ISSN Print: 2664-844X
ISSN Online: 2664-8458
IJAFS 2025; 7(1): 130-136
www.agriculturaljournals.com
Received: 15-11-2024
Accepted: 21-12-2024

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Optimization of level of incorporation of lutein, calcium and vitamin d for Shrikhand preparation

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DOI: https://doi.org/10.33545/2664844X.2025.v7.i1b.249

Abstract
Shrikhand was developed by incorporating tricalcium citrate tetrahydrate (TC) as calcium source, Vitamin D3 and lutein. Preliminarily the range of levels of TC and Vitamin D3 were fixed as 100 ppm-300 ppm and 200 IU/L to 600 IU/L of milk respectively. Chakka prepared from milk fortified using selected levels of TCC (200 ppm) and vitamin D (600 IU) was added with lutein in the range of 0.5 -2.5 mg/100 g serving for the preparation of shrikhand. Response surface methodology (RSM) was used to optimize the levels of three ingredients. Central Composite Rotatable Design using three variables and five responses consisting of sensory attributes was used for computation of optimized solution. The optimum values selected were at 162.78 ppm for tricalcium citrate tetrahydrate, 558.65 IU/L for vitamin D_3 and 0.60 for lutein. The predicted values for flavor, color and appearance, body and texture, sweetness and overall acceptability of lutein incorporated calcium and vitamin D_3 fortified shrikhand were 8.14, 8.18, 8.23, 8.03 and 8.17 respectively. RSM recommended with 94% acceptability for the optimum levels of ingredients. Product is prepared with an optimized solution and subjected to sensory evaluation.

Keywords: Shrikhand fortification, tricalcium citrate tetrahydrate (TC), vitamin D3 and lutein

Introduction

Milk and milk products have long been integral to the diet in India, valued for their rich nutritional content, including proteins, vitamins, and minerals. They play a vital role in traditional Indian cuisine and are widely consumed across all age groups, either in their natural form or as processed products. In India, dairy products such as curd, paneer, buttermilk, and lassi are essential components of everyday meals and are also used in religious and cultural rituals.

Lactic fermentation, a natural process involving lactic acid bacteria, is one of the oldest methods used to preserve milk and enhance its nutritional properties. During this process, milk is fermented into a variety of dairy products, including curd, yogurt, and other fermented milk products, which not only extend shelf life but also improve digestibility and offer health benefits. Lactic fermentation increases the availability of certain nutrients, enhances the probiotic content, and imparts unique flavors and textures to the final product, making it a popular preservation method in Indian dairy traditions.

Shrikhand is a popular traditional fermented dairy product, particularly cherished in the Indian states of Gujarat, Maharashtra, and Karnataka. Made from chakka (Thickened curd) and sweetened with sugar, Shrikhand is often flavored with cardamom, saffron, or other regional spices, making it a delectable dessert or side dish. Rich in protein, probiotics, and essential nutrients, Shrikhand offers numerous health benefits, such as improved digestion and enhanced gut health, while also being highly palatable and culturally significant.

In the Indian diet, Shrikhand is not just a treat for special occasions and festivals but is also consumed as a wholesome addition to everyday meals, often paired with puris. Its versatility, combined with its nutritional profile, makes it a favorite across various age groups. The growing demand for healthier, probiotic-rich foods has further contributed to the rise in Shrikhand consumption.

In recent years, the market for Shrikhand has seen a significant increase, driven by both domestic consumption and the growing interest in traditional, functional foods.

According to industry reports, the fermented dairy product market, including Shrikhand, is expanding rapidly due to changing consumer preferences towards nutritious and culturally rooted foods. The increasing market demand highlights Shrikhand's potential as a key player in the Indian dairy sector, offering opportunities for both traditional and modern variations of this beloved product.

Lutein, a member of the Xanthophylls family of carotenoids, is primarily extracted from the petals of marigold flowers (*Tagetes erecta*). Numerous studies have demonstrated its antioxidant and pigmentation properties, making it a valuable ingredient in the formulation of nutritional supplements, cosmetics, and the pharmaceutical industry (Landrum and Bone, 2001) [7]. Additionally, lutein-enriched foods have been shown to enhance human visual performance and help prevent age-related cataracts and adult macular degeneration (AMD) (Tokusoglu, 2013) [13].

