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Optimization of a novel probiotic-fermented pearl millet-based strained yoghurt-like functional dessert: physicochemical, microbial and sensory characterization†

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In this study, we developed a probiotic strained yogurt-like dessert using roasted pearl millet flour and *Hibiscus rosa-sinensis* extract. Ingredients (pearl millet: 2.5–8%, *Hibiscus*: 2.5–8%, and sugar: 25–45%) were optimized for probiotic viability, lactic acidity, sensory properties, and acceptability using Design Expert software. The optimal formulation (pearl millet: 4.86%, *Hibiscus*: 4.4%, and sugar: 29.47%) achieved a probiotic viability of 7.53 ± 0.33 $\log_{10}$ CFU g^{-1}, exceeding the recommended threshold. FTIR analysis revealed enriched nutritional profiles with functional peaks resulting from *Hibiscus* and probiotic metabolism. SEM analysis showed a porous microstructure, indicating functional enhancements due to pearl millet and *Hibiscus*. The dessert exhibited excellent nutritional, functional, and sensory attributes, making it a promising functional food innovation.

Sustainability spotlight

This research marks a significant advance in sustainable food technology by developing a probiotic strained yogurt-like dessert using roasted pearl millet and *Hibiscus rosa-sinensis* extract. Pearl millet, a climate-resilient grain, promotes agricultural biodiversity, while *Hibiscus* extract enhances nutritional value and provides an eco-friendly source. Using a central composite rotatable design (CCRD), the study optimized the formulation to achieve high probiotic viability (>6 $\log_{10}$ CFU g^{-1}), prolonged shelf life (28 days), and superior sensory attributes. This hybrid product combines the health benefits of dairy with plant-based innovation, offering a sustainable solution for large-scale production. The work highlights the potential of millet-based fermented foods in promoting healthy, eco-conscious diets and reducing food waste, with significant market potential.

1. Introduction

Yoghurt is a globally cherished fermented dairy product that is consumed as a probiotics, and is a good source of protein and calcium. It is prepared using the bacterial fermentation of milk, primarily using *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Shrikhand is a traditional sweetened yoghurt based fermented milk delicacy enjoyed in western India, known for its exquisite taste and lustrous, smooth semi-solid texture with a sweet flavor and delightful mouthfeel.[1]

Pearl millet emerges as a promising candidate owing to its robust nutritional profile: it is rich in vital elements such as magnesium, calcium, iron, and dietary fibre. Its incorporation into dairy products offers a transformative avenue for augmenting their nutritional diversity.[2] The nutritional benefits of pearl millet have spurred extensive research into the development of healthy, nutritious foods.[3] This is adeptly integrated into food products such as kheer,[4] kheer mix,[5] paneer,[6] and dairy by-products such as skimmed milk, buttermilk, and whey,[7] offering a transformative avenue for creating value-added products rich in nutritional benefits.

Currently, there has been a surge in interest in innovative functional foods, particularly those promoting digestive health and overall well-being. The rising demand for probiotics highlights their role in improving quality of life amid modern dietary and lifestyle changes.[8] This escalating demand for probiotic-enriched products underscores the significance of incorporating probiotics into daily regimens to enhance the overall quality of life.

Hibiscus rosa-sinensis, known as the rose mallow plant, China rose, gudhal, and japa, is well-known for its excellent antioxidant properties due to the presence of bioactive components such as anthocyanins, flavonoids, and phenolic acids.[9,10] These antioxidants neutralize the free radicals in the body and reduce oxidative stress, thus safeguarding against

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cellular damage.[11] Additionally, the unique color imparted by *Hibiscus* obviates the need for synthetic colors, aligning with consumer preferences for clean-label products. Owing to *Hibiscus*'s antioxidant properties, it is utilized as a functional ingredient in food products.[12]

Although various researchers have developed and characterized functional fermented product by incorporating ingredients such as aloe vera,[13] mango-banana,[14] stevia-carrot pulp,[15] custard apple,[16] ginger-honey,[1] millet vegetable milk,[17] beetroot puree,[18] okra,[19] and *Moringa olefira*,[20] none have ventured into the realm of creating a pearl millet based probiotic shrikhand enriched with *Hibiscus rosa-sinensis* or a similar combination. The combination of pearl millet and *Hibiscus rosa-sinensis* in shrikhand probiotic was undertaken for their complementary nutritional and functional benefits. Consumption of this novel probiotic product will harness the nutritional benefits of both prebiotic rich pearl millet and antioxidant-rich *Hibiscus rosa-sinensis*, thereby giving a synergistic effect. This improved formulation promises to deliver a stable source of probiotics, contributing to improved digestibility, enhanced gut health, bolstered immune function, and overall vitality leveraging sustainable and locally used ingredients.

2. Materials and methods

2.1 Proximate analysis

The moisture (%), fat (%), protein (%), crude fibre (%), sucrose (%), titratable acidity (% lactic acid), and pH were determined according to the protocol given by ref. 1. The antioxidant activity was determined as per the protocol adopted by ref. 12. The concentration of different minerals (*i.e.*, calcium, magnesium, iron, zinc, copper, manganese, and phosphorous) was determined using AAS (ECIL Model-4141, Electronics Corporation of India, India) as per the protocol described by ref. 21.

2.2 Methodology

The changes in responses for probiotic viability count, titratable acidity, color and appearance, body and texture, and overall acceptance were studied with three independent variables, *viz.* concentration of roasted pearl-millet flour (%), concentration of *Hibiscus rosa-sinensis* extract (%), and quantity of sugar (%) using a central composite rotatable design (CCRD). In this design, the actual experimental values were represented into coded values. The study included 19 experimental trials, featuring 5 central point experiments within a design encompassing linear, square, and quadratic interaction terms. This research investigated the impact of incorporating functional ingredients on several parameters: the survival of probiotic counts, lactic acidity, color and appearance, body and texture, and overall acceptance. The goal was to identify the best-fitting model by aiming for higher R^2 and adjusted R^2 values, as these metrics indicate a stronger relationship between the actual and predicted values.[21]

A quadratic polynomial model was applied to the observed results, and the following equation was generated by regression analysis for Y_{1-5} as the following responses:

$$Y_{1-5} = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2 \quad (1)$$

where Y_{1-5} are the responses, survival of probiotic count ($\log_{10}$ CFU g^{-1}), lactic acidity (%), color and appearance (hedonic rating 1–9), body and texture (hedonic rating 1–9), and overall acceptance (hedonic rating 1–9) respectively; b_0 to b_9 are the regression model coefficients.

A – coded values for the concentration of roasted pearl millet flour (%),

B – coded values for the concentration of *Hibiscus rosa-sinensis* extract (%),

C – coded values for quantity of sugar (%).

