International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 10-07-2026, Accepted: 09-08-2026, Published: 24-08-2026
Volume 11, Issue 3, 2026, Page No. 212-219

Development, optimization and storage study of a synbiotic Kesar mango smoothie

Tarusi[1], Gayatree R Jadeja[2*], B H Joshi[1], Amee Ravani[3], R B Modi[4]
[1] Department of Food Quality Assurance, College of Food Processing Technology and Bio-Energy, Anand Agricultural University, Gujarat, India
[2] Department of Food Science, Polytechnic in Food Science and Home Science, Anand Agricultural University, Gujarat, India
[3] Department of Food Plant Operation, College of Food Processing Technology and Bio-Energy, Anand Agricultural University, Gujarat, India
[4] Department of Food Processing Technology, College of Food Processing Technology and Bio-Energy, Anand Agricultural University, Gujarat, India
Corresponding Author: Gayatree R Jadeja

Abstract
Functional and synbiotic beverages are gaining rapid consumer acceptance owing to their combined probiotic and prebiotic health benefits, yet systematic, enzyme-assisted formulation of fruit-based synbiotic smoothies remains limited, particularly for regionally important cultivars such as Kesar mango (*Mangifera indica* L.). The present study aimed to develop and characterize a synbiotic Kesar mango smoothie through enzymatic liquefaction of mango pulp, incorporation of probiotic yogurt and inulin, and evaluation of its physicochemical, microbiological, sensory and storage attributes. Enzymatic liquefaction of Kesar mango pulp was optimized using a Central Composite Design (pectinase 100-150 IU, cellulase 2-8 IU, temperature 30-45 °C, time 5-15 min) under Response Surface Methodology (RSM), maximizing juice yield and overall acceptability, the optimum conditions (112.50 IU pectinase, 6.50 IU cellulase, 41.25 °C, 11.05 min) gave a validated juice yield of 83.71% and an acceptability score of 7.85 (9-point hedonic scale). The smoothie formulation (mango juice 20-40 g, yogurt 50-75 g, sugar 5-15 g, inulin 3% and xanthan gum 0.2% fixed) was optimized using a D-optimal mixture design, yielding an optimum formulation of 40 g mango juice, 50 g yogurt and 10 g sugar with a predicted acceptability of 8.35 (validated: 8.33). The optimized smoothie contained 75.95% moisture, 1.47% protein, 1.78% fat, 19.15% carbohydrate, 24.50 °Bx TSS, pH 4.46, 0.81% titratable acidity, 47.90% antioxidant activity and 240.60 mg GAE/100 mL total phenolics, with viable *Lactobacillus acidophilus* and *Bifidobacterium animalis* counts of 8.20 and 8.01 log CFU/mL, respectively, and no detectable yeast, mould or coliforms. During 27 days of refrigerated storage (5 ± 2 °C), overall acceptability declined from 8.33 to 5.62 while probiotic counts remained above the therapeutic minimum of 6 log CFU/mL throughout, indicating a sensorially acceptable and microbiologically safe shelf life of approximately three weeks. These findings demonstrate the feasibility of Kesar mango as a base for a nutritionally enriched, functionally active dairy-fruit beverage with commercial potential.

Keywords: Synbiotic smoothie, Kesar mango, enzymatic liquefaction, probiotic yogurt, inulin, response Surface methodology, refrigerated storage

Introduction
Consumer preference for foods and beverages has, over the past decade, shifted from mere hunger satisfaction toward products that simultaneously support health, convenience and wellness. Functional foods - those containing biologically active components capable of beneficially influencing target physiological functions when consumed as part of a normal diet - have consequently gained prominence, with fruit- and dairy-based matrices considered especially suitable owing to their inherent supply of vitamins, minerals, dietary fibre, antioxidants, fermentable substrates and beneficial micro-organisms (Essa *et al.*, 2023) [11]. Among functional foods, beverages occupy a privileged position because their liquid or semi-solid form permits convenient consumption and straightforward fortification with bioactive ingredients; probiotic beverages enriched with fibre, protein, plant extracts or prebiotics are increasingly formulated for digestive health, immunity and satiety (Gallina *et al.*, 2019) [15].