Calcium is essential for strong bones and teeth, muscle function, nerve transmission, and blood clotting. It plays a critical role in preventing bone-related diseases like osteoporosis, especially in postmenopausal women and older adults. In the Indian diet, calcium is primarily sourced from dairy products, leafy greens, and legumes. However, due to lactose intolerance and limited consumption of these foods in some regions, calcium deficiency is a concern in the country. Studies have shown that many Indians consume less calcium than the recommended levels, increasing the risk of bone health issues.

The Indian Council of Medical Research (ICMR) recommends a daily intake of 600 mg for children, 1000 mg for adults, and 1200 mg for women above 50 years and pregnant/lactating women. Ensuring sufficient calcium intake through diet or supplements is crucial for maintaining bone health and preventing calcium-related disorders in the Indian population

Vitamin D is crucial for the proper absorption of calcium and phosphorus, essential minerals for maintaining strong bones and a healthy immune system. It also plays a role in reducing inflammation and promoting cell growth. In India, vitamin D deficiency is widespread due to factors like limited sunlight exposure, which is the primary source of vitamin D, and dietary habits that lack sufficient vitamin D-rich foods. This deficiency has been linked to conditions such as rickets in children, osteomalacia and osteoporosis in adults, and an increased risk of chronic diseases like diabetes and cardiovascular conditions.

Research has shown that a significant proportion of the Indian population is deficient in vitamin D, with studies indicating that up to 70-90% of Indians may not meet the recommended levels. The lack of adequate sunlight exposure, especially in urban areas with high pollution, and low dietary intake further exacerbate this deficiency.

To address this, fortification of dairy products with vitamin D has been proposed as a viable solution. Dairy products are widely consumed across India, making them an effective vehicle for improving public health. Fortification can help bridge the gap in vitamin D intake, particularly in regions where dietary diversity is limited. Research studies have shown that vitamin D-fortified dairy foods, such as milk, yogurt, and ghee, can significantly enhance vitamin D status in the population. This approach has the potential to reduce the prevalence of deficiency-related diseases and improve overall health outcomes. Response Surface Methodology (RSM) is a powerful collection of mathematical and statistical techniques used for modeling and analyzing problems where a response of interest is influenced by multiple variables, and the goal is to optimize that response. In one application, the process optimization of bael-enriched shrikhand was conducted using RSM, where bael pulp powder and sugar were considered as the independent variables. The study measured responses including color, flavor, sweetness, body/texture, and overall acceptability. Similarly, Pathrikar *et al*. (2021) [9] employed the Central Composite Rotatable Design (CCRD), a subset of RSM, to develop a process for goat milk shrikhand with added kiwi fruit. In this case, the levels of kiwi fruit pulp and sugar were optimized as the key variables, with responses such as color, flavor, consistency, sweetness, and overall acceptability being evaluated.

Materials and Methods

Fresh whole buffalo milk was collected from University Dairy Plant, KVASU. Lutein was procured from Plant Lipids Pvt. Ltd, Kolenchery, Cochin. Vitamin D_3 premix was supplied by Pd-Navkar, Pvt. Ltd, Karnataka. Tricalcium citrate tetrahydrate purchased from Hi-Media Laboratories Mumbai was used as a calcium source for fortification. Yoghurt Culture (*Streptococcus thermophilus: Lactobacillus delbrueckii subsp. bulgaricus*) was procured from Dept. of Dairy Microbiology, VKIDFT, Mannuthy. Polystyrene cups (125 ml) with lids were obtained from Lyka Packaging Pvt. Ltd, Ernakulam.

Preparation of Shrikhand

Shrikhand was prepared following the method described by Aneja *et al*. (2002) [1], with slight modifications. Fresh whole buffalo milk was received, filtered, and standardized to 6% fat and 9% solid-not-fat (SNF). To the milk, tricalcium citrate tetrahydrate (162 ppm) and vitamin D3 (558 IU/L) were added, followed by thorough mixing. The milk was then heated to 85 °C for 30 minutes, after which it was cooled to 45 °C for inoculation with a starter culture at 1% concentration. The milk was incubated at 45 °C for 4-5 hours until a firm coagulum was formed.

Once the coagulum was formed, it was gently broken and transferred to a muslin cloth, where it was hung to allow complete whey drainage. The resulting semi-solid mass, known as chakka, was mixed with 40% sugar and lutein (0.59 mg/100 g). The mixture was kneaded thoroughly to achieve the desired consistency. It was then packed into polystyrene cups and stored under refrigeration.

