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Response Surface Methodology-Based Optimization of a Synbiotic *Pennisetum Glaucum-Carica Papaya* Granulate: Composition and Sensory Evaluation

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HIGHLIGHTS

- The optimized formulation contained 30 mL of papaya pulp and 1.25 g of probiotic blend.
- The optimized granulate exhibited high sensory acceptability and a desirability of 0.89.
- The formulation showed low moisture, low water activity, and high solubility.
- The optimized product demonstrated potential as a stable functional synbiotic food.

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Abbreviations
ANOVA= Analysis of Variance
CCD= Central Composite Design
CFU= Colony Forming Units
HCA= Hierarchical Cluster Analysis
OA= Overall Acceptability
RSM= Response Surface Methodology

ABSTRACT

Background: Synbiotic formulations are increasingly recognized for their ability to support gastrointestinal health, immune resilience, and nutrient utilization, particularly in individuals exposed to travel-related physiological stress. This study aimed to develop and optimize a millet-based synbiotic granulate enriched with *Carica papaya* pulp and probiotic strains (*Pediococcus acidilactici*, *Lactiplantibacillus plantarum*, and *Limosilactobacillus fermentum*) for improved sensory quality and desirable physicochemical properties.

Methods: A face-centered central composite design (CCD) within Response Surface Methodology (RSM) was employed to evaluate the combined effects of papaya pulp and probiotic blend concentrations on sensory attributes. Thirteen formulations were prepared and evaluated by 10 semi-trained panelists using a 9-point hedonic scale. Statistical analysis was performed using Design-Expert software version 13 (Stat-Ease Inc., Minneapolis, MN, USA). Regression analysis and analysis of variance (ANOVA) were used to evaluate the models and optimize the formulation. The optimized formulation was further characterized for moisture content, water activity, pH, titratable acidity, solubility, and bulk density.

Results: The optimum formulation contained 30 mL of papaya pulp and 1.25 g of probiotic blend, achieving the highest sensory acceptability with an overall desirability of 0.89. The optimized granulate exhibited low moisture content (3.85 ± 0.03%), contributing to improved storage stability; low water activity (0.34 ± 0.01); pH 4.52 ± 0.01; titratable acidity of 0.79 ± 0.01% lactic acid; high solubility (93.6 ± 0.4%); and bulk density of 0.51 ± 0.01 g/cm³.These properties could represent the physicochemical quality and storage stability of the synbiotic granulate. Statistical validation confirmed the adequacy of the optimization models for predicting the sensory responses.

Conclusion: The developed synbiotic granulate combined favorable sensory acceptance with desirable physicochemical characteristics, demonstrating its potential as a shelf-stable functional food. The formulation may provide a convenient nutritional option for long-distance travelers and other individuals exposed to travel-related physiological stress.

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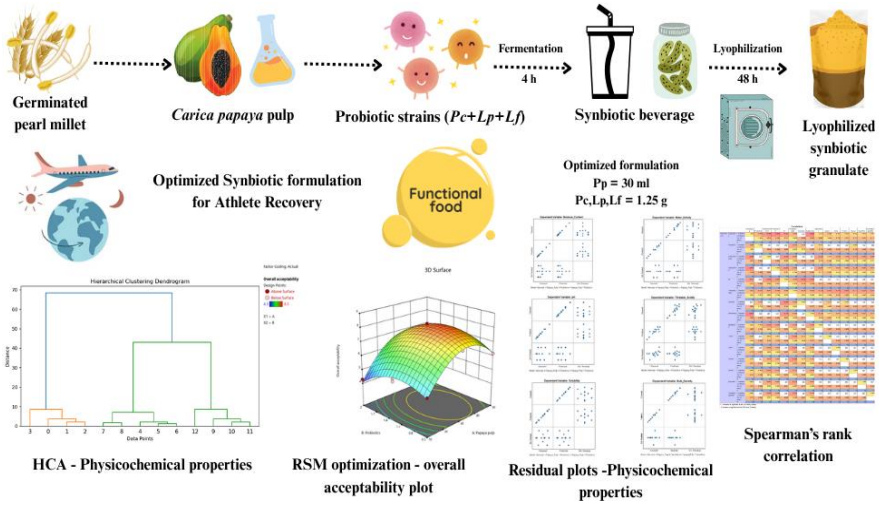
Graphical abstract



Introduction

In recent decades, there has been a shift in global food production and consumption trends. Food companies increasingly focus on developing nutritious products with gastrointestinal benefits through probiotics, prebiotics, and synbiotics to meet evolving consumer demands (Siddiqui et al., 2023). A synbiotic is a combination of probiotics (live beneficial microorganisms) and prebiotics (selectively utilized substrates that promote the growth and activity of beneficial gut microorganisms), designed to improve probiotic survival and enhance host health. Synbiotic formulations have gained considerable attention because they simultaneously support gut microbiota modulation, immune function, and nutrient utilization, making them attractive ingredients for functional food development. Although dairy contributes to over 74% of probiotic products (Taşkoparan, Altınay and Barbaros Özer, 2025), the rise in veganism and intolerance to lactose or gluten has driven demand for non-dairy and gluten-free alternatives (D'Almeida et al., 2024).

Pearl millet (*Pennisetum glaucum*) is a nutrient-dense cereal offering high energy, protein, vitamins, dietary fiber, and minerals (Sadh et al., 2024). Its resilience and prebiotic properties make it an ideal substrate for functional formulations. The inclusion of *Carica papaya* pulp further enhances nutritional content and health advantages, particularly beneficial for extended journeys.

Long-voyage travellers, including athletes, astronauts, army personnel, ocean voyagers, often face jet lag, gastrointestinal issues, exhaustion, and systemic inflammation affecting performance and recovery (Tsukahara et al., 2024). These effects are amplified by disruptions in circadian rhythms and limited access to healthy food. Synbiotic granulates combining probiotics and prebiotic-rich ingredients such as millet and papaya provide a novel dietary strategy for reducing physiological stress, helping maintain gut microbiota balance, boosting immune response, and supporting recovery (Tokarski, Tokarska and Bieganek, 2024).

Probiotics, containing beneficial microorganisms, are widely recognized for their ability to improve gastrointestinal health and immune function (Chandrasekaran, Weiskirchen and Weiskirchen, 2024). They can inhibit pathogens, enhance nutrient digestibility, and improve overall food quality—attributes especially beneficial during travel when access to fresh meals is limited (Sarita et al., 2025).

Papaya pulp is rich in vitamins A, C, and E; B-complex vitamins such as pantothenic acid and folate; and minerals such as magnesium and potassium. Its enzymes and fibers aid digestion and relieve gastrointestinal distress induced by stress or dietary alterations (Kumarasinghe et al., 2024). Integrating papaya and probiotics into millet-based formulations can significantly improve travelers' digestive well-being.

The synbiotic granulate may also benefit other travelers struggling with fatigue, inflammation, and digestive discomfort (Kearns et al., 2024). The formulation aims to support gut health and immune function, both critical for performance and recovery (Todorovic and Ostojic, 2025).

Response Surface Methodology (RSM) is a statistical tool that analyzes the interaction between variables in experimental design. It allows efficient optimization with fewer trials, often using software such as Design-Expert (Wang et al., 2024). Recent studies have found that the use of RSM in optimizing metabolite yields and functional properties of probiotic food systems is effective (Ren et al., 2024).

The primary objective of this study was to develop and

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optimize a synbiotic formulation using malted pearl millet, *Carica papaya* pulp, and probiotic strains through RSM to produce a nutritionally enhanced functional food.