2.3 Optimization

2.3.1 Probiotic culture and shrikhand curd preparation. Powdered refined sugar and pearl millet grains (86M80 variety), were procured from the local market of Jodhpur, India. Pearl millet grains were sorted and thoroughly washed. Thereafter, grains were boiled (grain to water ratio kept 1 : 1) for 30 minutes. Boiled grains were soaked in water overnight and dried the following day. Milled pearl millet grains were roasted (microwave oven) at 150 °C for 10 minutes and stored at refrigeration temperature (4 °C ± 1 °C). *Hibiscus rosa-sinensis* extract was prepared as per the protocol described by ref. 12. Chr. Hansen probiotic culture Nu-trish® BB-12TM (*Bifidobacterium animalis* subsp. *lactis*, BB-12®), *Lactobacillus bulgaricus* NCDC 09, and *Streptococcus thermophilus* NCDC 74 cultures were used to make shrikhand curd.

2.3.2 Development of pearl millet-based probiotic shrikhand incorporated with *Hibiscus rosa-sinensis* (PPHS). The cell biomass was prepared and activated as per the method followed by ref. 21. The activated culture was used to ferment pasteurized milk (90 °C for 10 minutes) containing 6% fat and 9% SNF. The pasteurized milk was uniformly mixed with roasted pearl millet flour, and the mixture was incubated at 37 °C for 12 h. The prepared curd was hung in a muslin cloth until the entire whey was removed and chakka (strained yoghurt) was obtained. The prepared chakka was kneaded with powdered sugar and then mixed with *Hibiscus* extract. The optimized product was used for bulk production of functional shrikhand (PPHS), packed in 9.5 × 9.5 cm polyethylene terephthalate (PET) cups in hygienic conditions and stored in the refrigerator at 4 °C ± 1 °C. The control sample was prepared using the same method but without the addition of pearl millet flour and *Hibiscus rosa-sinensis* extract. The comparison with the control ensured that the observed functional and sensory attributes of PPHS are specifically due to these two ingredients.

2.4 Measurement of responses

2.4.1 Probiotic viability count. The probiotic count was assessed using modified de Man, Rogosa, and Sharpe (MRS) agar supplemented with 0.05% cysteine hydrochloride to support the growth of *Bifidobacterium* species under anaerobic conditions.[22] The analysis ensured adherence to the essential

requirement for probiotics, which is a minimum count of 6.0 $\log_{10}$ colony forming units (CFU) per gram.

2.4.2 Lactic acidity and sensory attributes. The titratable acidity of milk and milk-based products is expressed as % lactic acidity and determined as per the protocol described by ref. 21. The sensory parameters, *viz.* color and appearance, body and texture, and overall acceptance were recorded on a hedonic scale ranging from 1 to 9 (1 for "dislike extremely" and 9 for "like extremely").[21] The sensory evaluation was conducted with the help of a selected panel of judges, with 30 semi-trained panelists conducting the sensory evaluation for different attributes of the product. The semi-trained panelists were the individuals who received preliminary training in sensory attributes of shrikhand. Additional training was given for palate cleansing to prevent carryover effects.

2.5 Optimization and validation

To obtain the most acceptable functional shrikhand (PPHS), various concentrations of ingredients were tested. The product optimization was based on three parameters: concentration of pearl millet flour (2.5–8%), concentration of *Hibiscus rosa-sinensis* extract (2.5–8%), and quantity of sugar (25–25%). Initial exploratory trials were conducted ranging from 0.5–16% for both pearl millet and *Hibiscus rosa-sinensis* extract to study the impact of ingredients on key quality parameters and sensory acceptance. For pearl millet, a concentration below 2.5% did not provide an appreciable amount of prebiotic substrate for growth of probiotics and more than 8% hindered fermentation. For *Hibiscus rosa-sinensis* extract, appreciable antioxidant activity was recorded above 2.5% and more than 8% reflected a decrease in probiotic count (possibly due to its antimicrobial nature). Thus, the range of 2.5–8% was selected to balance functional benefits and probiotic viability. The responses of these parameters were recorded in terms of probiotic viability count, lactic acidity, color and appearance, body and texture, and overall acceptance. The optimized formulation was determined by evaluating different combinations of parameters using Design Expert version 8.0.7.1. To study the adequacy of the fitted model, the lack of fit values of models was also evaluated.[19] The flow diagram for the preparation of functional shrikhand (PPHS) is shown in Fig. 1. The following criteria were considered for the optimization and selection of the product:

1. Probiotic viability counts greater than 6.0 $\log_{10}$ CFU g^{-1}.
2. Lactic acidity less than 1.4%.
3. Color and appearance (maximum score on 9-point hedonic scale).
4. Body and texture (maximum score on 9-point hedonic scale).
5. Overall acceptance (maximum score on 9-point hedonic scale).

2.6 Characterization of optimized product

2.6.1 Fourier transform infrared spectroscopy (FTIR). Fourier transform infrared spectroscopy (FTIR) analysis was conducted to identify the functional groups present in the functional shrikhand. The FTIR spectra were recorded using the KBr method in FTIR spectrometer (PerkinElmer Spectrum 100). The spectra of the samples were recorded between 4000–400 cm^{-1} with 25 scans and 4 cm^{-1} resolution. The FTIR spectra were plotted in OriginPro software (Version 2023).

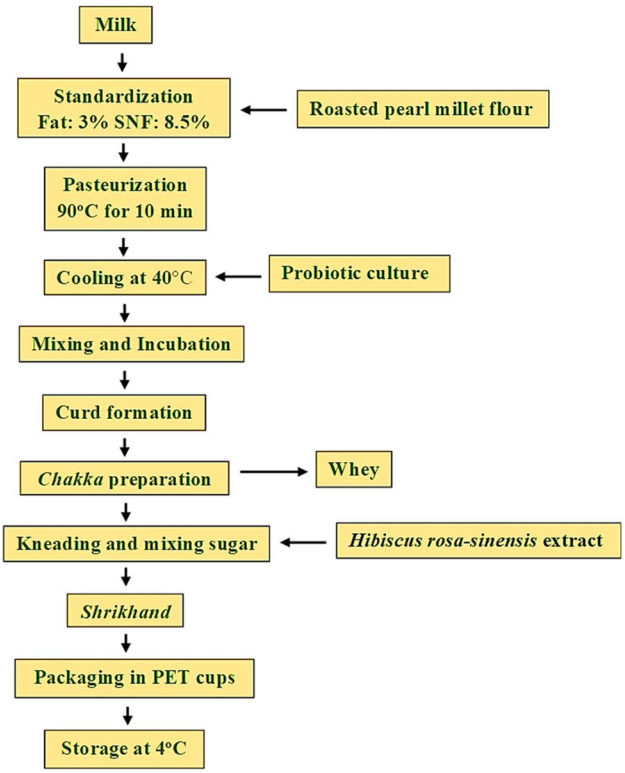

Fig. 1 Flow diagram for the preparation of pearl millet based probiotic shrikhand incorporated with *Hibiscus rosa-sinensis* extract.