Experimental Design

Optimization of lutein-enriched, calcium and vitamin D-fortified shrikhand was carried out using Central Composite Rotatable Design (CCRD) within the framework of Response Surface Methodology (RSM), utilizing Design-Expert® Software Version 9. Based on preliminary trials, the experimental parameters were carefully selected, focusing on three key variables: Calcium, vitamin D, and lutein. The response variables measured included flavor, color and appearance, body and texture, sweetness, and overall acceptability.

A total of twenty experiments were conducted following a second-order CCRD, with three independent variables each set at five different levels. The center point experiment was repeated six times to assess the reproducibility of the method. This design allowed for a comprehensive evaluation of the response patterns across the defined variables.

Product Optimization

The statistical analysis was performed using IBM SPSS Statistics 22. For all the standardized response values, Analysis of Variance (ANOVA) and multiple regression analysis were carried out using Design Expert Version 9.0 (Stat-Ease Inc., Minneapolis, USA) to determine the significance of the model terms. Non-significant terms were eliminated through backward elimination regression, and the polynomial model was recalculated accordingly.

To validate the model's adequacy, various statistical metrics were used, including F-values, lack-of-fit tests, R^2 (Coefficient of determination), PRESS values, and Adequate Precision Ratio (APR). The model equation used to fit the experimental data was:

$$
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{23}X_2X_3 + \beta_{13}X_3X_1 + \text{Error term}
$$

Where:

- Y is the predicted response,
- β_0 is the constant coefficient,
- β_1, β_2, β_3 are the linear coefficients,
- β_{11}, β_{22}, β_{33} are the quadratic coefficients,
- β_{12}, β_{23}, β_{13} are the interaction coefficients.

Three-dimensional response surfaces were generated using Design Expert Version 9.0, with both numerical and graphical optimization conducted via the same software.

Statistical Analysis of Data

For preliminary trial data, Friedman's test was used to assess statistical significance. For the optimization process involving calcium, vitamin D3, and lutein, data was analyzed using the Kruskal-Wallis test, followed by the Wilcoxon signed-rank test to compare groups. To examine variability in physico-chemical and microbiological properties over different time points, repeated measures ANOVA was applied. Sensory score variability between different measurement periods was analyzed using Friedman's test, followed by the Mann-Whitney U-test.

This approach ensured a robust and comprehensive analysis of all experimental parameters and responses.

Results and Discussion

Selection of minimum and maximum levels of tricalcium citrate tetrahydrate, vitamin D3 and lutein for the preparation of lutein incorporated calcium and vitamin D fortified shrikhand

Selection of Levels of Tricalcium Citrate Tetrahydrate (TCT)

Preliminary trials were conducted by preparing shrikhand from milk fortified with three different levels of TCT: 100 ppm (TC1), 200 ppm (TC2), and 300 ppm (TC3). The resultant products were compared with control shrikhand (T0) for sensory attributes, and the sensory scores were statistically analyzed. The chi-square ($\chi2$) values for attributes such as flavor, color and appearance, body and texture, sweetness, and overall acceptability were 7.18, 7.17, 13.07, 3.32, and 13.07, respectively (Table 1). These results revealed that responses related to body and texture, as well as overall acceptability, showed significant differences at the 1% level, while other attributes were non-significant ($p>0.05$). Although no significant difference was observed between TC1 and TC2 compared to the control (T0), TC3 (300 ppm) was found to result in poor consistency and was deemed unacceptable. Therefore, TC1 (100 ppm) and TC2 (200 ppm) were selected as the minimum and maximum levels of TCT to be added to shrikhand.

Table 1: Effect of different levels of tricalcium citrate tetrahydrate on sensory scores of shrikhand

Parameters	T1	TC1	TC2	TC3	Chi- square value
Flavour	8.17±0.11	7.83±0.17	7.75±0.11	7.67±0.11	7.18^{ns}
Colour and Appearance	8.08±0.15	7.67±0.17	7.50±0.22	7.42±0.15	7.17^{ns}
Body and Texture	8.00±0.13^{a}	7.67±0.17^{a}	7.58±0.15^{a}	6.83±0.17^{b}	13.07**
Sweetness	7.92±0.15	7.58±0.20	7.50±0.22	7.42±0.20	3.32^{ns}
Overall Acceptability	8.00±0.13^{a}	7.75±0.11^{a}	7.67±0.11^{a}	7.17±0.11^{b}	13.07**

