of yoghurt and to regain the textural properties, stabilizers can be added to the dried dahi, which when mixed with water can develop textural properties such as desirable mouth feel and viscosity. In any formulation more than one stabilizer are used to get required textural properties (Elfak *et al*., 1977; Christianson *et al*., 1981). Two stabilizers used in many food preparations and selected for this study include guar gum, obtained from guar beans

*Corresponding author.
Email: *routrayw@yahoo.com*

and locust bean gum, obtained from beans of carob tree. Both of them are non-ionic and water soluble (Elfak *et al*., 1977). The chemical structure of guar gum consists of β-1→4 linked D-mannopyranose units, with alternate mannose units carrying single α-1→6 linked D-galactopyranose units whereas locust bean gum contains a chain of β-1→4 linked D-mannopyranose units, but differs from guar gum in that the single α-1→6 linked D-galactopyranose side chains occur less frequently and their distribution is more irregular. Due to the differences in the solute-solute and solute-solvent interactions and also due to the influence of other constituents, the combined effect of the stabilizers is different from the individual effect. In previous study it has been observed that for guar gum the solute-solvent interactions are greater than for locust bean gum, but the solute-solute interactions are smaller. In many cases, the effect of more than one stabilizer is synergistic which leads to higher combined viscosity as observed in xanthan/guar gum mixture solutions (Casas *et al*., 2000; Tako and Nakamura, 1985). Other properties such as pH of solutions also affect the viscosity of guar gum, which has been reported to be decreasing with decrease of pH (O'Connor *et al*., 1981).

During this experimental setup, the variation of viscosity of reconstituted dahi drink with respect to the addition of two stabilizers guar gum and locust bean gum was studied. A central composite design (CCD) with two factors and five levels each, combined with response surface methodology (RSM) was used to finally obtain an optimum combination of the stabilizers for reconstituted drink, along with a regression model for the interpretation of variation of viscosity with respect to the addition of various quantities of the selected stabilizers (in the range fixed during the preliminary studies).

Materials and Methods

Dahi powder was used as the material with which the study was done. The entire process used for the study has been summarized briefly in this paragraph which has been further explained in the following paragraphs. The process of preparation of the dahi powder consisted of culture propagation and maintenance, preparation of dahi, preconcentration and freeze drying. Then the dahi powder obtained was reconstituted into dahi powder based drink using different combinations with guar gum and locust bean gum and viscosity for different combinations of stabilizers with dahi powder and water were measured. The experimental design used was CCD with RSM.

Preliminary experiments were done to fix the ratio of water to dahi powder, which was fixed as 10:1. The commercial dahi drink was kept at room temperature varying between 25 to 30ºC and the range of viscosity was measured using Brookfield digital viscometer (Model RVTD, DV I, Brookefield Engineering Lab. INC, LV4 (64) spindle at 0.5 rpm) during the preliminary experiments. At 1 min after the insertion of the spindle the viscosity measurement was noted for the drink sample. This was repeated three times. Based on these observations the range of the amount of stabilizers (guar gum and locust bean gum) to be used during the experiments was fixed between 0.22 g to 2.20 g. The guar gum was obtained from MERCK Specialities Pvt. Ltd. (Mumbai, India) and locust bean gum was obtained from SIGMA ALDRICH (St. Quentin Fallavier, France).

Sample preparation

Mixed mesophilic starter culture, NCDC-167 (EPS –ve), containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, *Lactococcus lactis* subsp. *cremoris* and *Leuconostoc citrovorum* (in pure freeze-dried form), was obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, India for dahi preparation. Ten percent non-fat dried milk (NFDM) was used as the medium for propagation of dahi culture. Ten gram of skimmed milk powder (Anik spray, standard grade, Nutricia India Pvt. Ltd., Mumbai, India) was reconstituted in 100 ml distilled water and sterilized in an autoclave using 15 psi pressure for 15 min. The milk was cooled to 30°C and then inoculated with dahi culture at the rate of 1-3%. The culture was maintained by transfer into sterile medium during regular intervals and incubation at 30°C.