2.6.2 Surface morphology. Surface morphology was studied to understand microstructure, texture, and consistency of functional shrikhand (freeze-dried) using a scanning electron microscope (SEM) (Model: Quanta 200 camera, Make: Thermo Fisher Scientific, USA). The sample was mounted on an aluminum stub using carbon conductive tape as an adhesive. The mounted sample was visualized under the microscope at an accelerating voltage of 20 kV. Observations were made at 1000×, 2000×, and 5000×.

2.6.3 Color analysis. Color analysis was performed to determine the color parameters of the functional shrikhand using a Hunter Lab ColorFlex spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA).[23] The color of the shrikhand was evaluated in terms of L^* (lightness), a^* (red-green), and b^* (yellow-blue) values. The hue angle and chroma were determined using the following formulas:[21]

$$\text{Hue angle } (H^\circ) = \tan^{-1}(b/a) \quad (2)$$

$$\text{Chroma} = (a^2 + b^2)^{1/2} \quad (3)$$

2.7 Storage studies

2.7.1 Physicochemical analysis. The titratable acidity (% lactic acid), pH, and total solids (%) were determined on the

0th, 7th, 14th, 21st, 28th and 35th day of storage. The pH analysis was measured using a HM digital pH-80 pH meter calibrated using a standard buffer of pH 7.0, and titratable acidity was measured using a previous protocol.[24] Total solids were determined as per the protocol used by ref. 18.

2.7.2 Microbial analysis. Probiotic counts, coliform count, standard plate count (SPC), and yeast and mold counts were conducted on the 0th, 7th, 14th, 21st, 28th, and 35th day of storage as per the protocol given by ref. 1.

2.7.3 Sensory analysis. The sensory evaluation was conducted with the help of a selected panel of judges and 30 semi-trained persons using a 9-point hedonic scale, as suggested by ref. 21.

2.8 Statistical analysis

The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test to determine significant differences between groups using IBM SPSS Statistics software, version 16.0. For all calculations, each parameter in the tests was measured in triplicate ($n = 3$) to ensure the reliability and reproducibility of the results. The values are expressed as mean ± standard deviation (SD), providing a clear representation of the data's variability.

3. Results and discussion

3.1 Proximate analysis of pearl millet flour and *Hibiscus rosa-sinensis* extract

The roasted pearl millet flour had 11.83% ± 0.6%, 5.91% ± 0.18%, 7.87% ± 0.03%, 4.46% ± 1.17%, and 2.32% ± 0.35% moisture, fat, protein, crude fibre, and ash content, respectively. The antioxidant activity of flour was found to be 44.68% ± 1.58%. The flour was free from any visible dust and abnormal smell. The *Hibiscus rosa-sinensis* extract used in the present study was prepared from fresh *Hibiscus rosa-sinensis* petals that contained 89.27% ± 0.68%, 0.06% ± 0.01%, 1.48% ± 0.08%, 0.42% ± 0.07%, and 1.43% ± 0.03% moisture, protein, crude fibre, fat, and ash, respectively. The antioxidant activity of the extract was 60.34% ± 1.24%. The extract has a pleasant smell resembling that of *Hibiscus* flowers.

3.2 Effect of independent variables on responses

The effect of independent variables *viz.* concentration of roasted pearl-millet flour (%), concentration of *Hibiscus rosa-sinensis* extract (%), and quantity of sugar (%) and observed experimental values on the five responses (probiotic viability count, lactic acidity, color and appearance, body and texture, and overall acceptance) are tabulated in Table 1.

The influence of independent variables on the responses was assessed using mean comparisons, aiming to observe higher *F*-values to reject the null hypothesis. The probiotic viability count ranged from 7.41 to 7.59 $\log_{10}$ CFU g^{-1}. It was significantly influenced by the concentration of *Hibiscus rosa-sinensis* extract (*B*) and the quantity of sugar (*C*) in linear terms, while the concentration of roasted pearl millet flour (*A*) showed a non-significant effect. Notably, all three independent variables significantly affected probiotic viability in their quadratic terms, highlighting the importance of quadratic effects. Interestingly, pearl millet flour and *Hibiscus rosa-sinensis* extract acted as prebiotics at lower concentrations, supporting microbial

Table 1 Effect of independent variables and observed experimental values of responses

	Independent variables			Responses[a]				
Trial run	Pearl-millet flour (*A*)	*Hibiscus rosa-sinensis* extract (*B*)	Sugar (*C*)	Y_1	Y_2	Y_3	Y_4	Y_5
1	0.63	5.25	35	5.95	0.83	5.74	6.42	5.3
2	2.5	2.5	25	6.86	0.69	6.2	6.76	5.8
3	2.5	8	45	5.22	0.57	4.9	5.9	5.68
4	2.5	2.5	45	7.16	0.83	5.82	5.6	6.3
5	2.5	8	25	5.65	0.73	4.3	5.5	6.43
6	5.25	0.63	35	6.31	0.94	6.24	6.16	6.88
7	5.25	5.25	35	7.49	1.04	6.46	7.5	7.64
8	5.25	5.25	35	7.38	1.09	6.11	7.6	7.38
9	5.25	9.87	35	5.44	0.53	4.4	6.3	5.63
10	5.25	5.25	35	7.54	1.18	6.29	7.8	7.5
11	5.25	5.25	51.82	6.25	0.81	4.2	6.52	4.56
12	5.25	5.25	35	7.46	1.18	6.21	7.4	6.94
13	5.25	5.25	35	7.23	1.08	6.87	8.5	7.26
14	5.25	5.25	18.18	7.24	1.11	5.5	5.4	6.24
15	8	8	25	6.69	0.79	5.02	5.8	5.58
16	8	2.5	25	6.61	0.93	5.84	6.2	4.33
17	8	8	45	5.68	0.75	3.89	5.33	4.89
18	8	2.5	45	5.72	0.72	4.47	5.8	5.43
19	9.87	5.25	35	5.58	0.52	4.12	5.76	4.88

[a] Where Y_{1-5} are the responses, survival of probiotic count ($\log_{10}$ CFU g^{-1}), lactic acidity (%), color and appearance (hedonic rating 1–9), body and texture (hedonic rating 1–9), and overall acceptance (hedonic rating 1–9) respectively.