Materials and methods

Acquisition of raw materials

Pearl millet and ripe papayas were procured from a local supermarket in Perungalathur, Chennai, India. Probiotic strains such as *Pediococcus* acidilactici (MTCC no. 13014), *Limosilactobacillus fermentum* (MTCC no. 903), and *Lactiplantibacillus plantarum* (MTCC no. 1325) were collected from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. All analytical-grade chemicals and reagents used in the study were procured from local laboratory suppliers in Chennai, Tamil Nadu, India.

Processing of raw materials

The synbiotic granulate was prepared using malted pearl millet, papaya pulp, and specific probiotic strains. A total of 13 treatment combinations were developed using RSM, keeping pearl millet flour (250 g) and sugar (3%) constant across treatments, based on a modified version of the method followed by Sharma and Sharma (2021).

Malting of pearl millet

Pearl millet was soaked and germinated at 27 ± 2 °C for 12 h, followed by sprouting for 48 h, oven-dried at 75 °C until the moisture content reached approximately 10–12%, then roasted at 70–80 °C and milled into flour (Dias-Martins et al., 2024). The malt extract was prepared by boiling the flour in 1500 mL of distilled water at 100 °C for 20 min, then filtered and reheated for 10 min with 3% sugar. The resulting malt solution was then cooled to room temperature.

Enhancing malt with papaya pulp and probiotics: fortification strategies

Papayas were washed with tap water, hand-peeled using a knife, and then processed to extract the pulp. The resulting fruit homogenates were placed into jars and pasteurized for 15 min at 95 °C (Saeed Ahmed and Mohamed El Zubeir, 2021). Probiotic fortification involved the addition of *Pediococcus* acidilactici (L1), *Lactiplantibacillus plantarum* (L2), and Limosilactobacillus fermentum (F3), either individually or in combination (10^8 CFU/mL), based on the RSM treatment matrix, followed by 4 h of ambient fermentation to enhance prebiotic–probiotic synergy. To ensure consistent microbial dissemination, each combination was stirred once each hour during ambient fermentation. During ambient fermentation, each treatment's pH and titratable acidity were measured hourly. Product temperatures were maintained below −20 °C until drying was completed, and lyophilization parameters were adjusted to preserve probiotic viability. After drying, the powder was kept in vacuum-sealed aluminum pouches at 4 °C for future use (Singh et al., 2022).

Experimental design and optimization

RSM with a face-centered Central Composite Design (CCD) was used to evaluate the effects of papaya pulp—CpP (X_1)—and probiotic blend—Pc + Lp + Lf (X_2)—on the quality of the synbiotic granulates (Wang et al., 2024). Table 1 presents the CCD design matrix along with the experimental results for sensory parameters of the probiotic beverage. Preliminary trials established acceptable upper and lower limits for these variables. Sensory attributes—appearance (Y_1), color (Y_2), taste (Y_3), flavor (Y_4), texture (Y_5), mouthfeel (Y_6), and overall acceptability (OA) (Y_7)—were selected as responses. The probiotic blend (X_2) consisted of *Pediococcus acidilactici*, *Lactiplantibacillus plantarum*, and *Limosilactobacillus fermentum* mixed in a ratio of 1:1:1 (w/w). The total amount of the probiotic blend (0.5–2.0 g) was varied according to the experimental design, while the proportion of each strain remained constant throughout the study. Data were fitted to a quadratic model for optimization. Five center points were included to estimate pure experimental error and to permit reliable lack-of-fit testing, thereby improving the precision and validity of the quadratic model.

$$Y = \beta + X_1 + X_2 + X_1^2 + X_2^2 + X_1X_2 \ldots\ldots\ldots \quad (1)$$

Where, Y = responses; β = constant; X_1, X_2 = linear regression; X_1^2, X_2^2 = quadratic regression; X_1X_2= interaction regression; X_1, X_2 = independent variables

Table 1: Variable levels used in RSM design

Independent Variables		Coded Value			
		-1	0	+1	Coding
Real value	Papaya pulp	10	30	50	Actual
	Probiotic strains	0.5	1.25	2.0	Actual

Note: *The quantities of probiotic strains ranged from 0.5 - 2.0g, and papaya pulp from 10 - 50 ml. Upper and lower bounds of these variables were defined after a preliminary process.

Validation of models

Model validation was based on the coefficient of determination (R^2), the p-value indicating lack of fit, and the coefficient of variance (CV% < 10%), indicating accuracy and repeatability (Joudi-Sarighayeh et al., 2023). The study used RSM to evaluate 13 formulations, examining the effects of papaya pulp and probiotic strains on sensory attributes such as visual appearance, color quality, taste perception, textural attributes, flavor characteristics, and general acceptability as dependent variables.

Sensory evaluation

Sensory assessment is commonly used by consumers to evaluate food quality (Rodrigues, Dias and Teixeira, 2024). Ten semi-trained panelists from the Department of Food Technology, Hindustan Institute of Technology and Science, evaluated the synbiotic granulate for appearance, color, taste, texture, flavor, mouthfeel, and OA using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Six samples were evaluated on Day 1, and the remaining seven samples on Day 2 by the same panel of assessors. The sensory scores reported for each attribute represent the mean of the individual ratings provided by the 10 panelists and were used for subsequent statistical analyses. The sensory evaluations were conducted over two consecutive days for practical reasons to minimize sensory fatigue. The same panelists, evaluation conditions, and sample presentation procedures were maintained on both days to ensure consistency. Therefore, day was not considered as a separate factor in the statistical analysis.

Statistical analysis

Sensory data were analyzed using Design-Expert software version 13 (Stat-Ease Inc., Minneapolis, MN, USA) with a face-centered CCD, incorporating two factors—*Carica papaya* pulp (X_1) and the probiotic strains (X_2)—at three levels with five center points. Although the 9-point hedonic scale is ordinal in nature, mean hedonic scores are widely accepted as approximating interval-scale data when averaged across panelists, permitting the application of parametric statistical methods such as analysis of variance (ANOVA) and regression analysis (Xia et al., 2020; Yeung et al., 2021). ANOVA, regression modeling, and lack-of-fit tests were performed to evaluate the significance and adequacy of the fitted quadratic model, while response surface plots were generated to visualize the interactions between the independent variables. Spearman's rank correlation was performed using IBM SPSS Statistics Version 27.0 to evaluate the relationships among sensory attributes. Hierarchical cluster analysis (HCA) was conducted using squared Euclidean distance as the similarity measure and Ward's linkage method as the clustering algorithm to assess relationships among sensory attributes and treatment clustering. Differences were considered statistically significant at $p < 0.05$.

Determination of functional properties of granulate

All physical property procedures were conducted using standardized and widely accepted laboratory protocols.

-Moisture content and water activity

Moisture content in raw samples was evaluated using the AOAC (2000) method. An AquaLab instrument (4TE, Decagon Devices, Pullman, USA) was used to measure water activity (aw) in the reconstituted samples at 25 °C. Both determinations were performed in triplicate.

-pH determination

The pH of each sample was determined using a calibrated tabletop digital pH meter (Model GECP40605V), manufactured by Global Engineering Corporation. The electrode was first allowed to stabilize, then immersed in the sample, and the pH value was recorded once the reading became steady (AOAC, 2000).

-Titratable Acidity

To calculate titratable acidity, an identified amount of material was titrated with a standardized 0.1 N sodium hydroxide (NaOH) solution. Phenolphthalein served as an indicator, turning pink at the endpoint (pH 8.3). The amount of NaOH required to reach this endpoint was noted. Subsequently, titratable acidity was computed and expressed as a percentage of lactic acid (AOAC, 2000).

-Solubility

The shake-flask method was used to determine solubility, which involved mixing an excess of the sample with a solvent and agitating at a regulated temperature until equilibrium was achieved. UV-Vis spectrophotometry was then used to analyze the supernatant after the solution had been filtered (Ali et al., 2020).