To prepare dahi the standard market milk (whole milk with 2% fat content) was obtained. The milk was heated to 60°C and subjected to mixing. Pasteurization was done at 80-90°C for 10 secs and the pasteurized sample was allowed to cool to 25-30°C. Then the milk was poured into the clean beakers and standard culture was added in the fixed amount to it and the dahi was left to set at room temperature (25-30°C) for 12-16 hours.

Pre-concentration of dahi was achieved through centrifugation using centrifuge (Make REMI and Model R 24). Dahi was centrifuged at 4000 rpm for 15 minutes and the pre-concentrated dahi was used for drying using the freeze dryer. The pre-concentrated dahi was spread as a layer of uniform thickness (3±1 mm) on the plates and the plates were pre-freezed overnight. Then the plates were put into the freeze drier (Lyodel freeze drier, Delvac pumps Pvt. Ltd.) and the samples prepared were dried for 12-14 hours until the moisture reached the required level (2-3%).

Experimental design

Experimental plan was prepared using two variables and five levels central composite design to obtain an optimised composition of the dahi powder based drink by analysing the effect of independent parameters, amount of guar gum and amount of locust bean gum on the dependent parameter viscosity. The experimental design consisted of four factorial points, four axial points and six centre points to increase uniformity of precision (Araujo and Brereton, 1997; Afoakwa *et al*., 2008). The coded form is represented as x and the real variable as X. If a_m and $-a_m$ are assigned as the maximum and minimum values of the coded variable x; X_{max} and X_{min} are assigned as the maximum and minimum for the real variable X, relationship between coded x and real X of the variables can be derived as

$$X_M = (X_{max} + X_{min})/2 \quad ......(1)$$

$$X_D = (X_{max} - X_M)/a_m \quad ......(2)$$

$$x = (X - X_M)/X_D \quad ......(3)$$

Real value of X was obtained from its coded value x by means of the equation

$$X = x X_D + X_M \quad ......(4)$$

where a_m = 1.41 for CCD with 2 variables, and X_D is the intermediate value calculated to obtain the relationship (which is generally the difference between the value of X at coded value 1 or -1 and X_m) (Das, 2005). Therefore, the relationship between the coded (GG and LB) and actual values of amount of guar gum (G) and locust bean gum (L) to be added were:

$$GG = (G - 1.21)/0.7 \quad ......(5)$$

$$LB = (L - 1.21)/0.7 \quad ......(6)$$

Table 1 shows the experimental plan along with the real and coded values of the parameters used. Experimental design required 14 trial runs (Table 2), as mentioned above. The trial runs were replicated twice at each level and the average was taken as the final response. From the observations obtained from the preliminary experiments, mixtures of the ratio of dahi powder to water (ratio of 1:10 i.e. 20:200) was fixed as mentioned before. To each of the 14 trial runs devised by the design, guar gum and locust bean gum were added (before adding water) as per the quantity suggested by the experimental design and were maintained at room temperature. The viscosity was measured after 30 mins using Brookfield digital viscometer (Model RVTD, DV I, Brookefield Engineering Lab. INC, LV4(64) spindle at 0.5 rpm), at 1 min after the insertion of the spindle.

Then the respective measurements of the viscosity obtained were used for further analysis and optimisation. The analysis of the data was performed using the PROC RSREG module of SAS version 9.2 and experimental data were fitted into a second order polynomial model which can be presented as equation (7), which is in terms of coded values of the independent variables,

$$\text{Viscosity} = \beta_0 + (\beta_1 \times GG) + (\beta_2 \times LB) + (\beta_3 \times GG^2) + (\beta_4 \times LB^2) + (\beta_5 \times GG \times LB) \quad ......(7)$$

where, there were the two independent variables GG and LB, influencing the response and the significance of the terms was evaluated by determining the F-value. The optimum point was intended to be at the minimum as the viscosity of a drink is generally expected to be low.