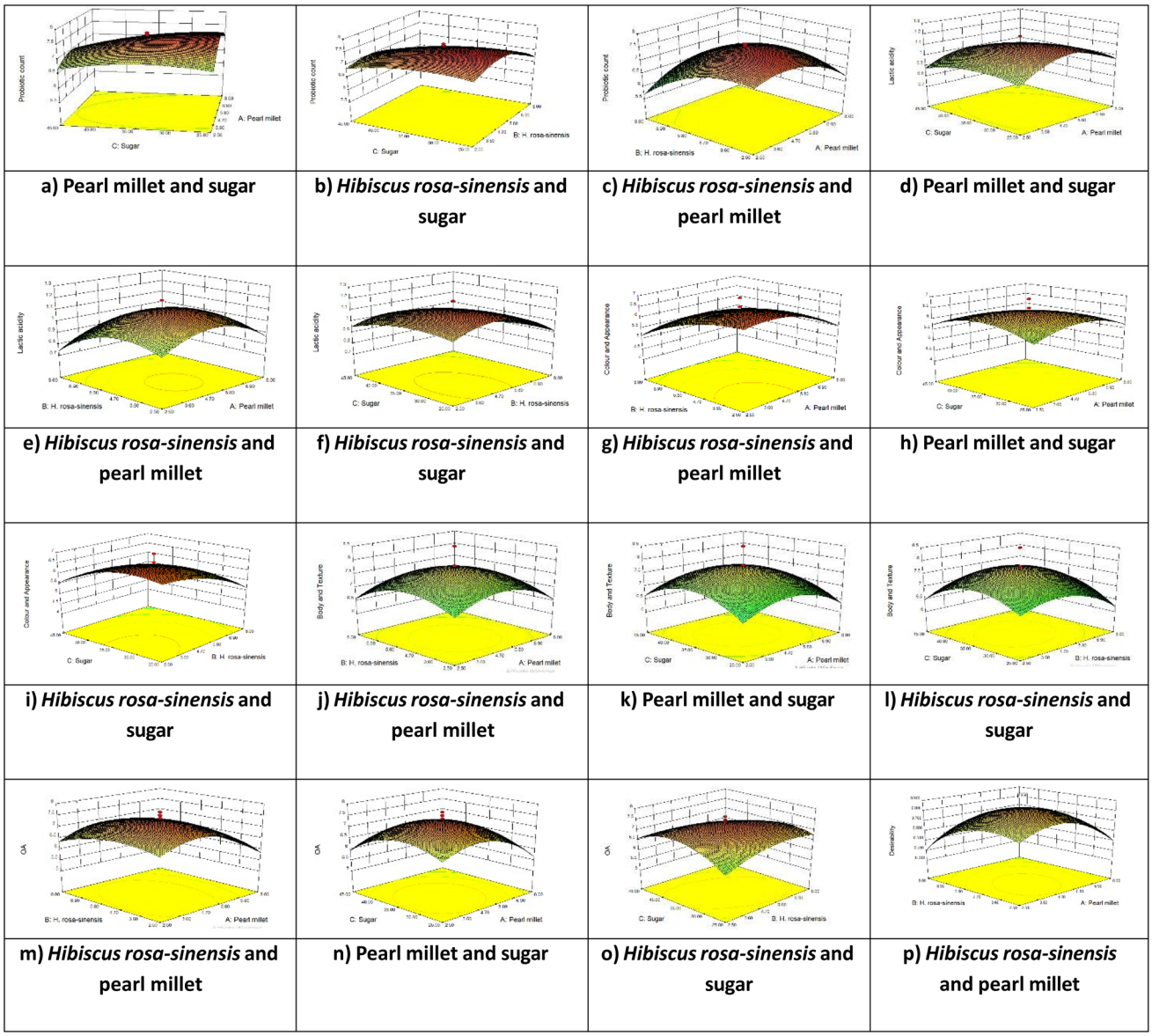

Fig. 2 Effect of independent variables on probiotic viability count (a)–(c), lactic acidity (d)–(f), color and appearance (g)–(i), body and texture (j)–(l), overall acceptance (m)–(o) and desirability (p).

growth and enhancing probiotic viability. However, they exhibited inhibitory effects on microbial growth at higher concentrations, as depicted in Fig. 2. This shift underscores the need to optimize concentrations to maximize probiotic viability and functionality in the formulation. Additionally, the significant interactions between *AB* and *AC* indicate a complex interplay between these variables, affecting probiotic viability. The results agreed with previous studies on raabadi powder,[21] lassi powder,[25] and yoghurt powder.[26]

The titratable acidity of the sample ranged from 1.08% to 1.14% lactic acid, indicating a narrow fluctuation in acidity levels. The model analysis revealed that the independent variables—roasted pearl-millet flour and *Hibiscus* extract—did not significantly affect the acidity in their linear or interaction terms, with only the quadratic terms (A^2 and B^2) being significant. This suggests that the quantities of pearl millet flour and *Hibiscus* extract had a more complex, non-linear impact on acidity. The buffering effect of pearl millet flour, likely due to its starch content, may have helped maintain stable acidity by preventing drastic pH changes. Similarly, the acidic compounds in *Hibiscus* extract, such as citric acid and anthocyanins, likely contributed to the observed acidity, with the quadratic significance indicating that the effect of *Hibiscus* extract on acidity becomes more pronounced at higher concentrations. The lack of significant linear and interaction effects implies that acidity remains relatively stable across varying levels of pearl millet flour and *Hibiscus* extract, with their effects becoming more evident at certain concentrations, thereby contributing to the overall stability of the product's acidity. The results of the study are in consonance with previous studies on tiger nut-based yoghurt[27] and fibre-enriched shrikhand.[28]

The color and appearance scores varied from 5.95 to 6.73 and were significantly affected by all three independent variables (*A*, *B*, and *C*) in linear terms, as well as the quadratic terms A^2, B^2, and C^2. Additionally, the interaction term *AC* was significant. This indicates that the concentration of roasted pearl millet flour, *Hibiscus rosa-sinensis* extract, and sugar all play crucial roles in determining the color and appearance of the product. The significant interaction between *A* and *C* suggests that these variables jointly influence color and appearance, likely due to their combined effects on the physical and chemical properties of the mixture. This finding is consistent with the studies on ginger-honey shrikhand,[1] inulin, psyllium, and partially hydrolyzed guar gum shrikhand,[29] and tiger nut-based yogurt,[27] where similar interactions between ingredients were observed to affect the color and sensory properties of dairy products, highlighting the role of ingredient combinations in influencing product characteristics.

The body and texture score ranging from 7.08 to 8.45 were significantly influenced by the quadratic terms A^2, B^2, and C^2, while the linear and interaction terms did not show significant effects. This suggests that the higher concentrations of the independent variables affect the body and texture of the product. The non-significant linear and interaction effects imply that these attributes are more sensitive to larger deviations in the concentrations of the independent variables. The results are consistent with the findings of ginger-honey shrikhand,[1] soy yogurt,[30] peanut-based yogurt-like fermented products,[31] and sugar-free yogurt.[32]

The overall acceptance score varied from 6.67 to 8.01 and was significantly influenced by the linear terms of the quantity of roasted pearl-millet flour (*A*) and the quadratic terms A^2, B^2, and C^2. The significant linear effect of *A* and its quadratic term suggests that both the amount and the squared amount of roasted pearl millet flour substantially affect overall acceptance. The significance of quadratic terms for all three variables highlights the importance of optimizing concentrations to achieve the best overall acceptance. Similar results were reported for ginger-honey shrikhand,[1] inulin, psyllium, and partially hydrolyzed guar gum shrikhand,[29] fibre enriched shrikhand,[28] and synbiotic soy yoghurt.[30]