-Bulk density

To assess the bulk density of synbiotic granulates, a conventional approach is to pour a known mass of granules into a graduated cylinder and record the volume without tapping. The formula used to determine the bulk density is:

$$Bulk\ Density\ \left(\frac{g}{mL}\right) = \frac{Mass\ of\ granules\ (g)}{Volume\ occupied\ (ml)} \dots\dots\dots\dots\dots\dots (2)$$

This approach gives insight into the granules' packing and flow features (Sadef *et al.*, 2022).

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Results and discussion

Model optimization and ANOVA

Thirteen synbiotic formulations were evaluated using RSM with *Carica papaya* pulp and probiotic strains as variables. Table 2 shows the mean sensory response values. Regression analysis confirmed significant quadratic effects of variables on all sensory responses, with optimal results at 30 mL of *Carica papaya* pulp and 1.25 g of probiotic blend. Sensory responses ranged from 4 to 8.5 across attributes. The CCD design enabled detailed evaluation of interactions between papaya pulp and probiotic levels.

Table 2: Mean sensory scores of synbiotic granulates

Independent variables			Dependent variables						
Run	**A: Papaya pulp**	**B: Probiotics**	**Appearance**	**Colour**	**Taste**	**Texture**	**Flavour**	**Mouthfeel**	**OA**
1	10	0.5	4.5	4	5	4.5	5.5	5	5
3	10	2	5	7.5	6.5	6.5	6	6	6.2
5	10	1.25	4	3.5	4.5	4	5	4.5	4.3
10	10	1.25	5.5	7	6	6.2	6.5	6	6.1
7	30	0.5	5	4.5	5.5	5	5.5	5.2	5.3
8	30	2	6	7.8	7	7.2	6.8	6.8	7
9	30	1.25	6.5	7	7.5	7.5	7	7	7.2
12	30	0.5	6	6	6.5	6.2	6.8	6.5	6.6
13	30	2	8	8.5	8.5	8.5	8.2	8.2	8.3
2	50	0.5	5	4	5	5	5.5	5.2	5.1
4	50	2	6	7.8	7	7.2	6.8	6.8	7
6	50	1.25	6.5	7	7.5	7.5	7	7	7.2
11	50	1.25	6	6	6.5	6.2	6.8	6.5	6.6

OA= Overall Acceptability

Regression coefficient for independent variables

Linear and quadratic terms of papaya pulp and the probiotic blend significantly influenced sensory responses ($p < 0.05$). The fitted regression models included interaction terms (X_1X_2), together with linear and quadratic effects, to describe the relationship between the independent variables and sensory responses (Supplementary material). Positive linear and negative quadratic coefficients suggested an optimal range for both factors. The sensory scores of synbiotic formulations were analyzed using a second-order polynomial regression model:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \ldots\ldots\ldots\ldots\ldots (3)$$

The regression coefficients showed that papaya pulp and probiotic blend levels had significant linear effects ($p < 0.05$). In addition, the quadratic terms were significant and negative, indicating curvature in the response surfaces and suggesting the existence of optimum formulation levels. The fitted quadratic models exhibited high coefficients of determination ($R^2 > 0.95$), indicating that a large proportion of the variability in the sensory responses was explained by the models. The ANOVA results further confirmed the statistical significance of the regression models, supporting the suitability of the fitted models for describing the experimental data. These findings are consistent with recent studies employing RSM for the optimization of fruit-based probiotic formulations, with minor differences likely due to formulation composition and probiotic strains (Hevlin, Raj and Gupta, 2024).

ANOVA of the response variables

Appearance, color, taste, texture, flavor, mouthfeel, and OA are important characteristics for evaluating product quality, with sensory analysis essential for understanding consumer preferences and product development (Rodrigues, Dias and Teixeira, 2024). ANOVA results (Supplementary material) show that *Carica papaya* pulp and probiotic strains significantly affected all sensory attributes ($p < 0.05$), with high F-values confirming model validity. R^2 values ranged from 0.94 to 0.97, indicating a strong correlation between predicted and actual results. Similar model significance has been reported in RSM-based synbiotic formulations (Ahmed et al., 2023), with differences likely due to formulation and processing conditions.

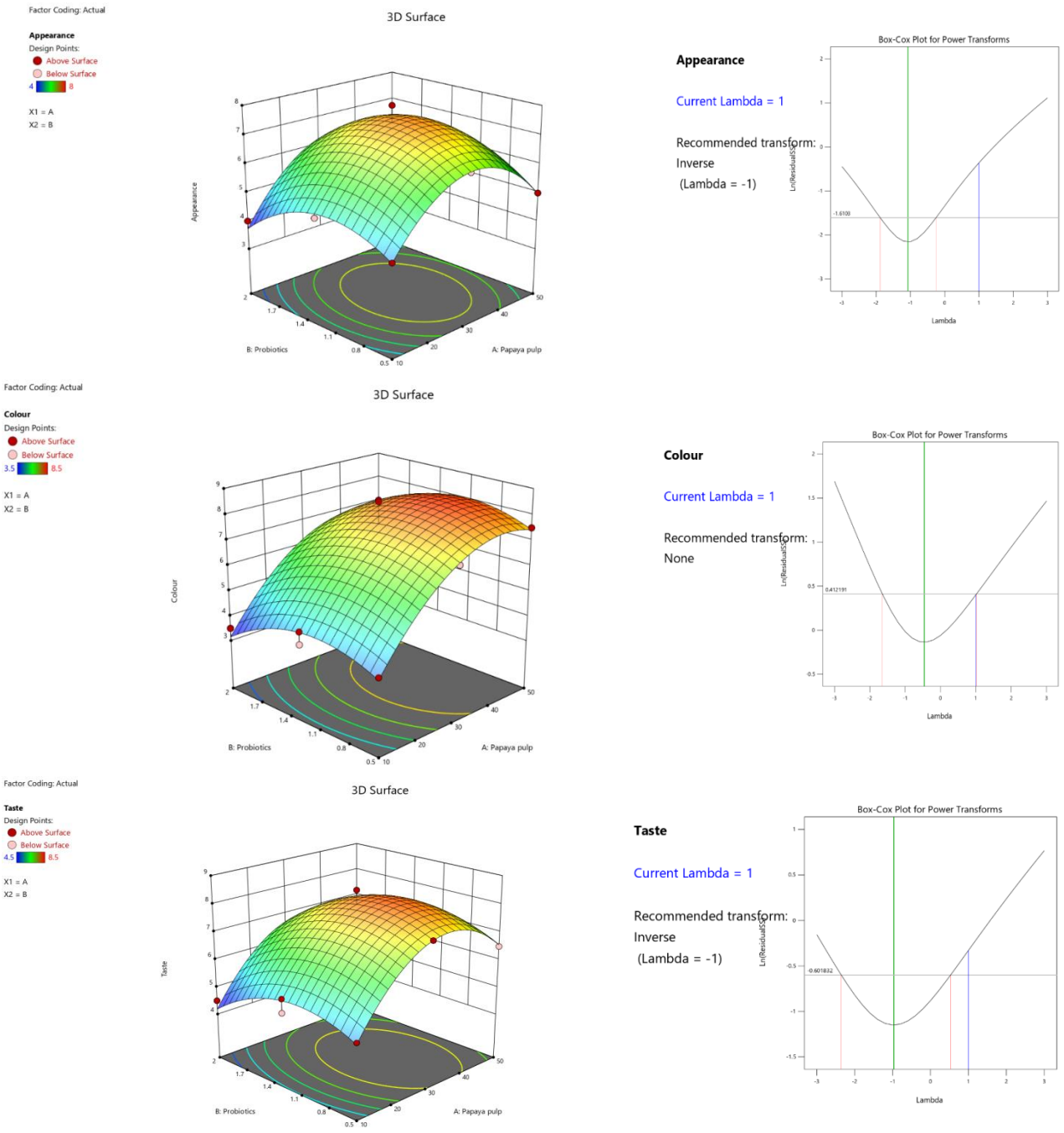
Sensory panel observations

Panelists provided qualitative evaluation in addition to their hedonic scores. Samples with more papaya pulp were consistently reported to taste fresher and have brighter colors. Panelists observed that probiotic-rich combinations, while tangier, could provide a chalkier mouthfeel if not balanced with enough pulp. The silky texture, mild acidity, and quick rehydration of optimal formulations were

commended for resembling fresh tropical fruit combinations.