Figures are Mean± Standard error of sensory scores by five selected judges in six replications, $^{a-b}$ Figures in a row with different superscripts differ significantly,**- significant at one per cent level ($p<0.01$), ^{ns}- non significant

Selection of Levels of Vitamin D3

Shrikhand fortified with vitamin D3 was prepared using milk fortified with a selected level of 200 ppm TCT. Three levels of vitamin D3 were tested: 200 IU/L (TV1), 400 IU/L (TV2), and 600 IU/L (TV3). The products were evaluated for sensory qualities, and the sensory scores were statistically analyzed. The chi-square ($\chi2$) values for sensory attributes such as flavor, color and appearance, body and texture, sweetness, and overall acceptability were 0.97, 4.40, 2.06, 2.35, and 5.51, respectively (Table 2). The results indicated no significant differences ($p>0.05$) between the control and vitamin D3-fortified samples in terms of sensory responses, aligning with the findings of Ganesan *et al.* (2011) [14], who reported that vitamin D fortification had no effect on cheddar cheese sensory properties. To optimize vitamin D content without affecting sensory attributes, TV2 (400 IU/L) and TV3 (600 IU/L) were selected as the minimum and maximum levels for fortification.

Table 2: Effect of different levels of vitamin D_3 on sensory scores of shrikhand

Parameters	T1	TV1	TV2	TV3	Chi- square value
Flavour	8.08±0.15	7.83±0.11	7.75±0.11	7.58±0.15	0.97^{ns}
Colour and Appearance	8.17±0.11	8.00±0.18	7.83±0.11	7.75±0.11	4.40^{ns}
Body and Texture	8.25±0.11	8.08±0.20	8.00±0.18	7.75±0.11	2.06^{ns}
Sweetness	8.00±0.13	7.83±0.17	7.75±0.11	7.75±0.11	2.35^{ns}
Overall Acceptability	8.17±0.17	7.92±0.15	7.83±0.11	7.83±0.11	5.51^{ns}

Figures are Mean± Standard error of sensory scores by five selected judges in six replications, ^{ns}- non significant

Selection of Levels of Lutein: Chakka prepared from milk fortified with 200 ppm TCT and 600 IU vitamin D3 was then incorporated with three different levels of lutein: 0.5 mg (TL1), 1.5 mg (TL2), and 2.5 mg (TL3) per 100 g serving. The prepared samples were evaluated by a panel of trained judges, and the sensory scores were statistically analyzed. The chi-square ($\chi 2$) values for attributes such as flavor, color and appearance, body and texture, sweetness, and overall acceptability were 7.09, 14.32, 15.56, 3.85, and 14.75, respectively (Table 3). The results showed that color and appearance, body and texture, and overall acceptability exhibited significant differences ($p<0.01$), while flavor and sweetness did not differ significantly ($p>0.05$). Found that yogurt samples with 2.5 mg lutein per 120 g serving had a more intense yellow color, which affected their appearance scores. Based on these findings, TL1 (0.5 mg/100g) and TL2 (1.5 mg/100 g) were chosen as the optimal levels for lutein fortification in shrikhand.

Table 3: Effect of different levels of lutein on sensory scores of Shrikhand

Parameters	T1	T2	T3	T4	Chi- square value
Flavour	8.17±0.11	7.83±0.11	7.75±0.11	7.67±0.17	7.09^{ns}
Colour and Appearance	8.00±0.13^{a}	7.83±0.11^{a}	7.75±0.11^{a}	7.00±0.13^{b}	14.32**
Body and Texture	7.92±0.15^{a}	7.67±0.11^{a}	7.50±0.13^{a}	6.83±0.11^{b}	15.56**
Sweetness	8.00±0.13	7.75±0.11	7.67±0.17	7.67±0.11	3.85^{ns}
Overall Acceptability	8.08±0.15^{a}	7.75±0.11^{a}	7.67±0.11^{a}	6.83±0.17^{b}	14.75**

Figures are Mean± Standard error of sensory scores by five selected judges in six replications, $^{a-b}$ Figures in a row with different superscripts differ significantly,**- significant at one per cent level ($p<0.01$), ^{ns}- non significant

Optimization of quantity of tricalcium citrate tetrahydrate, vitamin D3, and lutein by response surface methodology

Diagnostic Check of the Quadratic Model

The sensory responses, including flavor, color and appearance, body and texture, sweetness, and overall acceptability (Table 4), were analyzed through regression analysis to evaluate the impact of tricalcium citrate tetrahydrate (TCT), vitamin D3, and lutein on the sensory attributes of the experimental shrikhand. A second-order polynomial regression model was established to fit the experimental data for each response. The regression models derived from the experimental data demonstrated significance, with observed p-values indicating strong relationships. The partial regression coefficients for linear, quadratic, and interaction terms for each model, along with their R^2 values, are detailed in Table 5.

