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Eggnog: process optimization and characterization of a dairy-based beverage

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Abstract

Eggnog, a dairy-based beverage, comprises both milk and egg proteins. We aimed at optimizing the eggnog formulation using Box–Behnken design of response surface methodology. The combined effects of milk (60–75), cream (25–35) and eggnog base (6–8, all three as g 100/ml) were investigated on heat coagulation time, viscosity and thermal gelation temperature. ANOVA indicated that experimental data were well explained by a quadratic model with high check values ($R^2 > 0.94$) and non-significant lack of fit tests. Based on the responses, an optimized formulation of eggnog with 60.0 milk, 25.0 cream and 6.50 eggnog base (as g 100/ml), could be considered best for manufacturing eggnog with desired attributes. This optimized formulation was characterized for physico-chemical, microbial and sensory attributes and the results indicated significantly higher fat and protein content than control formulation, but lesser lactose and total sugar content. Significantly higher viscosity, heat stability and lower thermal gelation temperature were also observed for the optimized formulation. Coliform, yeast and mold, *E. coli* and *Salmonella* counts were not detected in any sample but a significantly lower total plate count was observed for the optimized formulation.

Milk and eggs are considered as a good source of essential nutrients including proteins, amino-acids, fatty acids, vitamins and minerals (Yilmaz *et al*., 2022) and their nutritional role has been extensively studied and well-documented (Górska-Warsewicz *et al*., 2019; Pal and Molnár, 2021). Besides this, their bioactive properties including antimicrobial, antihypertensive, anti-inflammatory, immunomodulatory, antioxidant, and opioid properties are of recent interest due to increased consumer demand for functional foods (Tonolo *et al*., 2020; Guha *et al*., 2021; Sharma *et al*., 2021a). Eggnog, a popular seasonal drink in the United States, United Kingdom and Canada, is a dairy beverage with, primarily, dairy and egg components (Ellis, 2021). Milk (preferably cow milk), partially skimmed milk, cream, or skimmed milk are the most frequent dairy ingredients used to prepare eggnog, either alone or in combination, while the egg components include egg yolk (liquid, frozen, or dried) and whole egg (liquid, frozen, or dried). It must have a minimum of 6.0 g 100/m milkfat, 1.0 g 100/ml egg yolk solids, and not less than 8.25 g 100/ml: milk solids not fat (FDA, 2020). While the origin of the eggnog is still unknown, some suggest a similarity to 'posset' (mixture of milk and alcoholic beverage with occasional addition of eggs) and 'egg flip' (blend of eggs and spirit without any milk or dairy ingredient: Graham, 2019) and in common usage eggnog can refer to either a non-alcoholic or an alcoholic beverage.

To the best of our knowledge, only one formulation is available in the literature related to ultra-high temperature processing of eggnog (Aggarwal, 1975) and no information is available regarding the method of preparation and the processing conditions. Also, this proposed formulation contained high sugar and water content with less protein, thus, there still exists the need to standardize the eggnog formulation and to optimize the processing methods and conditions which can further augment its commercialization on an industrial scale. Further, mixing egg components with milk components may pose certain challenges with heat stability, thermal gelation and other functional properties of the eggnog. In this regard, response surface methodology (RSM) is one of the most widely used optimization approaches in food science, because of its extensive theory, relatively high efficiency and simplicity (Hussain *et al*., 2016; Sreedevi *et al*., 2021; Torres Vargas *et al*., 2021). Bearing this in mind, the present study aimed to develop a response surface model to optimize the formulation of non-alcoholic eggnog by investigating the influence of the principle independent variables, namely, milk, cream, and eggnog base (consisting of egg yolk and albumen only) on the eggnog's heat stability and rheological characteristics. Consequently, the optimized formulation was evaluated and compared to control formulation (Aggarwal, 1975) for physico-chemical, rheological and sensory parameters.

Materials and methods

Ingredients and chemicals

Cow milk (3.2 and 8.2 g 100/g fat and SNF) and cream (40 g 100/g fat) were procured from the experiment dairy unit of ICAR-National Dairy Research Institute, Karnal, Haryana. Fresh eggs and spray skimmed milk powder (3.57 g 100/g moisture) (M/s Modern Dairies Ltd., Karnal) were purchased from the local store at Karnal, Haryana, India. All the chemicals and solvents were of analytical grade and purchased from commercial sources.