The substantial R^2 values (ranging from 0.8268 to 0.9770) and adjusted R^2 values (0.6535 to 0.9540) reflect that the models capture significant variability in the responses. Nonetheless, the predicted R^2 values suggest potential overfitting or the need for further refinement of the models, particularly for lactic acidity, body and texture, and overall acceptance. The significant *p*-values for the models indicate that the relationships between the independent variables and responses are statistically significant. In essence, the study revealed that the concentration of roasted pearl millet flour, *Hibiscus rosa-sinensis* extract, and sugar significantly affect the probiotic viability count, lactic acidity, color and appearance, body and texture, and overall acceptance of the product. The quadratic terms of these variables significantly influence the responses, highlighting the necessity to optimize their levels to achieve desirable product characteristics. Table 2 presents the analysis of variance (ANOVA) and model coefficients detailing the influence of the independent variables—concentration of pearl millet, *Hibiscus rosa-sinensis* extract, and sugar—on the responses. The effect of these independent variables on the various responses is described by the following quadratic polynomial equations:

$$\text{Probiotic count} = +7.41 - 0.059A - 0.34B - 0.27C + 0.40AB - 0.22AC - 0.11BC - 0.55A^2 - 0.52B^2 - 0.21C^2 \quad (4)$$

$$\text{Lactic acidity (\%)} = +1.11 - 0.011A - 0.075B - 0.057C + 0.014AB - 0.029AC - 0.016BC - 0.16A^2 - 0.14B^2 - 0.058C^2 \quad (5)$$

Table 2 ANOVA and model coefficients[a]

	$Y_i = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$				
Parameters	Probiotic count (log CFU g^{-1})	Lactic acidity (%)	Color and appearance (hedonic rating 1–9)	Body and texture (hedonic rating 1–9)	Overall acceptance (hedonic rating 1–9)
Intercept	7.41	1.11	6.38	7.77	7.34
A	−0.05948^{ns}	−0.01105^{ns}	−0.34604*	−0.12743^{ns}	−0.3434*
B	−0.33504*	−0.07467^{ns}	−0.53582*	−0.11688^{ns}	−0.10114^{ns}
C	−0.27055*	−0.05671^{ns}	−0.32703*	0.018585^{ns}	−0.19518^{ns}
AB	0.39875*	0.01375^{ns}	0.1775^{ns}	0.01125^{ns}	0.0875^{ns}
AC	−0.22125*	−0.02875^{ns}	−0.34*	−0.01375^{ns}	0.0825^{ns}
BC	−0.10625^{ns}	−0.01625^{ns}	0.1525^{ns}	0.18625^{ns}	−0.38^{ns}
A^2	−0.55403*	−0.1595*	−0.49397*	−0.61925*	−0.78093*
B^2	−0.51506*	−0.13824*	−0.35579*	−0.56965*	−0.36816*
C^2	−0.20639*	−0.05837^{ns}	−0.52113*	−0.66367*	−0.66954*
R^2	0.9770	0.8268	0.9576	0.8424	0.8737
Adj R^2	0.9540	0.6535	0.9153	0.6848	0.7475
Pred R^2	0.8500	−0.2507	0.8066	0.0738	0.1276
p value	<0.0001	0.0146	<0.0001	0.0100	0.0041
F value	42.46	4.77	22.6	5.35	6.92

[a] *p-value < 0.05, significant, ns = non-significant.

$$\text{Color and appearance} = +6.38 - 0.35A - 0.54B - 0.33C + 0.018AB - 0.34AC + 0.15BC - 0.49A^2 - 0.36B^2 - 0.52C^2 \quad (6)$$

$$\text{Body and texture} = +7.77 - 0.13A + 0.12B + 0.019C + 0.011AB - 0.014AC + 0.19BC - 0.62A^2 - 0.57B^2 - 0.66C^2 \quad (7)$$

$$\text{Overall acceptance} = +7.34 - 0.34A - 0.10B - 0.20C + 0.088AB + 0.082AC - 0.38BC - 0.78A^2 - 0.37B^2 - 0.67C^2 \quad (8)$$

The effect of independent variables on probiotic viability count, lactic acidity, color and appearance, body and texture, and overall acceptance is plotted using 3D surface response plots and shown in Fig. 2.

3.3 Optimization

The optimal levels of functional ingredients were established through numerical optimization following model fitting. Independent variables and responses were rated on a scale from 1+ for the least desirable to 5+ for the most desirable, with the probiotic viability count being prioritized with a rating of 5+. The model's accuracy was then confirmed by performing experiments in triplicate under the optimized conditions. The optimized conditions were determined to be a concentration of pearl millet flour at 4.86%, *Hibiscus rosa-sinensis* extract at 4.4%, and sugar at 29.47%. The optimized predicted values and corresponding experimental values are presented in Table 3. The predicted probiotic count was 7.53409 $\log_{10}$ CFU g^{-1}, with an experimental mean value of 7.28 ± 0.33 $\log_{10}$ CFU g^{-1}. The predicted lactic acidity was 1.13204%, while the experimental mean value was 1.05 ± 0.07%. For color and appearance, the predicted hedonic rating was 6.58286, compared with an experimental mean of 6.49 ± 0.14. The predicted value for body and texture was 7.57034, while the experimental mean was 7.36 ± 0.18. Lastly, the overall acceptance had a predicted value of 7.21846, with an experimental mean of 6.96 ± 0.21.

These optimized conditions and the subsequent experimental validation highlight the robustness and reliability of the model in predicting the desired responses. The good agreement between the predicted and experimental values confirms the efficacy of the optimization process. These findings are consistent with previous studies on raabadi powder,[21] ginger-honey shrikhand,[1] inulin, psyllium, partially hydrolyzed guar gum shrikhand,[29] fibre enriched shrikhand,[33] lassi powder,[25] tiger nut-based yoghurt,[27] soy yoghurt,[30] peanut-based yoghurt-like fermented product,[31] and sugar-free yoghurt.[32]

3.4 Proximate analysis of control and PPHS samples

The total solid content of the PPHS sample increased significantly (68.46% ± 0.70%) compared with the control sample (57.34% ± 0.55%). A similar increasing trend was observed for protein, fat, carbohydrates, and ash, wherein the increase was about 26.94%, 34.36%, 0.75%, and 11.39%, respectively, compared with the control sample. The significant improvement in nutritional profiling of functional shrikhand was attributed to the supplementation of pearl millet flour and *Hibiscus rosa-sinensis* extract.