In the recommended quadratic model, the F-values for all characteristics exceeded the critical F-value ($p<0.01$), confirming the significance of the developed model (Table 5). The coefficients of determination (R^2) for flavor, color and appearance, body and texture, sweetness, and overall acceptability were found to be 0.93, 0.95, 0.91, 0.93, and 0.96, respectively. This indicates that the quadratic model accounted for over 80% of the variability in the data. Additionally, the lack-of-fit test yielded a non-significant result, further confirming the model's authenticity for predicting the sensory properties of experimental shrikhand across varying levels of the specified variables.

The satisfactory precision ratio, with a signal-to-noise ratio greater than four for all five responses, further supports the suitability of the model for guiding the design of the shrikhand formulation.

Table 4: Sensory characteristics of experimental shrikhand with different levels of tricalcium citrate tetrahydrate, vitamin D_3 and lutein

Standard order	Response 1	Response 2	Response 3	Response 4	Response 5
	Flavour	Colour and appearance	Body and Texture	Sweetness	Overall acceptability
1	7.92	8.17	8.17	7.83	8
2	8.17	8.17	7.83	7.83	8.08
3	8	8.08	8	7.83	8
4	8	8.17	8.08	8.08	8.17
5	7.92	8.17	8.25	7.92	7.92
6	7.83	7.75	7.75	8	8.08
7	7.92	7.92	7.75	7.75	7.75
8	7.75	7.92	7.83	8	8.08
9	7.83	8.08	7.75	7.5	7.75
10	7.92	8.08	7.67	7.83	8.08
11	8.08	8.25	8	8	8.17
12	8.08	8.25	8.08	8.17	8.17
13	8.08	8	8.25	7.92	8.08
14	7.83	7.58	8.08	7.83	7.92
15	8.08	8.17	8.17	8	8.08
16	8.17	8.17	8.25	7.92	8.17
17	8.08	8.08	8.08	8	8.08
18	8.17	8.17	8.25	7.92	8.17
19	8.08	8.17	8.08	8	8.08
20	8.17	8.17	8.25	7.92	8.17

Effect on Flavour

The flavour scores, as shown in Table 4, were subjected to analysis, and the partial regression coefficients are listed in Table 5. The response surface plots (Figures 5a to 5c) illustrate how various factors influenced flavour. The model for flavour was found to be significant with an F-value of

15.73 ($p < 0.05$), a non-significant lack of fit, and a coefficient of determination (R^2) of 93%. The response surface equation for flavour is:

Flavour = 8.13 +0.010*A - 0.012*B - 0.080*C - 0.041*AB - 0.064*AC +0.001*BC -0.093*A^2 - 0.021*B^2 - 0.065*C^2

The results suggest that TCT (A) and vitamin D3 (B) have non-significant effects on flavour (p>0.05), while lutein (C) significantly affects flavour ($p<0.01$). The interaction terms AB and AC had significant negative effects, while BC showed a non-significant positive effect. Also found that calcium, vitamin D3, and dietary fiber fortification did not negatively affect sensory acceptance.

Table 5: Estimated parameters of model for sensory attributes and responses of experimental shrikhand