Effect of independent variables on sensory attributes with model fit and transformation analysis

Predictive models for millet-based synbiotic granulate qualities, based on *Carica papaya* pulp and probiotic strains, are presented in Equations 4–17, with graphical interactions shown in Figure 1. RSM revealed a positive, synergistic effect of these factors on sensory scores. Box–Cox plots showed lambda (λ) values near 1, confirming that no data transformation was needed and validating the normality of residuals. These results demonstrate the reliability of the RSM models in predicting sensory responses accurately.



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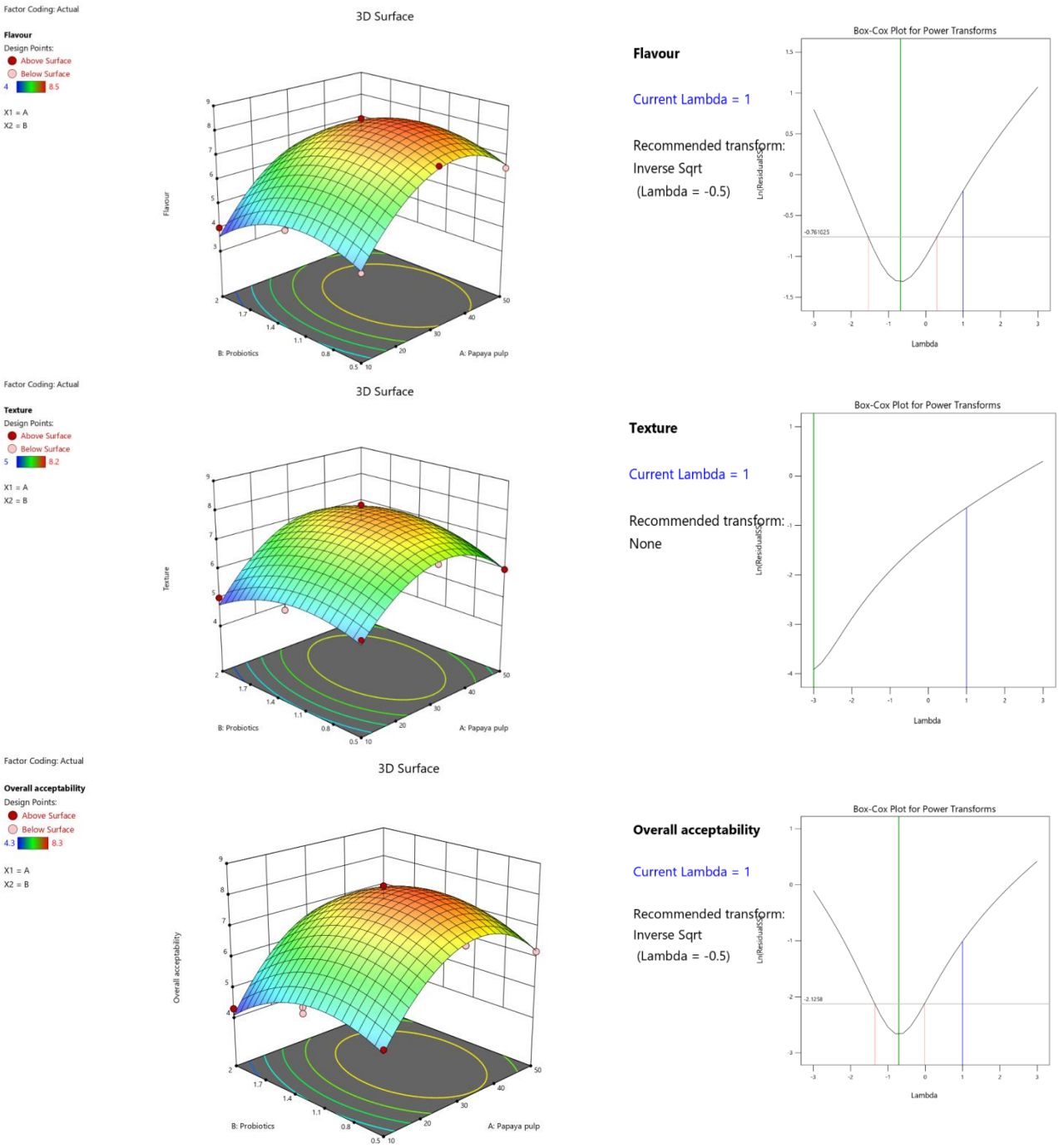


Figure 1: 3D surface and Box-Cox plot showing the effect of the independent variables on the OA score.

-Statistical optimization and model evaluation of appearance response

The appearance of the granulate was significantly ($p < 0.05$) affected by quadratic models A, A^2, and B^2, with the model showing an F-value of 24.27, indicating strong significance and only a 0.03% chance of this result arising from noise. The model's predicted R^2 is 0.7429, slightly lower than the adjusted R^2 of 0.9065, while the signal-to-noise ratio of 17.250 exceeds the acceptable threshold of 4, confirming model adequacy. The appearance response had an R^2 of 94%. The optimized formulation of 30 mL of *Carica papaya* pulp and 1.25 g probiotic strains achieved a mean score of 7.4 for appearance. The model effectively guides exploration of the design space. An equation based on coded factors:

$$Appearance = 7.43 + 0.5000X_1 - 0.125X_2 - 1.79X_1^2 - 1.04X_2^2 + 0.25X_1X_2 \ldots\ldots\ldots (4)$$

An equation based on actual factors:

$$Appearance = 0.61 + 0.27X_1 + 3.93X_2 - 0.004X_1^2 - 1.84X_2^2 + 0.016X_1X_2 \ldots\ldots\ldots (5)$$

-Statistical optimization and model evaluation of colour response

Color was significantly affected by quadratic terms A, A², and B². The model showed strong significance with an F-value of 30.41 and Prob > F < 0.0500. The predicted R² (0.8125) is close to the adjusted R² (0.9246), with an R² of 95% for the color response. A signal-to-noise ratio of 15.439 confirms model adequacy, making it effective for exploring the design space. The mean score for color was 7.6.