The titratable acidity (% lactic acidity) of the PPHS sample increased significantly (0.92% ± 0.06%) compared with the control sample (0.86% ± 0.03%), reflecting a rise of 6.98%. Antioxidant activity (% DPPH), which was absent in the control sample, was observed at 42.35% ± 0.05% in the PPHS sample. A slight decrease in the pH value was observed in the PPHS (pearl millet-based probiotic shrikhand incorporated with *Hibiscus rosa-sinensis*) sample (4.3 ± 0.23) compared with the control (plain shrikhand) (4.5 ± 0.21). This decrease can be attributed to the enhanced fermentation activity of the probiotics, which produce organic acids, particularly lactic acid, during storage. Additionally, the incorporation of *Hibiscus rosa-sinensis*, which contains organic acids such as citric acid, may have contributed to the overall acidification of the PPHS sample, resulting in a slight reduction in pH compared with the control. The crude fibre content, which was absent in the control sample, was detected at 5.89% ± 0.04% in the PPHS sample. The mineral content showed significant enhancements in the PPHS sample compared with the control. Calcium increased by 22.33% (220.34 ± 5.88 mg kg^{-1} *vs.* 180.13 ± 4.56 mg kg^{-1}), magnesium by 66.38% (150.26 ± 0.5 mg kg^{-1} *vs.* 90.33 ± 6.34 mg kg^{-1}), iron by 424.89% (12.23 ± 6.03 mg kg^{-1} *vs.* 2.33 ± 6.05 mg kg^{-1}), zinc by 106.98% (5.33 ± 6.31 mg kg^{-1} *vs.* 2.58 ± 7.45 mg kg^{-1}), copper by 300% (1.44 ± 7.02 mg kg^{-1} *vs.* 0.36 ± 5.27 mg kg^{-1}), manganese by 406.73% (2.63 ± 0.05 mg kg^{-1} *vs.* 0.52 ± 0.02 mg kg^{-1}), and phosphorus by 47.22% (295.76 ± 5.02 mg kg^{-1} *vs.* 200.93 ± 3.04 mg kg^{-1}). The antioxidant activity of the PPHS sample was found to be 42.35% ± 0.05%, which is a substantial improvement compared with the control sample, which exhibited no detectable antioxidant activity (0.0% ± 0.00%). The improvement in shrikhand's properties of acidity, antioxidant activity, crude fibre, mineral content, and overall functionality

Table 3 Optimized processing conditions of responses

S. no.	Response	Predicted value	Experimental value (mean ± SD, $n = 3$)	% error
1	Probiotic count ($\log_{10}$ CFU g^{-1})	7.53409	7.28 ± 0.33	3.37
2	Lactic acidity (%)	1.13204	1.05 ± 0.07	7.24
3	Color and appearance (hedonic rating 1–9)	6.58286	6.49 ± 0.14	1.41
4	Body and texture (hedonic rating 1–9)	7.57034	7.36 ± 0.18	2.78
5	Overall acceptance (hedonic rating 1–9)	7.21846	6.96 ± 0.21	3.44

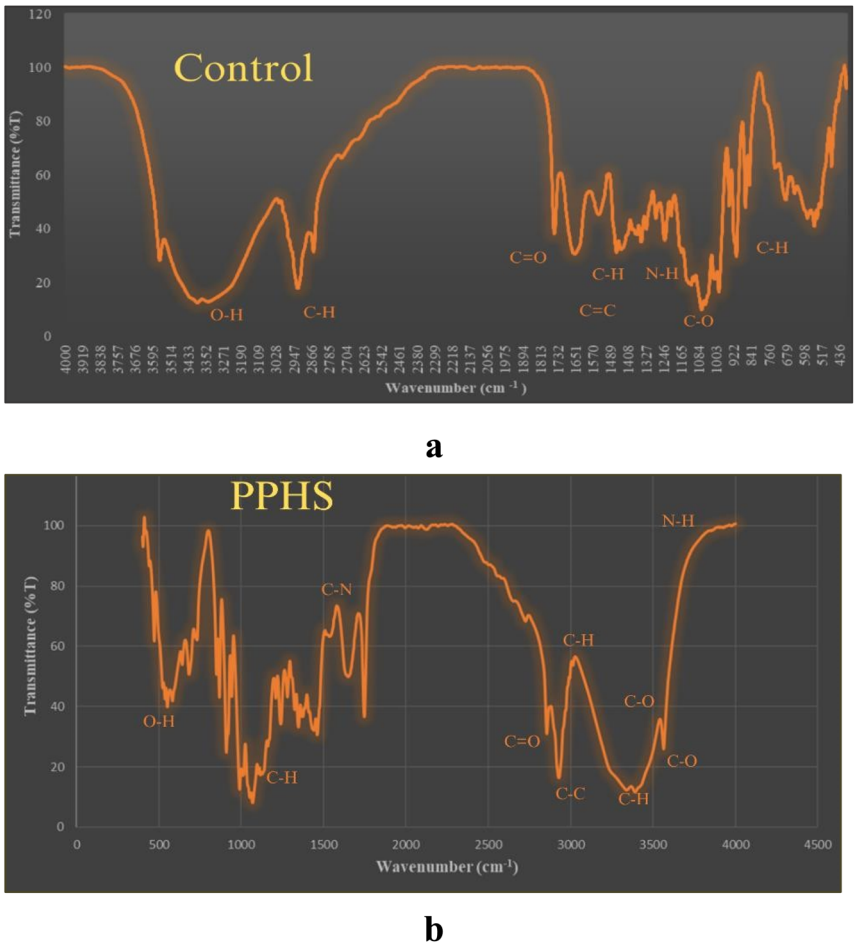

Fig. 3 FTIR spectra of shrikhand samples showing characteristic peaks corresponding to functional groups. (a) Control shrikhand sample with peaks at 3700–3200 cm^{-1} (–OH stretching), 3400 cm^{-1} (–NH stretching), 3000–2800 cm^{-1} (–CH stretching), 1730 cm^{-1} (–C═O stretching), and 1650–1500 cm^{-1} (amide bands), indicating the presence of moisture, proteins, and fatty acids; (b) PPHS sample exhibiting similar peaks with an additional peak at 1400 cm^{-1} attributed to *Hibiscus* extract or probiotic by-products, and peaks in the range of 1200–1000 cm^{-1} (C–O, C–C stretching for carbohydrates) and 900–700 cm^{-1} (C–H bending for organic molecules), indicating the enriched nutritional profile from pearl millet, *Hibiscus*, and probiotics.

is due to the addition of *Hibiscus rosa-sinensis* extract and pearl millet flour. The *Hibiscus* extract is rich in anthocyanins, flavonoids, and phenolic acids, and plays a key role in enhancing its nutritional and therapeutic value.

3.5 Product characterization

3.5.1 FTIR analysis. The FTIR spectra of control and PPHS samples are presented in Fig. 3. The FTIR spectra were recorded to evaluate the chemical composition and functional groups of samples.

The FTIR spectra of both samples are nearly similar, except for a few functional differences found in the case of functional dessert. The FTIR peak observed in both the samples at 3700–3200, 3400, 3000–2800, 1730, and 1650–1500 cm^{-1} may be due to stretching vibrations of –OH (due to the presence of moisture), –NH (due to protein), –CH (due to fatty acids), –C═O (due to fats and proteins) and amide bands of functional groups, respectively. The additional functional peak at 1400 cm^{-1} in the PPHS sample may be due to the fortification of *Hibiscus* extract or the formation of by-products by probiotic bacteria. In addition, the peaks in the 1200–1000 cm^{-1} range correspond to C–O and C–C stretching, which indicates the carbohydrate content of samples, and the peak at 900–700 cm^{-1} is due to C–H bending vibrations that represent the organic molecules of samples. These differences highlight the enriched nutritional profile of PPHS shrikhand, attributed to the pearl millet, *Hibiscus*, and probiotic cultures.