Partial Coefficients	Sensory characteristics				
	Flavour	**Colour and Appearance**	**Body and Texture**	**Sweetness**	**Overall Acceptability**
Intercept	**8.13**	**8.15**	**8.18**	**7.96**	**8.13**
A:Tricalcium citrate tetrahydrate (ppm)	0.010^{ns}	-0.024^{ns}	-0.060*	0.083**	0.095**
B:Vitamin D_3 (IU/L)	-0.012^{ns}	-0.012^{ns}	-0.015^{ns}	0.027^{ns}	-0.006^{ns}
C:Lutein(mg/100g	-0.080**	-0.112**	-0.058*	-0.004^{ns}	-0.050**
AB	-0.041*	0.064**	0.125**	0.053*	0.033*
AC	-0.064**	-0.064**	-0.02^{ns}	0.010^{ns}	0.030*
BC	0.001^{ns}	0.001^{ns}	0.063^{ns}	-0.053*	-0.033*
A^2	-0.093**	-0.024^{ns}	0.166**	-0.097**	0.077**
B^2	-0.021^{ns}	0.036*	-0.050*	0.052**	0.013^{ns}
C^2	-0.065**	-0.127**	-0.006^{ns}	0.023^{ns}	-0.047
Lack of fit	0.78^{ns}	3.35^{ns}	0.88^{ns}	1.82^{ns}	0.15^{ns}
Model F value	15.73*	19.60*	11.45*	14.12*	23.75*
R^2	0.93	0.95	0.91	0.93	0.96
Press	0.10	0.20	0.29	0.15	0.03
Adeq. Press	11.99	17.50	11.40	16.43	16.69

Effect on Colour and Appearance

The colour and appearance scores are summarized in Table 11, and the regression coefficients are in Table 5. The model was significant with an F-value of 19.60 ($p<0.05$), a non-significant lack of fit, and an R^2 of 95%. The surface equation for colour and appearance is:

Colour and appearance = 8.15 -0.024*A - 0.012*B - 0.112*C +0.064*AB - 0.064*AC +0.001*BC -0.024*A^2 + 0.036*B^2 - 0.127*C^2

The results show that lutein (C) has a significant effect ($p<0.01$), whereas TCT (A) and vitamin D3 (B) were non-significant (p>0.05). All ingredients (A, B, and C) negatively impacted the colour and appearance at quadratic levels. The interaction terms AB and BC positively influenced the scores, similar to previous findings where fortifications affected the appearance without compromising acceptance.

Effect on Body and Texture

Table 4 presents the body and texture scores, and Table 5 shows the regression coefficients. The model was significant with an F-value of 11.45 ($p<0.05$), a non-significant lack of fit, and an R^2 of 91%. The equation for body and texture is:

Body and texture = 8.18 -0.060*A - 0.015*B - 0.058*C +0.125*AB - 0.02*AC +0.063*BC +0.166*A^2 - 0.050*B^2 - 0.006*C^2

The analysis revealed that TCT (A) and lutein (C) significantly affected body and texture ($p<0.05$), while vitamin D3 (B) was non-significant (p>0.05). Ingredient A and C negatively influenced body and texture, and the AB interaction had a significant impact on scores.

Effect on Sweetness

Sweetness scores (Table 4) and partial regression coefficients (Table 5) were analyzed, yielding a significant model with an F-value of 14.12 ($p<0.05$), a non-significant lack of fit, and an R^2 of 93%. The response surface equation for sweetness is:

Sweetness = 7.96 +0.083*A +0.027*B - 0.004*C +0.053*AB + 0.010*AC -0.053*BC -0.097*A^2 +0.052*B^2 + 0.023*C^2

The results show that TCT (A) significantly influenced sweetness ($p<0.01$), while vitamin D3 (B) and lutein (C) were non-significant (p>0.05). The AB interaction had a positive effect, while BC negatively impacted sweetness.

Effect on Overall Acceptability

The overall acceptability scores (Table 4) and partial regression coefficients (Table 5) were analyzed using a quadratic model. The model was significant with an F-value of 23.75 ($p<0.05$), a non-significant lack of fit, and an R^2 of 96%. The surface equation for overall acceptability is:

Overall acceptability = 8.13 +0.095*A -0.006*B - 0.050*C +0.033*AB + 0.030*AC -0.33*BC +0.077*A^2 +0.013*B^2 - 0.047*C^2

Both TCT (A) and lutein (C) significantly influenced overall acceptability ($p<0.01$), while vitamin D3 (B) had no significant effect. Positive interactions were observed for AB and AC, with all interaction effects being significant at the 5% level. Found similar results in calcium-fortified products, where sensory acceptability remained high with fortification. The analysis of lutein incorporated calcium and vitamin D3 fortified shrikhand revealed that lutein had the most significant influence on sensory parameters such as flavour, colour, and texture, while TCT had a positive effect on sweetness and overall acceptability. Vitamin D3, on the other hand, had no significant impact on any sensory attribute. Most interaction terms between factors showed significant influences, either positive or negative, indicating the complex relationships between fortification ingredients. These results align with other studies, confirming that fortification does not necessarily compromise sensory qualities.