Experimental design

Preliminary trials were conducted for eggnog preparation using milk, cream, eggnog base (consisting of egg yolk and albumen), skimmed milk powder and sugar. Based on preliminary results, skimmed milk powder and sugar levels were fixed at 4 g 100/ml and the selected minimum and maximum levels (g 100/ml) for milk (X_1), cream (X_2) and eggnog base (X_3) were 60 and 75, 25 and 40, 5 and 8, respectively (detailed in online Supplementary Table S1). The response surface methodology was used to determine the optimum formulation of eggnog. Based on Box–Behnken design, 17 experimental runs including 5 centre points, 6 axial and factorial points were performed with three independent variables (X_1, X_2, and X_3) (Table 1). The dependent responses used for optimization of eggnog were heat coagulation time (s), dynamic viscosity (mPa s) and thermal gelation temperature (°C). The best formulation was predicted by generating a second-order polynomial regression model equation of the quadratic model as expressed in the (Eq. 1):

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} b_{ij} X_i X_j \qquad (1)$$

where b_0, b_i, b_{ii}, b_{ij} are the regression coefficient. (b_o is the constant term, b_i is a linear effect term, b_{ii} is a squared effect term, and b_{ij} is an interactive effect term) and Y is the predicted response. The experiment was repeated three times and all the parameters were performed in triplicate.

Process for eggnog preparation

Briefly, pre-warmed cow milk at 35°C and cream (40 g 100/g fat) were mixed followed by the addition of skimmed milk powder. Egg white and yolk contents were separated carefully and yolk was mixed with part of the sugar (50% of the total quantity) followed by whipping. The whole milk mixture was then mixed with the whipped egg yolk and sugar mix and subjected to pasteurization. Simultaneously, egg white was whipped with the remaining part of the sugar and the mixture was separately pasteurized at 56.7°C for 3.5 min (FDA, 2002). The detailed methodology for the preparation of eggnog is given in online Supplementary Fig. S1. Control eggnog was prepared using skimmed milk powder (10.0 g 100/ml), water (60 g 100/ml), cream with 40% fat (15.0 g 100/ml), eggnog base (6.0 g 100/ml) and sugar (9.0 g 100/ml) (Aggarwal, 1975).

pH, acidity and proximate composition

The pH of the eggnog samples was measured at 25°C using a calibrated pH meter (Eutech Instruments, Cyberscan pH 2100UK). Acidity of the samples was determined by titration method using 0.1 N NaOH (AOAC, 2000). Total solids and ash content of eggnog samples were estimated by drying the samples to a constant weight at the temperature of 102 ± 1 and 550 ± 10°C, respectively. Fat content of the samples was estimated by a modified Mojonnier method (AOAC, 2000) and protein content by Kjeldahl method (AOAC, 2000). The lactose content of the eggnog was determined by the Lane–Eynon method (IS:SPPartXI, 1981). All these parameters were determined on the day of experiment.

Heat coagulation time

Heat coagulation time (HCT) of eggnog samples was determined on the next day of the experiment as per the methodology described previously (Saipriya *et al.*, 2021) with some modifications. Eggnog was adjusted to various pH in the range of 5.5–7.5 by adding 0.1 M HCl or 0.1 M NaOH. 2 ml of sample was placed in the glass tube of 4.0 ml capacity and 12.2 cm in length made from precision-bore Pyrex tubing (0.9 cm outside diameter and 0.65 cm bore). The tubes were closed with a silicon–rubber stopper and clamped on the carriage, which was immersed in the hot liquid paraffin to a depth of 4 cm. HCT was determined in a thermostatically controlled oil bath at 120°C temperature.

Microbiological analysis

Microbial count of the eggnog samples including total plate count (ISO 4833 : 2003), coliforms (ISO 4832 : 2006), Yeast and Moulds (ISO 6611 : 2004), *Salmonella* (IS 5887 (Part 3):1999) and *Escherichia coli* (IS 5877 (Part I):1976) was conducted on the next day using standard protocols.

Rheological characteristics: dynamic viscosity

The dynamic viscosity of eggnog was measured on the same day of the experiment on a dynamic rheometer (MCR 52, Anton Par, Ostifildern, Germany) using cone and plate configuration (CP-75; 75 mm diameter) at 20°C (Chand *et al.*, 2021). Viscosity measurements were performed at a shear rate of 1000/s and at a temperature of 20°C using the probe CP-75.

Rheological characteristics: thermal gelation

The rheological characterization of the gelation process was studied on the same day of the experiment from 20 to 80°C with a heating rate of 2°C per minute using Rheometer (MCR 52, Anton Paar, Germany) (Lucey *et al.*, 2001).

Sensory analysis

Sensory evaluation of the eggnog samples was carried out by 10 volunteers aged between 25 and 45 years (six male and four females) on the same day of preparation of the samples using the composite score card (Chand *et al.*, 2022). Sensory scores of all seventeen runs of eggnog samples were subjected to principal component analysis (Sharma *et al.*, 2021b).