The present study aligns with previous studies on non-fat yoghurt gel syneresis by incorporating heat-unfolded whey proteins,[34] sea buckthorn enriched probiotic yoghurt,[29] yoghurt prepared from different commercial starters[35] and inulin extract from chicory based low-fat synbiotic yoghurt.[36]

3.5.2 Surface morphology. The SEM analysis clearly illustrated that incorporating pearl millet and *Hibiscus rosa-sinensis* into shrikhand significantly alters its microstructure. While the control sample maintained a smooth, dense, and uniform protein matrix, the PPHS sample exhibited a more heterogeneous and porous structure. These microstructural variations

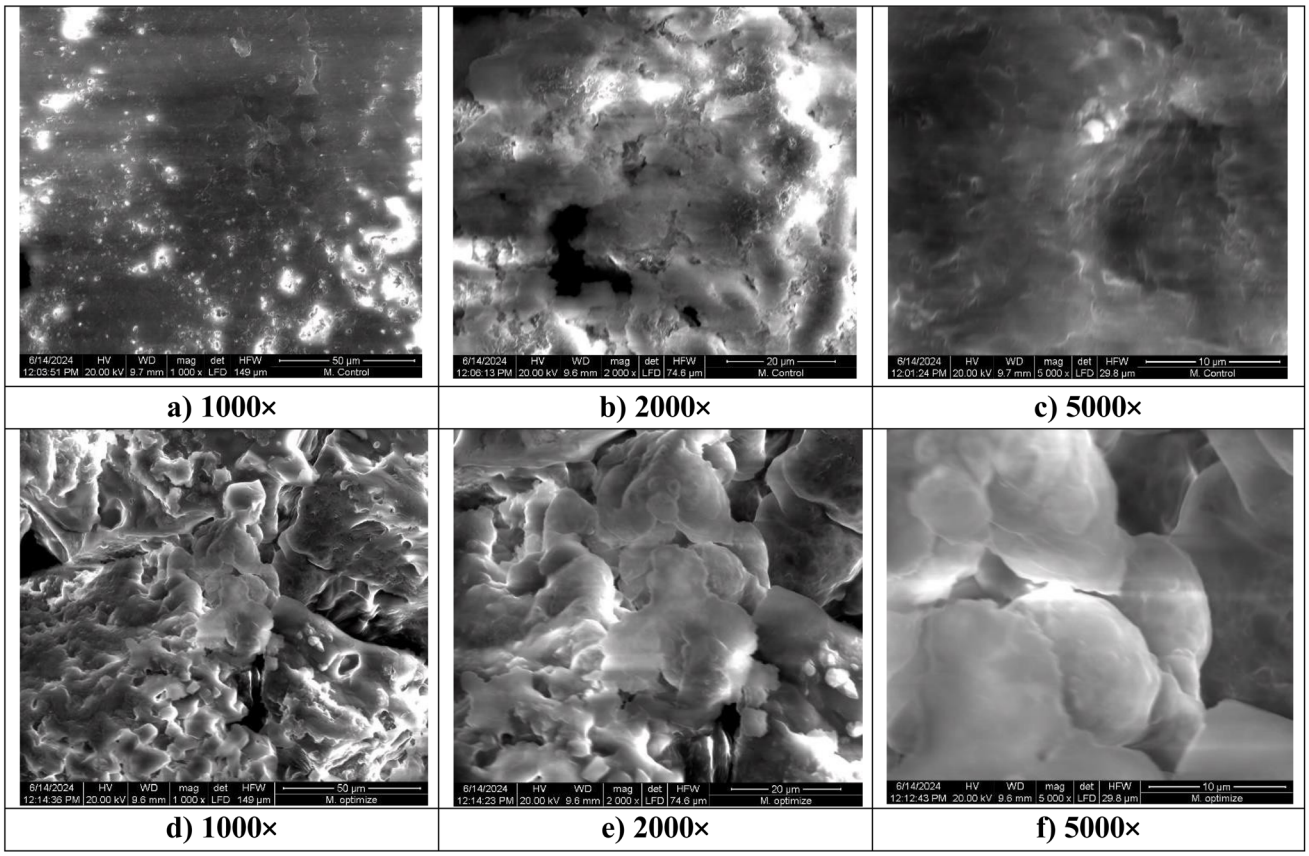

Fig. 4 Scanning electron microscope (SEM) images of shrikhand samples highlighting microstructural differences. (a)–(c) Control sample at 1000×, 2000×, and 5000× magnifications, showing a smooth, dense, and uniform protein matrix; (d)–(f) PPHS sample at 1000×, 2000×, and 5000× magnifications, revealing a heterogeneous and porous structure due to the incorporation of pearl millet and *Hibiscus rosa-sinensis*, along with visible fibres and bioactive compounds, resulting in a less compact protein network with increased air pockets, which influence textural and functional properties.

remain important as they directly influence the textural attributes and potentially the organoleptic characteristics of the product. SEM analysis at 1000, 2000, and 5000× evidently demonstrated that the inclusion of pearl millet and *Hibiscus* introduced beneficial fibres and bioactive compounds, thereby enhancing the nutritional profile and functional properties of the product. However, these additions resulted in a less compact protein network and an increased presence of air pockets, which can be seen in Fig. 4. The porous and less compact texture of PPHS is likely to result in a softer and lighter mouthfeel with improved spreadability, making it more palatable. The presence of fibres make texture slightly chewy and grainy. These findings are consistent with previous studies on raabadi powder,[21] protein glutaminase-based yogurt,[37] sweet potato-based yogurt,[38] and inulin extract from chicory based low-fat synbiotic yogurt.[39]

3.5.3 Color analysis. Color analysis depicted that the L^* value decreased from 95 ± 0.2 in the control sample to 23.51 ± 0.1 in the PPHS sample, the a^* value increased from 0.5 ± 0.05 to 18.89 ± 0.03, and the b^* value increased from 1.3 ± 0.5 to 4.5 ± 0.02. Chroma values also increased from 1.39 to 19.42, while the hue angle decreased from 68.96° to 13.40°, which is due to the incorporation of dark pigments from pearl millet and *Hibiscus rosa-sinensis*. These findings are in agreement with previous studies on raabadi powder[19] and functional shrikhand.[18]