An equation based on coded factors

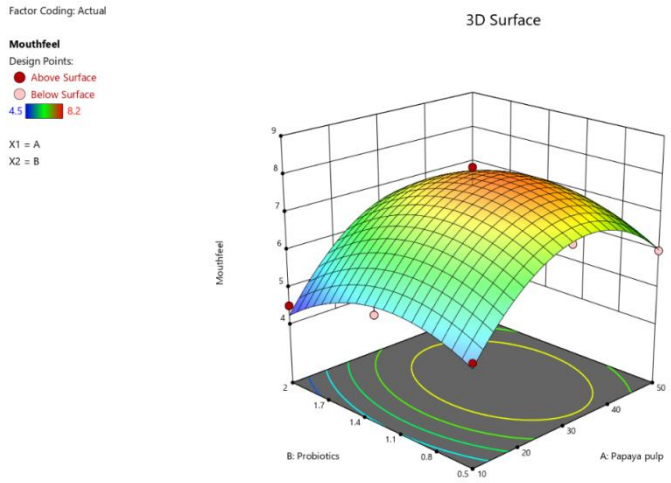
$$Colour = 7.66 + 1.76X_1 - 0.375X_2 - 1.42X_1^2 - 0.94X_2^2 + 0.000X_1X_2 \ldots\ldots\ldots\ldots (6)$$

An equation based on Actual factors:

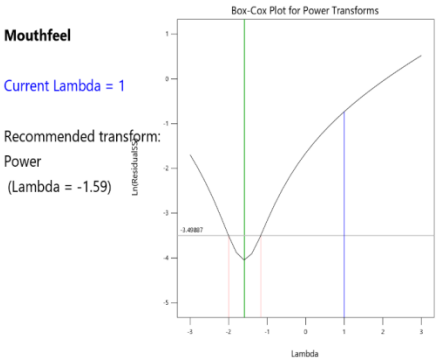
$$Colour = -0.188 + 0.30X_1 + 3.706X_2 - 0.003X_1^2 - 1.68X_2^2 - 1.806X_1X_2 \ldots\ldots\ldots\ldots (7)$$

-Statistical optimization and model evaluation of taste response

Models A, B, A², and B² significantly impacted taste, with an F-value of 29.92 and Prob > F < 0.05, confirming model significance. The lack-of-fit F-value (6.33) was non-significant, indicating minimal unexplained variation (Mai et al., 2022).



(a) Mouthfeel



The model's predicted R² (0.7985) is close to the adjusted R² (0.9234), with an overall R² of 95%. A precision ratio of 17.5132 suggests a sufficient signal. The mean score for taste was 8, and the model is effective for navigating the design space. The optimized formulation achieved a taste score of 8.0, which is comparable to recent probiotic fruit-based formulations that showed that the balanced use of fruits enhanced taste acceptability (Cao, Fang and Hekmat, 2025).

An equation based on coded factors:

$$Taste = 8.00 + 0.81X_1 - 0.37X_2 - 1.75X_1^2 - 0.875X_2^2 + 0.000X_1X_2 \ldots\ldots\ldots\ldots (8)$$

An equation based on actual factors:

$$Taste = 1.03 + 0.303X_1 - 3.38X_2 - 0.004X_1^2 - 1.55X_2^2 - 2.1002X_1X_2 \ldots\ldots\ldots\ldots (9)$$

-Statistical optimization and model evaluation of flavour response

Models A, B, A², and B² significantly affected flavor, with an F-value of 34.85 and Prob > F < 0.05, confirming model significance. The predicted R² (0.7796) is close to the adjusted R² (0.9338), and the R² is 96%. The adequate precision ratio of 18.8288 suggests an adequate signal. The mean score was 8.01, and the model effectively supports design space exploration. The flavor score (8.01) was similar to those reported for probiotic yogurts supplemented with fruit ingredients: moderate addition of fruit ingredients did not negatively affect consumer acceptability but had a positive effect on the flavor score (Kanca, Onaran Acar and Albayrak Delialioğlu, 2024).

An equation based on coded factors:

$$Flavour = 8.01 + 1.08X_1 - 0.42X_2 - 1.79X_1^2 - 1.04X_2^2 + 0.05X_1X_2 \ldots\ldots\ldots\ldots (10)$$

An equation based on actual factors:

$$Flavour = 0.304 + 0.318X_1 + 3.96X_2 - 0.004X_1^2 - 1.85X_2^2 + 0.003X_1X_2 \ldots\ldots\ldots\ldots (11)$$

-Statistical optimization and model evaluation of Texture response

Models A, A², and B² significantly influenced texture (p < 0.05), with an F-value of 22.89 and Prob > F < 0.0500. The predicted R² (0.7384) is close to the adjusted R² (0.9012), and the R² for the texture response is 94%. A precision ratio of 15.76 confirms model adequacy. The mean texture score was 7.6, and the model supports effective design space exploration.

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An equation based on coded factors:

$$Texture = 7.69 + 0.57X_1 - 0.05X_2 - 1.41X_1^2 - 0.65X_2^2 + 0.25X_1X_2 \ldots\ldots\ldots\ldots (12)$$

An equation based on actual factors:

$$Texture = 2.54 + 0.218X_1 + 2.35X_2 - 0.003X_1^2 - 1.168X_2^2 + 0.016X_1X_2 \ldots\ldots\ldots\ldots (13$$

-Statistical optimization and model evaluation of Mouthfeel response

Models A, A², and B² significantly influenced mouthfeel ($p < 0.05$), with an F-value of 35.21 and Prob > F < 0.05. The predicted R^2 (0.8182) and adjusted R^2 (0.9344) indicate good model fit, with an R^2 of 96% for mouthfeel. A precision ratio of 19.62 confirms signal adequacy. The mean score was 7.7, supporting the model's utility in design space optimization.

An equation based on coded factors:

$$Mouthfeel = 7.73 + 0.71X_1 - 0.18X_2 - 1.61X_1^2 - 0.8607X_2^2 + 0.125X_2 \ldots\ldots\ldots\ldots (14)$$

An equation based on actual factors:

$$Mouthfeel = 1.269 + 0.26X_1 + 3.32X_2 - 0.004X_1^2 - 1.53X_2^2 + 0.008X_1X_2 \ldots\ldots\ldots\ldots (15)$$

-Statistical optimization and model evaluation of OA response

Models A, B, A², and B² influenced OA ($p < 0.05$), with a strong model F-value of 55.98 and Prob > F < 0.05. The predicted R^2 (0.8876) closely aligns with the adjusted R^2 (0.9582), and the R^2 value is 97%. OA across 13 distinct concentrations ranged from 4 to 8, with a mean score of 7.9 for the optimal combination (30 mL of *Carica papaya* pulp and 1.25 g of probiotic strains: Pc + Lp + Lf). The precision ratio of 24.6564 confirms model adequacy for navigating the design space. The optimized formulation had an overall score of 7.9, which is comparable to that reported for synbiotic mango yogurt optimized by RSM, in which fortification with the fruit had a significant effect on consumer acceptability (Minj and Vij, 2025).

An equation based on coded factors:

$$Overall\ acceptability = 7.90 + 0.82X_1 - 0.25X_2 - 1.70X_1^2 - 0.90X_2^2 + 0.15X_2 \ldots\ldots (16)$$

An equation based on actual factors:

$$Overall\ acceptability = 1.12 + 0.28X_1 + 3.36X_2 - 0.004X_1^2 - 1.60X_2^2 + 0.01X_1X_2 \ldots.(17)$$

All independent variables showed an optimal effect on *Carica papaya* pulp and probiotic strains. Interactive terms for appearance, color, taste, flavor, texture, mouthfeel, and OA were positively influenced by Cp and Pc + Lp + Lf. Among sensory attributes, taste exhibited a non-significant lack of fit. All quadratic model terms were statistically significant ($p < 0.05$).