Optimized Solutions and Validation

Numerical optimization was performed with the aim of obtaining the best formulation of TCT, vitamin D3, and lutein for producing a high-quality lutein incorporated calcium and vitamin D3 fortified shrikhand. During the optimization process, all factors were placed within a specific range, and the sensory attributes, except for sweetness, were maximized (Table 6). The solutions obtained using the RSM software are detailed in Table 7. The desirability of the optimized solution was 0.94, indicating a well-balanced combination of ingredients.

The optimal formulation suggested by RSM was 162.78 ppm of tricalcium citrate tetrahydrate, 558.65 IU/L of vitamin D3, and 0.60% lutein. The predicted sensory scores for the optimized shrikhand were as follows: flavour 8.14, colour and appearance 8.18, body and texture 8.23, sweetness 8.03, and overall acceptability 8.17 (Table 5).

Table 6: Constraints and criteria for optimization of lutein incorporated calcium and vitamin D fortified shrikhand

Constraints	Goal	Lower Limit	Upper Limit
Tricalcium citrate tetrahydrate (ppm)	In range	100	200
Vitamin D (IU/L)	In range	400	600
Lutein(mg/100g)	In range	0.5	1.5
Flavour	Maximize	7.75	8.17
Colour and Appearance	Maximize	7.58	8.25
Body and Texture	Maximize	7.67	8.25
Sweetness	In range	7.5	8.17
Overall Acceptability	Maximize	7.75	8.17

Table 7: Solution obtained after response surface analysis

So. No.	Tricalcium citrate tetrahydrate (ppm)	Vitamin D_3 (IU/L)	Lutein (mg/100g)	Desirability
1	162.77	558.68	0.60	0.943

Verification of the Optimum Formulations

To validate the optimized solution, shrikhand was prepared using the recommended levels of TCT, vitamin D3, and lutein, and the sensory attributes were evaluated. A t-test was conducted to compare the observed sensory values with the predicted values. As shown in Table 8, there was no significant difference (p>0.05) between the observed and predicted values for all sensory attributes, demonstrating that the model was effective in predicting the quality of the lutein incorporated calcium and vitamin D3 fortified shrikhand. The close match between the observed and predicted values confirms the reliability and accuracy of the RSM optimization.

Table 8: Verification of the optimum formulation

Attributes	Predicted value by RSM	Observed value	t-value
Flavour	8.14	8.18±0.05	0.80 ^{ns}
Colour and Appearance	8.18	8.16±0.05	0.37 ^{ns}
Body and Texture	8.22	8.16±0.05	1.06 ^{ns}
Sweetness	8.03	8.06±0.04	0.74 ^{ns}
Overall Acceptability	8.17	8.14±0.04	0.60 ^{ns}

Sensory Evaluation

The mean sensory scores of both control and optimized shrikhand samples were compared using the Mann-Whitney test, with the results summarized in Table 9. The statistical analysis revealed significant differences ($p<0.05$) between the control and optimized shrikhand in terms of colour and appearance, body and texture, and overall acceptability. However, the differences in flavour and sweetness were not statistically significant (p>0.05).

Table 9: Sensory attributes of control and optimized shrikhand

Sensory attributes	Control Shrikhand	Optimized Shrikhand	Mann-Whitney U
Flavour	7.75±0.11	8.05±0.05	21.00^{ns}
Colour and appearance	7.70±0.09	8.10±0.06	23.50*
Body and Texture	7.85±0.06	8.20±0.09	23.00*
Sweetness	7.85±0.06	8.05±0.09	19.50ns
Overall Acceptability	7.80±0.09	8.25±0.08	24.00*

The Mann-Whitney U values for the sensory attributes were as follows: flavour (21.00), colour and appearance (23.50), body and texture (23.00), sweetness (19.50), and overall acceptability (24.00). Across all attributes, the sensory scores for the optimized shrikhand were higher compared to the control, indicating that the optimized formulation enhanced the sensory properties without causing any undesirable effects. This result suggests the feasibility of value addition to shrikhand.

Similar findings were reported by Singh (2003) [15], who observed that dahi prepared from calcium gluconate-fortified cow milk also showed higher sensory scores compared to control dahi. This further supports the potential for fortification to improve sensory qualities in dairy products.

Conclusion

Based on the study, it can be stated that by optimising the additives, dairy products can be fortified without experiencing a substantial change in their sensory qualities.

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