Response surface modelling and model adequacy

The data obtained for the different responses were analyzed by the Design Expert® software version 13.0.5.0. The value of the

Table 1. Experimental design with the level of three factors according to the Box–Behnken design of response surface methodology for the preparation of eggnog and mean values of responses for different parameters

	Factors			Responses		
Run	X_1 (%)	X_2 (%)	X_3 (%)	Heat coagulation time (s)	Viscosity (mPa s)	Thermal gelation temperature (°C)
S1	60.00	25.00	6.50	314	3.68	50.10
S2	75.00	25.00	6.50	379	3.54	51.57
S3	60.00	40.00	6.50	323	8.23	45.27
S4	75.00	40.00	6.50	327	6.14	51.20
S5	60.00	32.50	5.00	393	7.88	48.22
S6	75.00	32.50	5.00	325	5.55	52.63
S7	60.00	32.50	8.00	339	7.06	47.86
S8	75.00	32.50	8.00	383	6.29	50.95
S9	67.50	25.00	5.00	454	3.47	53.01
S10	67.50	40.00	5.00	297	9.49	47.77
S11	67.50	25.00	8.00	414	8.67	51.24
S12	67.50	40.00	8.00	363	6.12	48.47
S13	65.00	30.00	6.00	535	6.73	50.40
S14	67.50	32.50	6.50	540	6.90	50.47
S15	69.00	32.50	6.00	532	6.81	50.38
S16	63.50	30.00	6.50	505	6.70	50.36
S17	62.00	31.00	6.00	485	6.52	50.43

#Experiments were conducted randomly; X_1: Milk, X_2: Cream, and X_3: Eggnog base.

desirability index (D) was determined by the following equation (Chakraborty *et al.*, 2015):

$$D = (d_1^{r1} \times d_2^{r2} \times d_3^{r3})^{(1/r1+r2+r3+r4+r5)} \quad (2)$$

where r is the relative index and d_i is the desirability index. Here i varies from 1 to 3 and desirability ranges from 0 to 1. Further, the Student's t-test was applied using SPSS (IBM® SPSS®, 23) to compare the predicted values and the actual values and thus, the efficiency of the model (Deshwal *et al.*, 2020).

Statistical analysis

Box–Behnken design of response surface methodology was used for the optimization of the levels of ingredients (milk, cream and eggnog base). A total of 17 trials were conducted for the optimization of eggnog formulation. The data generated for different responses were analyzed by using the Design Expert® software (13.0.5.0 version) (Stat-Ease., 2021 E. Hennepin Avenue, Minneapolis, USA). Statistical significance was evaluated by F value, and the effect of independent variables on the individual responses was described at $P < 0.01$ and $P < 0.05$. Results of sensory scores were analyzed using the principle component analysis. Further variation among the control and optimized eggnog samples was analyzed using the independent t-test. All the experiments for observing the differences between control and optimized eggnog were conducted three times and the measurements for the parameters were taken in triplicate for reducing experimental variation.

Results and discussion

Optimization of eggnog using response surface methodology

The formulations obtained from experimental design and experimental mean values obtained for HCT, thermal gelation temperature (TGT) and dynamic viscosity of eggnog after analysis are presented in Table 1.

Diagnostic check of the quadratic model

A value of the coefficient of multiple determination (R^2) more than 0.8 indicates a good fit model (Chakraborty *et al.*, 2015). In the present study, it could be observed that quadratic models were the best-fitted model for each response since the R^2 value for HCT (Y_1), dynamic viscosity (Y_2) and TGT (Y_3), was 0.97, 0.95 and 0.99 respectively (Table 2). Besides this, the coefficient of variance in a well-fitted model should be less than 10%. For the obtained model, this requirement was also fulfilled with the value ranging from 0.79 to 8.70%. Moreover, the adequate precision value (which gives a measure of signal to noise and should be greater than 4) was in the range of 12.55–25.83, which also indicates the adequacy of the quadratic model. From these results, we can suggest that the obtained model can be used for this design.