Multi-response optimization through desirability function methodology

A multi-response optimization combining RSM and the desirability function was used to optimize sensory attributes of the synbiotic powder by converting multiple responses into a common desirability scale from 0 (totally undesirable) to 1 (entirely desirable). Overall desirability (D) was calculated as the geometric mean of individual desirabilities:

$$D = \left(\prod_{i=1}^{n} d_i^{w_i}\right)^{1/\sum w_i}$$

Where, d_i = individual desirability of the ith response, w_i = weight or importance of the ith response, n = number of responses considered (Shojaeimeher *et al.*, 2024). In the present study, all response weights (w_i) were set to 1, indicating equal weighting of the sensory responses during numerical optimization, while the response priorities were assigned according to the optimization objectives in Design-Expert software. This approach ensures balanced optimization, penalizing low scores in any response (Supplementary material). The goal was to maximize sensory qualities (color, mouthfeel, texture, flavor, taste, and general acceptability) while keeping formulation factors within a practical range. The RSM optimization identified the optimal formulation as 30% *Carica papaya* pulp and 1.25% probiotic strains, achieving high sensory ratings (taste, 8.0; flavor, 8.0; texture, 7.6; mouthfeel, 7.7; OA, 7.9) and a total desirability of 0.897, indicating a highly optimized formulation. The desirability value obtained in the present study is comparable to values reported for optimized functional food formulations developed using RSM, where desirability values above 0.89 generally indicate excellent agreement between predicted and experimental responses. Similar optimization efficiencies have been reported for probiotic yogurt and fruit-based functional beverages, confirming the robustness of RSM as a tool for multi-response optimization (Gamero-Salinas and López-Fidalgo, 2025).

Characterization of physicochemical properties in synbiotic formulation

The physicochemical properties of the synbiotic granulates were analyzed using factorial ANOVA, and significant differences among treatment means were further compared using Tukey's HSD test ($p < 0.05$).

-Moisture content and water activity

Moisture content ranged from 3.85% to 4.47%, highest in the 10% papaya pulp and 2.0% probiotic concentration, showing that a greater probiotic dose improves moisture retention due to cellular water-binding capacity

(Albuquerque et al., 2024). Water activity (aw) varied from 0.34 to 0.42, staying below the microbiological stability threshold (0.6), indicating good shelf-life potential. Lower aw was associated with increased solubility and lower moisture, as observed in the 30% papaya pulp and 1.25% probiotic formulation, aligning with the best drying techniques used in encapsulated tropical fruit products (Fuentes et al., 2023). These findings are also comparable to those reported by Sandhya et al. (2025) for millet-based synbiotic microcapsules, which showed a moisture content of 3.95%, water activity of 0.40, and water solubility of 95.4%, confirming that low moisture and water activity contribute to improved microbial stability and prolonged shelf life of probiotic formulations. The slight variation in moisture content and water activity between the two studies may be attributed to differences in probiotic strains, formulation composition, and freeze-drying conditions.

-*Titratable acidity and pH*

Titratable acidity showed an inverse connection with pH, ranging from 4.49 to 4.62 and 0.70% to 0.88%, respectively. Lactic acid generation resulted in increased acidity and decreased pH as probiotic concentration increased. This supports previous findings with papaya–probiotic snacks and fortified yogurts (Sarker, Jung and Siddiqui, 2023). The agreement is likely due to the production of organic acids by lactic acid bacteria during fermentation. Minor differences among studies may result from variations in fermentation time, inoculum concentration, and substrate composition. The combination of acidity from lactic fermentation and natural acids in papaya improves both preservation and sensory quality.

-*Solubility*

The formulation with 30% pulp and 1.25% probiotic had the highest solubility, which varied from 89.2% to 93.6%. Fruit-derived carbohydrates likely increased rehydration capability. The same high solubility has been observed for freeze-dried synbiotic powders. The lower solubility in the present study could be attributed to the presence of a higher amount of fiber in pearl millet, which may not be completely dissolved in the powder compared with fruit-based powders (Fuentes et al., 2023).

-*Bulk density*

Bulk density (0.51–0.59 g/cm³) increased with probiotic addition, due to the compact cellular structure of microbes. This is similar to what Ali et al. (2020) found—bulk density of probiotic powders increased with tighter packing of particles after drying. Differences between studies may have been related to particle size distribution or composition of the formulations. This supports efficient packaging and flowability, critical for food granulates intended for reconstitution over long journeys or space missions.

Across the 13 formulations (Runs 1–13), increased probiotic levels generally resulted in higher titratable acidity and bulk density. Run 3 (10% pulp, 2.0% probiotic) had the highest bulk density (0.59 g/cm³). According to the study, the proportions of probiotics and papaya pulp had an impact on bulk density, moisture, pH, acidity, and solubility (Table 3). These qualities are crucial for assuring the shelf life, reconstitution performance, and sensory quality of synbiotic granulates designed for functional food applications.

Table 3: Means followed by different superscripts (a,b,c) within a column indicated statistically significant differences ($p<0.05$), as determined by Tukey's HSD test

Run	Papaya pulp (mL)	Probiotics (g)	Moisture Content (%)	Water Activity (a_w)	pH	Titratable Acidity (%)	Solubility (%)	Bulk density (g/cm³)
1	10	0.5	4.10 ± 0.03^{bc}	0.40 ± 0.01^{bc}	4.55 ± 0.02^{ca}	0.78 ± 0.01^{ab}	89.5 ± 0.6^{ca}	0.56 ± 0.01^{ac}
2	50	0.5	3.95 ± 0.02^{bb}	0.35 ± 0.01^{ba}	4.62 ± 0.01^{ca}	0.70 ± 0.01^{aa}	92.0 ± 0.6^{ca}	0.52 ± 0.01^{aa}
3	10	2.0	4.47 ± 0.04^{cc}	0.42 ± 0.01^{cc}	4.52 ± 0.01^{aa}	0.85 ± 0.02^{ca}	89.2 ± 0.5^{aa}	0.59 ± 0.01^{bc}
4	50	2.0	4.30 ± 0.03^{bc}	0.41 ± 0.01^{cc}	4.49 ± 0.01^{aa}	0.88 ± 0.02^{cc}	92.2 ± 0.5^{aa}	0.56 ± 0.01^{ba}
5	10	1.25	4.00 ± 0.02^{ca}	0.38 ± 0.01^{ac}	4.53 ± 0.02^{ba}	0.81 ± 0.01^{bb}	90.2 ± 0.6^{ba}	0.55 ± 0.01^{ac}
6	50	1.25	3.88 ± 0.02^{ba}	0.34 ± 0.01^{aa}	4.51 ± 0.01^{ba}	0.82 ± 0.01^{bb}	93.0 ± 0.5^{ba}	0.53 ± 0.01^{aa}
7	30	0.5	3.90 ± 0.02^{ab}	0.36 ± 0.01^{bb}	4.58 ± 0.01^{ca}	0.73 ± 0.01^{ab}	91.0 ± 0.6^{ca}	0.54 ± 0.01^{ab}
8	30	2.0	4.10 ± 0.02^{ac}	0.40 ± 0.01^{cb}	4.50 ± 0.01^{aa}	0.86 ± 0.01^{cb}	91.5 ± 0.5^{aa}	0.57 ± 0.01^{bb}
9	30	1.25	3.85 ± 0.03^{aa}	0.34 ± 0.01^{ab}	4.52 ± 0.01^{ba}	0.79 ± 0.01^{ab}	93.6 ± 0.4^{ba}	0.51 ± 0.01^{ab}
10	10	1.25	4.02 ± 0.03^{ca}	0.39 ± 0.01^{ac}	4.54 ± 0.01^{ba}	0.80 ± 0.01^{ba}	90.0 ± 0.5^{ba}	0.56 ± 0.01^{ac}
11	50	1.25	3.90 ± 0.03^{ba}	0.34 ± 0.01^{aa}	4.50 ± 0.01^{ba}	0.83 ± 0.01^{bb}	93.2 ± 0.4^{ba}	0.53 ± 0.01^{aa}
12	30	0.5	3.92 ± 0.02^{ab}	0.36 ± 0.01^{bb}	4.57 ± 0.01^{ca}	0.74 ± 0.01^{ab}	91.0 ± 0.6^{ca}	0.54 ± 0.01^{ab}
13	30	2.0	4.11 ± 0.03^{ac}	0.41 ± 0.01^{cb}	4.50 ± 0.01^{aa}	0.87 ± 0.01^{cb}	91.6 ± 0.5^{aa}	0.57 ± 0.01^{bb}

Note: Values are Mean ± Standard Deviation. aw, water activity. Superscripts a-c within a column indicates statistically significant variation ($p<0.05$), according to Tukey's HSD test.