Table 1. Levels and codes of independent variables in the experiment to optimise the composition of dahi drink using stabilisers

Factors	Code	Coded values				
		-1. 41421	-1	0	1	1. 41421
Guar gum (g)	GG	0.22	0.51	1.21	1.91	2.20
Locust bean gum (g)	LB	0.22	0.51	1.21	1.91	2.20

Table 2. Experimental design to optimise the composition of dahi powder based drinks using stabilisers

Experiment no.	Amount of Guar gum to be added		Amount of Locust bean gum to be added	
	Coded	Real (g)	Coded	Real (g)
1	-1	0.51	1	1.91
2	1	1.91	1	1.91
3	0	1.21	0	1.21
4	-1	0.51	-1	0.51
5	0	1.21	0	1.21
6	0	1.21	0	1.21
7	1	1.91	-1	0.51
8	0	1.21	0	1.21
9	-1.41421	0.22	0	1.21
10	0	1.21	0	1.21
11	1.41421	2.20	0	1.21
12	0	1.21	0	1.21
13	0	1.21	1.41421	2.20
14	0	1.21	-1.41421	0.22

Results and Discussion

The full design layout with the observed viscosities is presented in Table 3 and the analysis of variance (ANOVA) of this experiment is reported in

Table 3. Values of response i.e. viscosity with the corresponding values of independent variables guar gum and locust bean gum

Run	Guar gum		Locust bean gum		Viscosity (Pa-s)
	Coded (GG)	Uncoded (G, (g))	Coded (LB)	Uncoded (L, (g))	
1	-1	0.51	+1	1.91	4.48
2	+1	1.91	+1	1.91	19.60
3	0	1.21	0	1.21	6.40
4	-1	0.51	-1	0.51	2.98
5	0	1.21	0	1.21	6.96
6	0	1.21	0	1.21	6.88
7	+1	1.91	-1	0.51	10.48
8	0	1.21	0	1.21	5.36
9	-1.41	0.22	0	1.21	0.96
10	0	1.21	0	1.21	5.48
11	+1.41	2.20	0	1.21	20.88
12	0	1.21	0	1.21	5.20
13	0	1.21	+1.41	2.20	10.84
14	0	1.21	-1.41	0.22	3.44

GG, G = Guar gum; LB, L = Locust bean gum

Table 4. ANOVA table presenting the different statistical parameters associated with the regression model obtained and dependent and independent variables of the Dahi drink formulation

Source of variation	Degree of Freedom	Sum of Squares	R-square	Mean squares	F value	P value
Linear	2	378.0423	0.8431		208.04	<0.0001
Quadratic	2	48.5423	0.1003		26.71	0.0003
Bilinear	1	14.5161	0.0324		15.98	0.0040
Total Model	5	441.1007	0.9838		97.10	<0.0001
Error	8					
Lack of Fit	3	4.1056		1.3685	2.16	0.2108
Pure Error	5	3.1629		0.6326		
Total	13	7.2685		0.9086		
Response mean		7.8529	R-square		0.9838	
Root mean square		0.9532	Coefficient of variation		12.1381	

Table 4.

From ANOVA results (Table 4), it was observed that a significant model (P<0.0001) was obtained that described the system and gave combination of stabilizers that corresponds to the optimum viscosity. The insignificance of lack of fit (P<0.2108) and high R^2 value (98.38%) indicated the reliability and adequacy of the model to represent the system. In detail, the model obtained was composed of linear (GG and LB), quadratic (GG^2 and LB^2) and bilinear (GG×LB) terms and all of these were found to be significant at α = 0.05. The linear and quadratic terms presented the individual significant effects of the stabilizers whereas the bilinear term presented the significant effect of the interaction of the two components on the final viscosity.