Effect of different variables on heat coagulation time

Heat treatment induces certain changes in the heat stability of protein beverages when subjected to a high temperature for few seconds. In the present study, HCT values varied between 297 and 540 s. Maximum HCT (540 s) was obtained for the formulation

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Table 2. Regression coefficients and analysis of variance of quadratic model for heat coagulation time, viscosity and thermal gelation temperature of eggnog prepared with each formulation

Partial coefficients	Responses: Heat coagulation time	Viscosity	Thermal gelation
Intercept	540.00	6.90	50.47
Linear			
X_1	5.63^{NS}	−0.6662*	1.86**
X_2	−31.37*	1.33**	−1.65**
X_3	3.75^{NS}	0.2187^{NS}	−0.3887*
Interaction			
X_1X_2	-15.25^{NS}	-0.4875^{NS}	1.11**
X_1X_3	28.00^{NS}	0.3900^{NS}	-0.3300^{NS}
X_2X_3	26.50^{NS}	−2.14**	0.6175*
Quadratic			
X_1^2	−113.13**	−0.8725*	−0.5713*
X_2^2	−91.13**	-0.6300^{NS}	-0.3638^{NS}
X_3^2	−66.87**	0.6675*	0.0136^{NS}
Lack of fit	NS	NS	NS
Model *F* value	23.05	15.44	42.02
C.V. (%)	6.18	8.70	0.7943
R^2	0.97	0.95	0.98
Adequate precision	12.5495	15.9837	25.8287

**Significant ($P<0.01$);* Significant ($P<0.05$); NS – non-significant ($P>0.05$); X_1 – milk; X_2 – cream; X_3 – eggnog base; C.V. – coefficient of variation; R^2 – co-efficient of determination.

comprised of 67.50, 32.50 and 6.50 g 100/ml milk, cream and eggnog base, indicating that a higher quantity of cream decreased the HCT (Fig. 1a) significantly at both linear ($P<0.05$) and quadratic levels ($P<0.01$). Interaction effect of milk and cream also decreased the HCT (Table 3). The coefficient of determination for HCT of eggnog samples (at quadratic level) was high enough ($R^2=0.97$) to estimate the parameter with the regression equation (Table 3). Figure 1a also reflects the effect of interaction of cream and eggnog base as well as milk and eggnog base and it is noteworthy that both cases present a similar effect with higher values for HCT at an intermediate level of variables. Additionally, it can be observed that cream had significantly higher impact on the heat coagulation time followed by eggnog base and milk. A higher concentration of milk and cream is reported to dissociate kappa-casein, thereby increasing the concentration of hydrogen ions and calcium ions and resulting in lower heat stability (Dumpler *et al.*, 2020). However, at the higher concentration of the eggnog base, it is speculated that the susceptibility of the milk and egg proteins to denaturation might increase. Our results suggested the heat-induced gelation of the eggnog during the heat stability test could be attributed to the proteins and lipids present in the eggnog.

Effect of different variables on dynamic viscosity

Physico-chemical and sensory properties such as creaminess are highly correlated with the viscosity of the product (Flamminii *et al.*, 2020). The dynamic viscosity values for eggnog samples varied from 3.54 to 9.49 mPa s. A regression equation which has a good fitting capacity for dynamic viscosity of eggnog samples with high coefficient of determination ($R^2=0.95$) was constructed (Table 4). All the variables had a significant influence (linear and quadratic). The dynamic viscosity of the eggnog significantly decreased with increased level of milk (Fig. 1b) owing to dilution effect upon addition of higher quantity of milk in the formulation. However, cream has a greater effect ($P<0.01$) on the dynamic viscosity of the eggnog samples. Increased cream level and fat content might have been shown to increase the viscosity of cheese due to cold agglutination and churning effects (Kamath *et al.*, 2022). The functional properties of the egg proteins such as foaming, gel formation, and emulsification might also have played key role in regulating dynamic viscosity.

Effect of different variables on thermal gelation temperature

Thermal gelation is heat-induced gelation during which proteins are activated by increasing the temperature and undergo several non-covalent interactions to form a network of a hydrogel (Kharlamova *et al.*, 2019). The quadratic model revealed that the regression coefficients for all the variables had a significant effect on the thermal gelation values of eggnog at a linear level wherein, increased level of milk significantly increased the thermal gelation temperature ($P<0.01$), whilst cream and eggnog decreased it ($P<0.05$). Only one variable (milk) could positively influence the thermal gelation at quadratic level ($P<0.01$) (Fig. 1c). It has been reported that addition of high level of milk in cream (having ~83.0% water and 3.5% protein), significantly decreased the protein concentration, which regulates the gel formation and gel strength (Totosaus *et al.*, 2002). Regression equation with high coefficient of determination ($R^2=0.98$) was obtained for thermal gelation temperature of eggnog suggesting the suitability of the model to predict the parameter.