The optimized synbiotic granulate exhibited a moisture content of 3.85% and solubility of 93.6%, indicating excellent storage stability and reconstitution characteristics. These findings are comparable to those reported by Pande, Dharmaraj and Veerapandian (2024) for pearl millet-based food products, where appropriate moisture regulation produced high-quality products with a water solubility index of approximately 94–95%, demonstrating that optimized processing conditions improve the functional properties and reconstitution behavior of pearl millet formulations.

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Biofunctional implications

Biofunctionally, the synbiotic granulate provides more than just nutritional support; it may modify host microbiota and lower systemic inflammation during times of severe stress (Chandrasekaran, Weiskirchen and Weiskirchen, 2024). The lactic acid-producing bacteria included in this composition have previously shown immunomodulatory and anti-pathogenic properties, which may benefit long-distance travelers recovering from strenuous physical activity (Sarita et al., 2025). Furthermore, the millet and papaya matrix contains fermentable fibers and polyphenols that promote the colonization and activity of probiotic bacteria during gastrointestinal transit (Sadh et al., 2024).

Although probiotic viability was not evaluated in the present study, previous investigations have demonstrated that freeze-drying, when combined with suitable formulation and drying conditions, can effectively preserve probiotic viability while maintaining desirable physicochemical properties. Therefore, future studies should evaluate the survival of probiotic cells during storage to further validate the functional performance of the developed synbiotic granulate (Albuquerque et al., 2024).

Model adequacy and residual analysis

Residual plots for moisture content, water activity, pH, titratable acidity, solubility, and bulk density (Figure 2) revealed that predicted and experimental values were nearly identical, with residuals randomly distributed around zero. This shows that the regression assumptions of linearity, homoscedasticity, and independence were reasonably satisfied, supporting the adequacy of the fitted models (Verma, 2025). For the OA model, ANOVA showed a significant regression model ($F = 55.98$, $P < 0.0001$), while the lack-of-fit test yielded an F-value of 23.00 ($p = 0.0055$), as presented in the supplementary material.

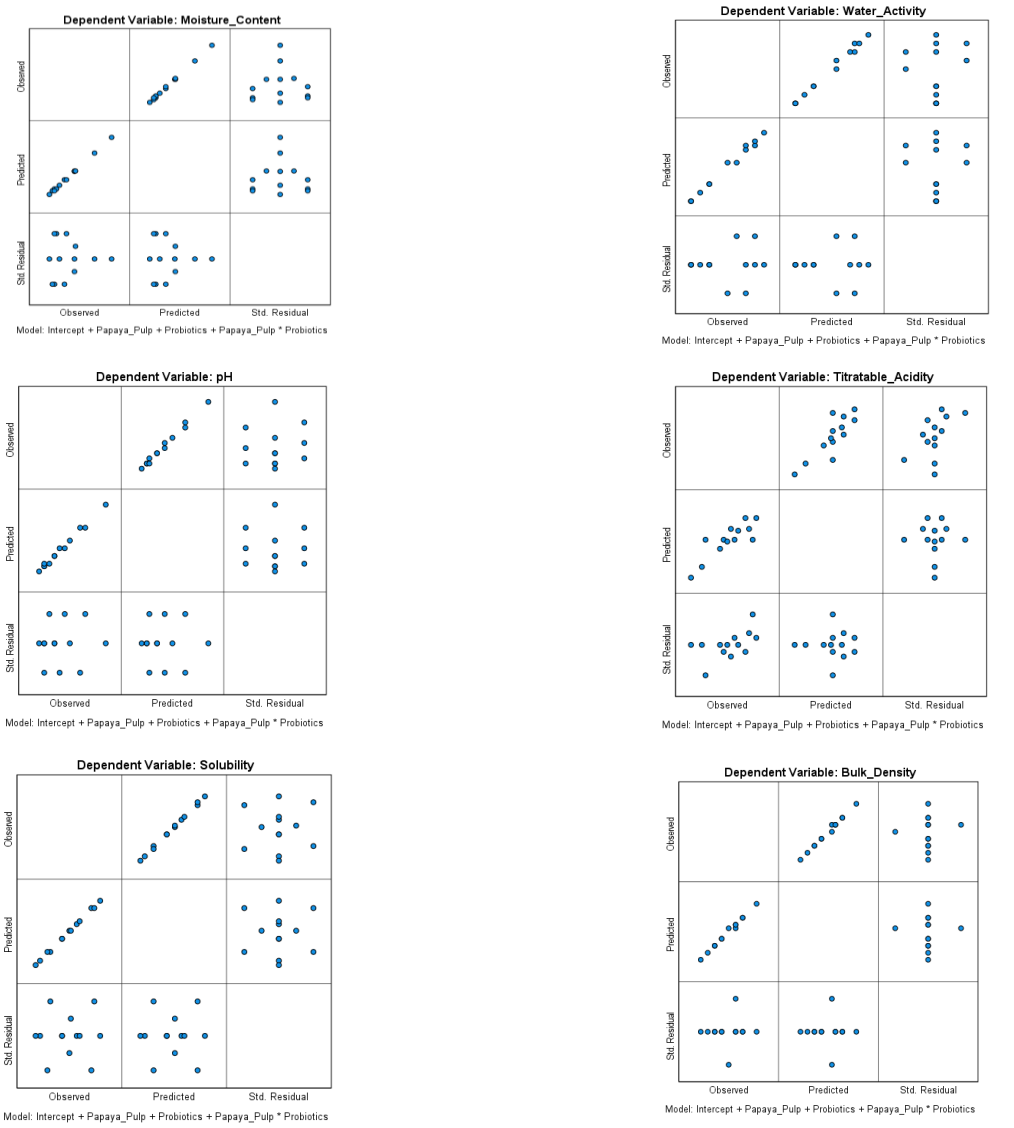

Figure 2: Residual diagnostic plots for the regression models of individual physicochemical parameters of the synbiotic granulate.

HCA of physicochemical properties

Formulations can be grouped according to similarities in their physicochemical features, as seen in the dendrogram (Supplementary material). Three primary clusters showed significant differences between formulations' moisture, pH, solubility, and other characteristics. The clustering of samples with comparable quantities of probiotics or papaya pulp indicated the role of composition. Similar clustering patterns have been reported in probiotic formulations (Vahdat et al., 2024). The optimized formulation formed a distinct cluster with low moisture (3.85%), low water activity (0.34), high solubility (93.6%), and high OA (7.9), demonstrating its physicochemical and sensory properties.

Spearman's rank correlation analysis

Spearman's rank correlation analysis showed significant correlations between physicochemical and sensory parameters of the synbiotic granulates (Figure 3). The results indicated positive correlations between probiotic concentration and mouthfeel, flavor, texture, and OA, which indicated that increased probiotic concentration led to better flavor, texture, and acceptability. There was a negative correlation between pH and several sensory attributes, suggesting that pH affects the perception of sensory attributes. Water activity and moisture content had a strong positive relationship, while solubility was positively related to some sensory properties. In addition, appearance and color were highly correlated, indicating that visual quality plays a significant role in consumers' acceptance. In general, the correlations observed confirm the strong relationship between physicochemical characteristics and sensory quality, which supports the optimization of synbiotic formulations (Cao, Fang and Hekmat, 2025).