3.6 Studies on storage

3.6.1 Chemical analysis. Over 35 days, PPHS consistently showed higher titratable acidity compared with the control, starting at 0.92% ± 0.06% and reaching 1.58% ± 0.02%, indicating enhanced fermentation due to prebiotic and probiotic components. This increase in acidity correlated with a progressive decline in pH, which occurred more rapidly in PPHS, reflecting increased acid production by probiotics and other fermentative microbes. Total solids were higher in PPHS from the start (68.46% ± 0.07%) and remained stable, suggesting minor moisture changes. This stability in total solids indicates that there was minimal moisture loss in the fermentation process, likely due to the relatively low rate of evaporation and the absence of significant added water. The fermentation primarily led to the production of fermentation byproducts, such as lactic acid, without causing a notable reduction in the non-volatile components, maintaining a steady total solid content. The pH levels decreased over time, with PPHS dropping more rapidly, reflecting increased acid production. Studies

done on 35th day of storage reflected formation of lactic acid due to bacterial action. Thus, the pH levels declined concomitantly from 4.30 ± 0.23 (0 day) to 3.7 ± 0.05 (35 day). The findings of current study align with previous studies.[1,12,23,40]

3.6.2 Microbial analysis. Microbial counts in functional shrikhand during storage are critical for assessing its microbiological stability, safety, and shelf life. Standard plate count (SPC), yeast and mold count, and probiotic viability influence product quality. Increased microbial load can indicate spoilage, while a decline in probiotic count affects functional benefits. Microbial populations exhibited significant changes over time. The standard plate count (SPC) increased from 2.85 ± 0.02 to 7.38 ± 0.03 $\log_{10}$ CFU g^{-1}, indicating active microbial metabolism, which corresponded to rising titratable acidity and decreasing pH. Coliforms were detected by day 35 (3.78 ± 0.05 $\log_{10}$ CFU g^{-1}), suggesting possible post-processing contamination. Yeast and mold counts showed a marked increase after day 21, reaching 2.89 ± 0.05 $\log_{10}$ CFU g^{-1} by day 35, signaling spoilage and reduced product stability. Initial probiotic viability counts in the optimized samples were found to be 7.53 ± 0.33 $\log_{10}$ CFU g^{-1}. During the storage, there was no significant ($P < 0.05$) reduction in probiotic counts up to 14 days, but they decreased significantly after 21, 28, and 35 days of storage. The probiotic counts for 28 and 35 days of storage were found to be 6.08 ± 0.03 and 5.84 ± 0.04 $\log_{10}$ CFU g^{-1}, respectively. The shelf life of PPHS was considered 28 days because counts reduced below the acceptable limit of 6 $\log_{10}$ CFU g^{-1} after this time period. The decline in probiotic viability was likely due to increased acidity, which creates a less favorable environment, and competition from other microbial populations. Initially the coliform and yeast and mold counts were not reported until the 14th day; however, the increased counts on further evaluation could be due to possible contamination while packaging (since the manual packaging method was adopted). Environmental factors such as temperature fluctuations, humidity, and oxygen exposure likely influenced the microbial growth. Temperature variations could have accelerated microbial metabolism, affecting the rate of acid production and pH decline. Oxygen exposure may have contributed to oxidative reactions, particularly affecting bioactive components from *Hibiscus rosa-sinensis*, potentially leading to altered antioxidant properties over time. Additionally, humidity control was critical in preventing unwanted moisture absorption, which could otherwise lead to variations in water activity, impacting microbial stability and metabolic activity. These findings indicate that PPHS maintained its functional and chemical integrity for up to 28 days, while further storage beyond this period led to increased acid accumulation, which may have implications for sensory acceptability and probiotic viability. Strict refrigeration, oxygen barrier packaging, and humidity-controlled storage could further enhance product stability and extend shelf life. These trends align with findings from previous studies,[1,12,23,35,36] which similarly reported microbial dynamics, spoilage indicators, and challenges to probiotic viability in fermented dairy products over extended storage periods.

3.6.3 Sensory analysis. Sensory evaluation is crucial for quality control, product development, and consumer preference analysis. The sensory attributes of the product declined over 35 days. Color and appearance scores dropped from 6.49 ± 0.14 to 5.12 ± 0.06, and body and texture from 7.36 ± 0.18 to 5.34 ± 0.06, reflecting microbial growth with concomitant chemical changes. Sweetness decreased from 7.64 ± 0.06 to 5.79 ± 0.06 due to sugar conversion by microorganisms. Flavor scores fell from 8.07 ± 0.06 to 6.07 ± 0.05, linked to acidic and off-flavor compounds. Overall acceptance declined from 6.96 ± 0.21 to 5.23 ± 0.06. Due to a significant reduction in the L^* value (lightness), consumers showed less interest in accepting the product because of their habitual behavior to consuming light-colored shrikhand. However, some consumers showed keen interest in the new formulation and considered the color attractive. A 35 day analysis clearly showed that the shelf life of the product could be up to 28 days as a significant reduction in viable microbes occurs after 28 days. This is supported by the proliferation of coliforms and yeast and mold results after this time period. Further, the acidity of the product increased considerably after 28 days, resulting in low scores in sensory tests. These observations align with studies by ref. 1, 23 and 35–37 indicating consistent sensory quality degradation over storage.

4. Conclusions

The development of a pearl millet-based probiotic strained yogurt-like functional dessert enriched with *Hibiscus rosa-sinensis* extract and probiotic culture (*Bifidobacterium animalis* subsp. *lactis*, BB-12®) successfully enhanced the nutritional profile and probiotic viability of this product. Through the use of a central composite rotatable design (CCRD), the optimization of functional ingredients led to significant improvements in key parameters, such as probiotic viability count, lactic acidity, color, texture, and overall acceptance. The final product demonstrated high protein content, rich antioxidant levels, and essential minerals while maintaining a probiotic viability of over 6 $\log_{10}$ CFU g^{-1} with satisfactory physico-chemical properties for up to 28 days. This novel dessert not only leverages the nutritional benefits of dairy, millet grains, and *Hibiscus* but also provides a delicious option for supporting digestive health and overall well-being. Its appealing flavor and prolonged shelf life enhance its appeal to health-conscious consumers seeking functional and nutritious food choices. Functional yogurt's scalability and market potential depend on raw material availability, fermentation optimization, and regulatory compliance. With increasing consumer demand for functional, gluten-free, and gut-health-promoting foods, this product will offer a novel, nutrient-dense alternative to conventional dairy and plant-based yogurts. Challenges include ensuring probiotic stability, optimizing texture, and enhancing consumer acceptance. Strategic market positioning, efficient processing, and innovative distribution channels can facilitate the commercial viability of this functional food. The successful enhancement of this dessert variant underscores its potential as a functional food and suggests promising avenues for future research and development in probiotic-enriched, millet-based food products.

Ethical statement

Informed consent has been taken from the human individual for the sensory evaluation test.

Data availability

The data generated during the study will be made public after publication.

Author contributions

Conceptualization: Manvik Joshi, Arun Kumar and Kamalesh Kumar Meena; investigation: Manvik Joshi; supervision: Arun Kumar and Kamalesh Kumar Meena; software; Manvik Joshi and Kamalesh Kumar Meena; writing–original draft preparation, Manvik Joshi; writing–review and editing: Kamalesh Kumar Meena, and Sunil Meena. All the authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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