Optimization of independent variables and model prediction

The criteria for dependent variables (maximum for HCT and thermal gelation, in range for dynamic viscosity) chosen to find optimized formulation are outlined in online Supplementary Table S2. From the RSM, the optimized formulation with highest desirability (0.82) was obtained for better quality eggnog preparation with milk, cream, and eggnog base at the level of 60.0, 25.0 and 6.50 g 100/ml, respectively. Comparison of the predicted values with experimental values for HCT, dynamic viscosity and thermal gelation temperature was conducted and the results revealed no significant difference between these two values (online Supplementary Table S3). Hence, it could be inferred that the optimized formulation of eggnog just defined (together with 4 g 100/ml each of skimmed milk powder and sugar) could be considered best for manufacturing eggnog with desired attributes.

Principal component analysis for sensory analysis of the eggnog samples

Principal component analysis was conducted for the sensory scores of all the seventeen trials of the eggnog and it was observed that a total of four principal components with a cumulative variance of 89.45% were extracted. PC1, PC2, PC3 and PC4 showed 40.47, 24.18, 14.71, and 10.10% of the variance, respectively.

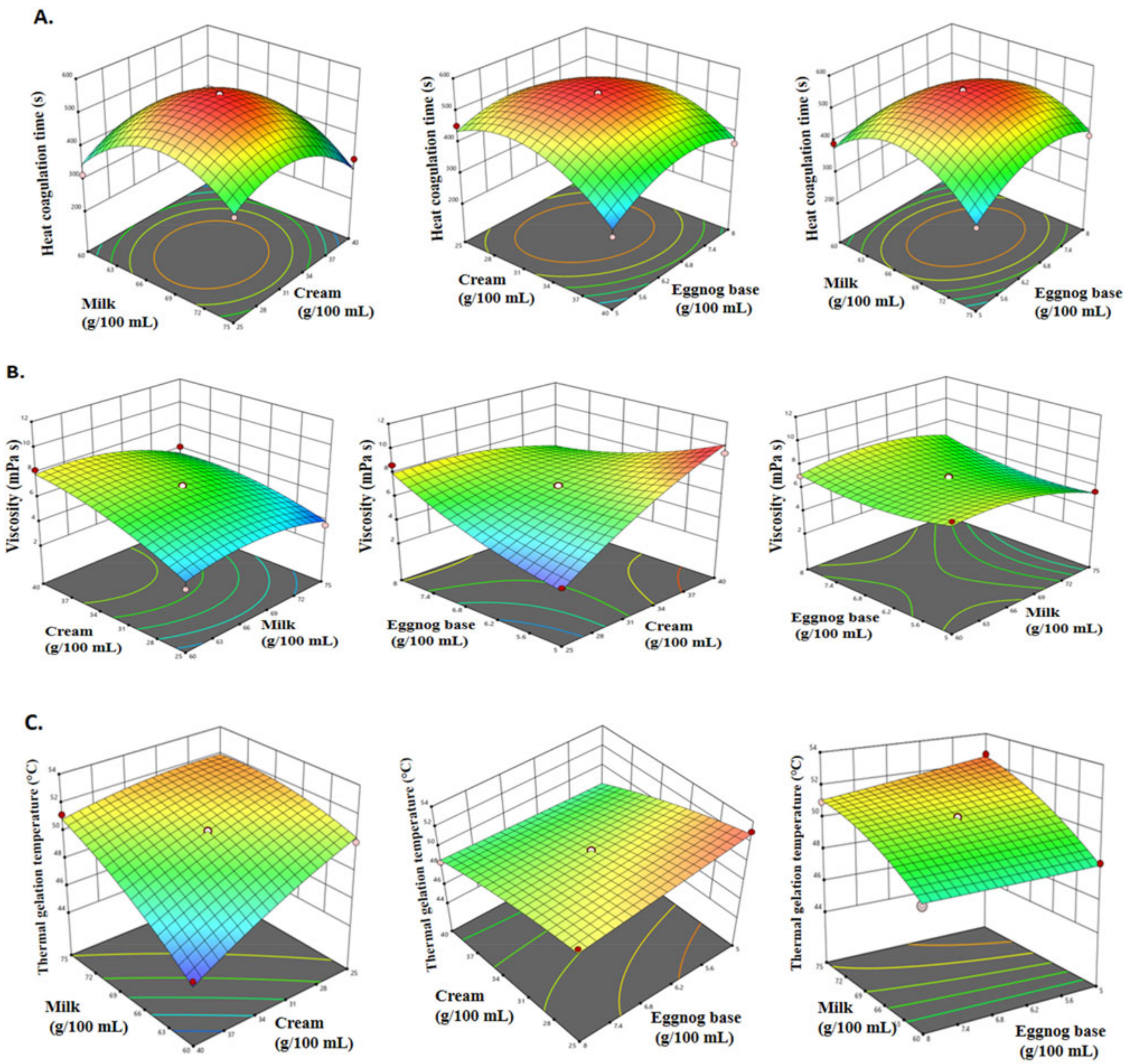

Fig. 1. Three-dimensional representation of the effect of independent variables (milk, cream and eggnog base) on (a) heat coagulation time (s), (b) viscosity (mPaS), and (c) thermal gelation temperature (°C).