The coefficient (Coded and Actual) and significance level or critical P value of the individual terms are presented in Table 5 and based on them, it was inferred that the model of viscosity is highly affected by GG, GG^2, GG×LB and LB terms. Though the quadratic terms were together found to be significant, LB^2 individually was found to have no significant impact on the model even at α = 0.1. Therefore, by considering only the terms significant at α = 0.1, the model could be written in coded (Equation 8) form as shown below.

$$\text{Viscosity}=6.05+(8.99\times GG)+(3.73\times LB)+(5.05\times GG^2)+(3.81\times GG\times LB) \quad ......(8)$$

The model obtained was a second order polynomial model with an optimum point of minimum. SAS output indicated that the predicted optimum viscosity (2.003 Pa-s) could be achieved with optimum combination of guar gum and locust bean gum at 0.445 g and 0.905 g (rounded up to the third decimal).

Table 5. Regression coefficients and significance value for all terms present in the final regression model

Factor	Coefficients		P value
	Coded	Uncoded/Actual	
GG	8.99	-8.11	0.0054
LB	3.73	-4.08	0.0943
GG^2	5.05	5.15	< 0.0001
LB^2	1.27	1.30	0.1076
GG×LB	3.81	3.89	0.0040

GG = Guar gum; LB = Locust bean gum

The effects of guar gum and locust bean gum on viscosity could also be visualized from the response surface plot in Figure 1.

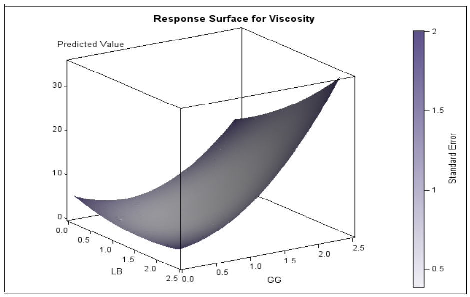

Figure 1. Response surface plot for viscosity of the reconstituted Dahi drink as a function of amount of guar gum added and amount of locust bean gum added. (GG = Guar gum in grams; LB = Locust bean gum in grams)

It was observed that as the concentration of guar gum and locust bean gum increased, the viscosity increased however the relative increase in viscosity due to guar gum was found to be higher than the relative increase due to locust bean gum. This meant that the addition of guar gum makes the drink more viscous compared to the addition of locust bean gum under similar conditions. This was also supported by canonical analysis in SAS output, where it showed that guar gum is having stronger effect on the system compared to locust bean gum. In previous studies, locust bean gum has been found to be less soluble and attain lower viscosity than guar gum as it has fewer galactose branch points and the value of reduced viscosity (ratio of the relative viscosity increment to the mass concentration of the polymer) was found to be higher in dilute solutions of guar gum than locust bean gum. With increasing concentration the difference in case of guar gum from locust bean gum was found to decrease (Elfak *et al*., 1977). Along with the individual effects of the stabilizers, the increase in viscosity can also be attributed to the increase of the total solids content, which has been observed to affect the viscosity significantly (Deysher *et al*., 1944). The combined effect of the stabilizers can be attributed to the synergistic effects and also to differences in solute-solute and solute-solvent interactions (Elfak *et al*., 1977). It was observed that the viscosity obtained by the individual higher amount of the guar gum was higher as compared to the combined higher amount of both the stabilizers which can be attributed to the effect of pH on the comparatively lower concentration of guar gum as compared to the individual higher concentration, because in case of guar gum lower pH lowers the viscosity (as mentioned before) and in this case, the reconstituted drink was observed to have pH 4.26, which is quite low.

Conclusion

The effect of the two stabilizers, guar gum and locust bean gum combination, on the viscosity of the dahi drink was studied in this project. The optimum lowest viscosity of 2.003 Pa-s could be obtained by incorporating 0.445 g guar gum and 0.905 g of locust bean gum with 20 g of dahi powder and 200 g of water, to obtain the Indian yoghurt (dahi) drink. A reliable and highly significant (P<0.0001) second order polynomial model was obtained that represented the system which was composed of GG, GG^2, LB and GG×LB terms. The individual effect of guar gum and locust bean gum could be visualised in the surface plot from which it was interpreted that guar gum is having stronger effect on viscosity of the drink as compared to locust bean gum. The combined effect of the two stabilizers was also found to be significant. Therefore, a reconstituted drink with the desirable property (low viscosity) was obtained.

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