Correlations

			Papayapulp	Probiotics	Moisturecontent	Wateractivity	pH	Titratableacidity	Solubility	Bulkdensity	Appearance	Colour	Taste	Texture	Flavour	Mouthfeel	Overallacceptability
Spearman's rho	Papayapulp	Correlation Coefficient	1.000	0.000	-0.368	-0.476	-0.303	0.052	.840**	-0.529	0.522	0.159	0.397	0.357	0.442	0.475	0.461
		Sig. (2-tailed)		1.000	0.216	0.100	0.313	0.865	0.000	0.063	0.067	0.604	0.179	0.231	0.131	0.101	0.113
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Probiotics	Correlation Coefficient	0.000	1.000	.565*	.556*	-.858**	-0.131	0.105	.609*	0.415	.834**	.609*	.662*	0.468	.554*	.566*
		Sig. (2-tailed)	1.000		0.044	0.049	0.000	0.670	0.733	0.027	0.159	0.000	0.027	0.014	0.106	0.049	0.044
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Moisturecontent	Correlation Coefficient	-0.368	.565*	1.000	.967**	-0.300	-0.041	-.556*	.897**	-0.228	0.433	-0.028	0.039	-0.155	-0.093	-0.086
		Sig. (2-tailed)	0.216	0.044		0.000	0.320	0.893	0.049	0.000	0.454	0.140	0.928	0.899	0.614	0.763	0.781
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Wateractivity	Correlation Coefficient	-0.476	.556*	.967**	1.000	-0.265	-0.100	-.617*	.941**	-0.200	0.471	0.024	0.080	-0.126	-0.061	-0.043
		Sig. (2-tailed)	0.100	0.049	0.000		0.381	0.745	0.025	0.000	0.513	0.105	0.938	0.795	0.682	0.842	0.889
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	pH	Correlation Coefficient	-0.303	-.858**	-0.300	-0.265	1.000	0.008	-0.429	-0.338	-.592*	-.740**	-.703**	-.683*	-.640*	-.680*	-.688**
		Sig. (2-tailed)	0.313	0.000	0.320	0.381		0.979	0.143	0.259	0.033	0.004	0.007	0.010	0.018	0.011	0.009
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Titratableacidity	Correlation Coefficient	0.052	-0.131	-0.041	-0.100	0.008	1.000	-0.039	-0.028	-0.126	-0.183	-0.316	-0.363	-0.171	-0.221	-0.251
		Sig. (2-tailed)	0.865	0.670	0.893	0.745	0.979		0.901	0.928	0.681	0.549	0.292	0.222	0.576	0.467	0.408
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Solubility	Correlation Coefficient	.840**	0.105	-.556*	-.617*	-0.429	-0.039	1.000	-.647*	.698**	0.213	.569*	0.524	.634*	.645*	.628*
		Sig. (2-tailed)	0.000	0.733	0.049	0.025	0.143	0.901		0.017	0.008	0.486	0.042	0.066	0.020	0.017	0.021
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Bulkdensity	Correlation Coefficient	-0.529	.609*	.897**	.941**	-0.338	-0.028	-.647*	1.000	-0.136	0.517	0.081	0.125	-0.054	-0.007	0.014
		Sig. (2-tailed)	0.063	0.027	0.000	0.000	0.259	0.928	0.017		0.658	0.071	0.792	0.685	0.861	0.982	0.964
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Appearance	Correlation Coefficient	0.522	0.415	-0.228	-0.200	-.592*	-0.126	.698**	-0.136	1.000	.708**	.946**	.912**	.989**	.980**	.972**
		Sig. (2-tailed)	0.067	0.159	0.454	0.513	0.033	0.681	0.008	0.658		0.007	0.000	0.000	0.000	0.000	0.000
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Colour	Correlation Coefficient	0.159	.834**	0.433	0.471	-.740**	-0.183	0.213	0.517	.708**	1.000	.846**	.881**	.742**	.809**	.816**
		Sig. (2-tailed)	0.604	0.000	0.140	0.105	0.004	0.549	0.486	0.071	0.007		0.000	0.000	0.004	0.001	0.001
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Taste	Correlation Coefficient	0.397	.609*	-0.028	0.024	-.703**	-0.316	.569*	0.081	.946**	.846**	1.000	.985**	.963**	.987**	.994**
		Sig. (2-tailed)	0.179	0.027	0.928	0.938	0.007	0.292	0.042	0.792	0.000	0.000		0.000	0.000	0.000	0.000
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Texture	Correlation Coefficient	0.357	.662*	0.039	0.080	-.683*	-0.363	0.524	0.125	.912**	.881**	.985**	1.000	.929**	.969**	.972**
		Sig. (2-tailed)	0.231	0.014	0.899	0.795	0.010	0.222	0.066	0.685	0.000	0.000	0.000		0.000	0.000	0.000
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Flavour	Correlation Coefficient	0.442	0.468	-0.155	-0.126	-.640*	-0.171	.634*	-0.054	.989**	.742**	.963**	.929**	1.000	.983**	.978**
		Sig. (2-tailed)	0.131	0.106	0.614	0.682	0.018	0.576	0.020	0.861	0.000	0.004	0.000	0.000		0.000	0.000
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Mouthfeel	Correlation Coefficient	0.475	.554*	-0.093	-0.061	-.680*	-0.221	.645*	-0.007	.980**	.809**	.987**	.969**	.983**	1.000	.997**
		Sig. (2-tailed)	0.101	0.049	0.763	0.842	0.011	0.467	0.017	0.982	0.000	0.001	0.000	0.000	0.000		0.000
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
	Overallacceptability	Correlation Coefficient	0.461	.566*	-0.086	-0.043	-.688**	-0.251	.628*	0.014	.972**	.816**	.994**	.972**	.978**	.997**	1.000
		Sig. (2-tailed)	0.113	0.044	0.781	0.889	0.009	0.408	0.021	0.964	0.000	0.001	0.000	0.000	0.000	0.000	
		N	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Figure 3: Spearman's rank correlation matrix among physicochemical, Functional and Sensory properties of Synbiotic granulate.

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Conclusion

This study developed and optimized a pearl millet-based synbiotic granulate including *Carica papaya* pulp and probiotic strains (*Pediococcus acidilactici*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*), developed as a functional synbiotic formulation intended for individuals experiencing travel-related fatigue and jet lag. The formulation was optimized using RSM and a CCD for sensory and physicochemical characteristics. The ideal formulation, consisting of 30 mL of papaya pulp and 1.25 g of probiotic blend, attained excellent sensory acceptability along with desirable physicochemical characteristics, such as low moisture content, reduced water activity, high solubility, and stable acidity. The optimized granulate had a desirability score of 0.89, indicating a well-balanced formulation suitable for long-term storage and reconstitution. This synbiotic granulate offers a shelf-stable, nutritionally enhanced functional meal that helps maintain gut health, enhances hydration, and supports recovery during extended travel or in non-refrigerated environments. Further research should investigate its probiotic viability over time, bioavailability, and functional efficacy in real-world settings.

Despite the formulation's promising sensory and physicochemical qualities, this study did not replicate gastrointestinal survival or evaluate the probiotics' long-term survivability during storage. Future research should include in vitro digestion models, shelf-life stability testing over long periods, and in vivo trials in athletic populations to evaluate the functional benefits under real-world travel stress settings.

Author contributions

J.B. conceptualized the study, developed the methodology, conducted the investigation, and wrote the original draft; A.S.A.O. supervised the research, validated the study, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Ethical consideration

The study did not require ethics committee approval, as it was conducted in accordance with institutional and national guidelines. All procedures were carried out in accordance with the ethical standards of Hindustan Institute of Technology and Science, and informed consent was obtained from all participants prior to their participation in the sensory evaluation.

Data availability statement

All data generated or analyzed during this study are included in this manuscript and its supplementary materials.

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