Table 3. Quadratic model in term of coded and actual factors

Response	Coded equation
Heat coagulation time	$540 + 5.63*X_1 - 31.37*X_2 + 3.75*X_3 - 15.25*X_1*X_2 + 28.00*X_1*X_3 + 26.50*X_2*X_3 - 113.13X_1^2 - 91.13X_2^2 - 66.87X_3^2$ ($P < 0.0001$)
Dynamic viscosity	$+6.90 - 0.67*X_1 + 1.33*X_2 + 0.22*X_3 - 0.49*X_1X_2 + 0.39*X_1\_X_3 - 2.14X_2X_3 - 0.87X_1^2 - 0.63X_2^2 + 0.67X_3^2$ ($P < 0.05$)
Thermal gelation	$+50.47 + 1.86*X_1 - 1.65*X_2 - 0.39*X_3 + 1.11*X_1X_2 - 0.33*X_1\_X_3 + 0.62X_2X_3 - 0.57X_1^2 - 0.36X_2^2 + 0.02X_3^2$ ($P < 0.05$)

(* indicating multiplication).
X_1: Milk, X_2: Cream and X_3: Eggnog base.

It is worth mentioning that all principal components showed a positive correlation with viscosity, mouth feel, and creamy flavor. Variance values obtained in the present study are in accordance with reported values (35–65%) (Sharma *et al*., 2021b). The loading factor with an absolute value of >0.6 indicate a strong influence (Table 4) (Sharma *et al*., 2021b). In the case of PC1, the absolute correlation of milky, creamy flavor, mouth feel, viscosity and color was found to be more than 0.6, whereas the absolute correlation of cooked flavor was −0.935, indicating their strong influence on the sensory attributes (Fig. 2).

Table 4. Eigen values, percentage variance and variable loadings for varimax rotated principal components

Loading factors	PC1	PC2	PC3	PC4
Eigen values	4.86	2.90	1.76	1.21
Variance (%)	40.47	24.18	14.71	10.10
Cumulative variance (%)	40.47	64.65	79.36	89.45
Attributes				
Milky flavor	**0.951***	−0.088	−0.071	0.145
Cooked flavor	**−0.935***	0.287	0.053	−0.093
Creamy flavor	**0.908***	0.105	0.049	0.229
Colour	**0.898***	−0.107	0.359	−0.097
Mouthfeel	**0.633***	**0.600***	0.250	0.209
Viscosity	**0.606***	0.245	0.143	**0.600***
Metallic aftertaste	0.082	**−0.912***	0.210	0.170
Overall acceptability	−0.011	**0.752***	0.447	0.380
Smoothness	−0.222	**0.723***	−0.343	0.391
Bland flavor	−0.055	−0.020	**−0.929***	0.107
Egg flavor	0.136	−0.430	**0.705***	0.468
Sweet flavor	0.177	0.088	−0.031	**0.899***

PC, Principal component.
Loadings with an absolute value >0.600 are shown as* marked.

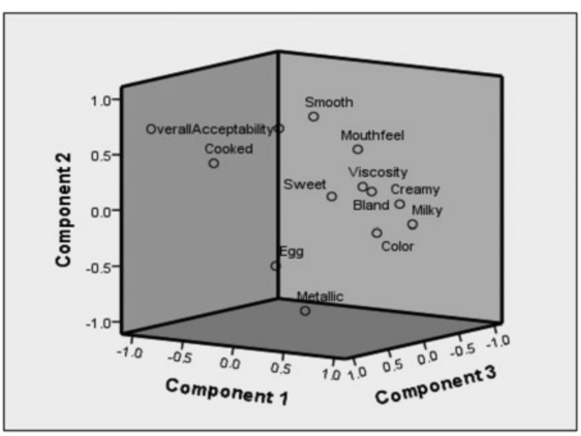

Fig. 2. Three-dimensional plot showing factor scores of eggnog samples on varimax rotated PC axes (PC1, PC2, and PC3).

Characterization of optimized eggnog

Physico-chemical properties of optimized eggnog

As is evident from Table 5, significantly higher fat, protein, and total solids content differentiated the optimized formulation from the control eggnog due to the presence of 60 times more cow milk and nearly double the amount of cream in the latter formulation. On the other hand, higher amounts of cow milk (average 3.4 g 100/g protein), cream, eggnog base, along with skimmed milk powder could be the reason for higher protein content and thus, increased total solids ($P < 0.05$) (Bharti *et al.*, 2021). Various health benefits of a product containing higher protein and lower carbohydrates have been well documented (Kumar *et al.*, 2015; Goswami *et al.*, 2017; Yarar-Fisher *et al.*, 2019). Moreover, the optimized formulation fulfilled the requirements recommended by the U.S. Food & Drug Administration (FDA, 2020), wherein, eggnog must have a minimum of 6.0 g 100/ml milk fat, 1.0 g 100/ml egg yolk solids, and not less than 8.25 g 100/ml milk SNF.

Table 5. Physico-chemical, rheological and microbial characteristics of the control and optimized eggnog formulation

Parameters	Control sample	Optimized sample
pH	7.06 ± 0.01[a]	7.09 ± 0.01[b]
Fat (g 100/ml)	6.74 ± 0.79[a]	10.45 ± 0.26[b]
Protein (g 100/ml)	4.586 ± 1.24[a]	9.73 ± 0.87[b]
Total solids (g 100/ml)	25.25 ± 0.54[a]	27.14 ± 0.19[b]
Ash (g 100/ml)	0.80 ± 0.06[a]	0.77 ± 0.04[a]
Total sugar (g 100/ml)	14.61 ± 0.43[b]	8.21 ± 0.25[a]
Lactose (g 100/ml)	5.55 ± 0.08[b]	4.02 ± 0.15[a]
Viscosity (η, mPaS at 50/s, 20°C)	7.15 ± 0.24[a]	8.77 ± 0.15[b]
Thermal gelation temperature (TGT) (°C)	67.70 ± 5.14[b]	48.53 ± 3.02[a]
Heat stability (s)	464.33 ± 8.34[a]	523.00 ± 2.51[b]
Total plate count (log CFU /ml⁻)	4.87 ± 0.48[b]	3.07 ± 0.61[a]

All the values are Mean ± S.D ($n = 9$).
[ab]Mean values in a row with at least one similar superscript do not differ significantly ($P >$ 0.05).
#Coliform, yeast and mold, *E. coli* and *Salmonella* count was not observed in these samples.

HCT of the optimized and control eggnogs was strongly affected by pH with the optimized formulation having higher HCT at all of the studied pH range, except pH 7.1 (online Supplementary Fig S2a). Majority of liquid milks exhibit type A behaviour (Singh, 2004). However, in the present study, type B behaviour (increase in HCT as a function of pH) of both the samples would be attributed to the added egg base which might have increased the heat stability in the range of the minimum (i.e. ~pH 6.9) (Tziboula *et al.*, 1994). In our study, the lower pH of the control eggnog might have been the contributory factor for its lower heat stability as ionic calcium concentration enhances at reduced pH resulting in neutralization of casein micelles charges, thereby augmenting the aggregation process (Wu *et al.*, 2020).

Rheological characteristics of optimized eggnog

Higher amounts of fat, protein or total solids are responsible for increased viscosity (Bienvenue *et al.*, 2003). The viscous nature of milk is mainly attributable to fat content as people generally correlate the consistency of milk with fat content (Ghanimah, 2018). These might be the primary factors contributing to almost 18–20% higher dynamic viscosity (mPaS) ($P < 0.05$) of the optimized eggnog (8.77 ± 0.15 *vs.* 7.15 ± 0.24: Table 5). Results of RSM also demonstrated that fat content in the formulation produced marked impact on the viscosity of the eggnog samples. Further, both the samples exhibited a typical shear-thinning behavior (decrease in apparent viscosity with increasing shear rate) (online Supplementary Fig. S2b). Thermal gelation temperature (cross over temperature of G' and G") was observed to be significantly higher ($P < 0.05$) for the control eggnog (67.70 ± 5.14 *vs.* 48.53 ± 3.02: online Supplementary Fig. S2c). Two stages are mainly

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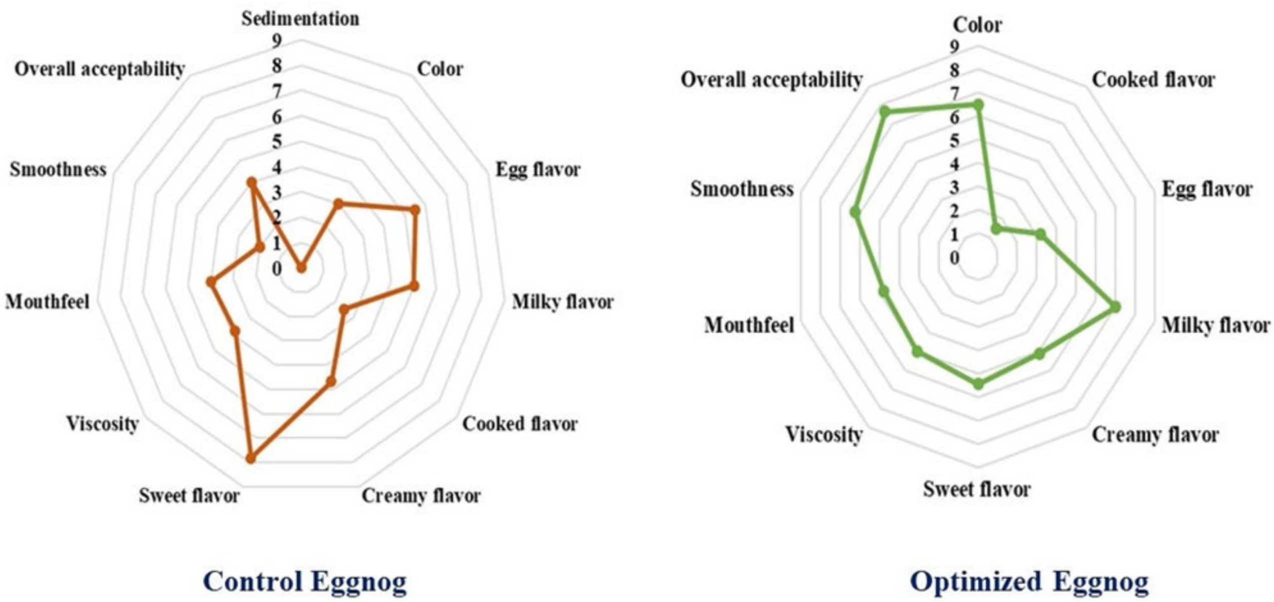

Fig. 3. Effect of composition of control and optimized eggnog on sensory attributes.

involved in controlling the gelation rate, namely protein denaturation and protein aggregation (Kharlamova *et al.*, 2019). Therefore, increased protein content in eggnog might have resulted in decreased thermal gelation temperature as protein content mainly regulates the transition of sol–gel (Schäfer *et al.*, 2018).

Microbiological characteristics of optimized eggnog

Total plate count (Log CFU/ml) for the control and optimized eggnogs was 4.87 ± 0.48 and 3.07 ± 0.61, respectively, indicating the lower count in the latter (Table 5). Yeast and mold count, coliform count, *Escherichia coli* count, and *Salmonella* count were not detected in any sample, which might be due to the pasteurization of egg and milk (Sharma *et al.*, 2019, 2020). Grade A pasteurized milk should not have more than 4.3 $\log_{10}$ CFU/ml and less than 10 coliforms/ml and it is worth noting that the optimized eggnog had comparable microbial count to the recommendation count.

Sensory attributes of optimized eggnog

Even upon the addition of cream (with 40 g/100 g fat) to the optimized eggnog, no significant difference ($P > 0.05$) was observed in the scores of appearances, mouthfeel and the creamy flavor (online Supplementary Table S4). This might be due to the preference and perception of sensory panelists to the creamy taste (Upadhyay *et al.*, 2020). However, it should be remembered that only ten panelists were included in the analysis, so further work is required to verify these sensory conclusions. Egg and sweet flavor were perceived significantly higher ($P < 0.05$) in the case of the control eggnog (4.67 ± 2.56 and 7.83 ± 0.69, respectively, Fig. 3) which corroborates with our results of lower lactose and total sugar content in the optimized formulation. Viscosity and smoothness of the products varied significantly ($P < 0.05$), being higher in the optimized formulation (Fig. 3). This might be due to higher protein and fat content in the former (Jørgensen *et al.*, 2019). Overall acceptability is generally influenced by the culmination of the responses of all the sensory attributes (Sharma *et al.*, 2021c). In the present study, overall acceptability was significantly higher ($P < 0.05$) for the optimized formulation (7.66 ± 0.94 *vs.* 4.91 ± 1.02).

In conclusion, we were able to optimize the formulation of eggnog using RSM (Box-Behnken Design). Second order polynomials were well fitted for the experimental data. In general, all the independent variables significantly affected HCT, dynamic viscosity and TGT. Based on the responses, optimal levels of milk, cream and eggnog base were observed to be 60, 25 and 6.5 g 100/ml, respectively. This optimized formulation of eggnog contained significantly higher fat, protein and total solids than the control eggnog which resulted in higher dynamic viscosity and heat stability but lower TGT.

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