The synbiotic concept - the deliberate combination of live micro-organisms and selectively fermentable substrates that jointly confer host benefit - carries a specific technological meaning in food systems: the probiotic organism must remain viable throughout processing and storage, and the prebiotic component must not compromise flavour, texture or gastrointestinal tolerance. Fermented milk, yogurt, fruit juice and smoothie matrices employing *Lactobacillus acidophilus, Lactobacillus casei*, *Bifidobacterium spp.* and *Streptococcus thermophilus* have been most extensively studied, with prebiotic inulin frequently added to enhance functional value while modulating mouthfeel, total solids and microbial survival (Swanson *et al.*, 2020) [42]. Probiotic efficacy is strain-specific and governed by viable count, acid tolerance and survival under the acidic, oxygenated conditions typical of fruit-containing beverages during refrigerated storage (Boyte *et al.*, 2023) [5], while inulin, as a soluble, mildly sweet and bifidogenic fibre, interacts with milk proteins and hydrocolloids to influence viscosity and serum stability in smoothie systems (Teferra, 2021) [44].

Mango (*Mangifera indica* L.) is among the most economically and nutritionally significant tropical fruits grown in India, prized for its colour, aroma, sweetness, carotenoids, vitamin C and phenolic content. The Kesar cultivar, closely identified with Gujarat and neighbouring growing regions, is particularly valued for its saffron-yellow pulp and distinctive flavour (Rakholiya *et al.*, 2016) [31], making it an attractive base for smoothie development from sensory, nutritional and commercial standpoints. Its phytochemical profile - including mangiferin, quercetin derivatives, gallic acid derivatives and carotenoids - contributes antioxidant capacity, while its total soluble solids, acidity and viscosity govern the texture and acceptability of derived products (Gupta *et al.*, 2022) [16]. Given the seasonality and perishability of fresh mango, value addition through pulp, nectar, purée, yogurt or smoothie manufacture offers both diversification and commercial opportunity (Gupta *et al.*, 2022) [16].

Smoothies - blended beverages typically combining fruit pulp or juice with milk, yogurt, sweeteners and stabilizers - retain a portion of the fruit fibre matrix and are perceived as more satiating than conventional juices, though they remain a comparatively nascent category in the Indian market relative to lassi, buttermilk (chaas) and fruit juices (Škegro *et al.*, 2021) [40]. The Indian functional beverage sector has nonetheless shown strong growth, with the probiotic drinks segment projected to expand at a compound annual growth rate of approximately 16.7% between 2025[18] and 2033, driven by urbanization, cold-chain expansion, organized retail and rising gut-health awareness (IMARC Group, 2025) [18]. Traditional fermented dairy beverages provide a favourable cultural platform for yogurt-based smoothie development; however, commercial uptake remains constrained by high cost, refrigeration dependence, limited shelf life and a scarcity of products supported by validated functional claims (Raman *et al.*, 2019) [32].

Formulating a fruit-yogurt-probiotic smoothie is technically demanding: fruit acidity, yogurt flavour, sugar level, stabilizer concentration, inulin dose and probiotic culture must be jointly balanced to avoid pulp separation, excessive viscosity, post-acidification and flavour deterioration during storage (Ribeiro *et al.*, 2017) [35], while microbial safety and probiotic viability must be maintained against the risk of yeast and mould proliferation under refrigerated, moderately acidic, sugar- and moisture-rich conditions (Boyte *et al.*, 2023) [5]. Despite the existing body of work on probiotic, fruit-yogurt and fibre-fortified dairy beverages, a specific gap persists regarding the systematic, enzyme-assisted formulation and optimization of a synbiotic smoothie based on Kesar mango pulp: prior studies have generally addressed non-dairy or generic fruit-probiotic systems, examined probiotic viability and prebiotic incorporation in isolation, or omitted enzymatic pulp liquefaction as a pre-processing step, leaving the combined optimization of liquefied pulp, yogurt, probiotic culture and prebiotic fibre within a single Kesar mango-based product largely unexplored (Gallina *et al.*, 2019) [15].

The present study was therefore undertaken to develop and characterize a synbiotic Kesar mango smoothie incorporating enzymatically liquefied Kesar mango pulp, probiotic yogurt, inulin, xanthan gum and sugar, packaged and stored under refrigerated conditions. The specific objectives were to: (i) characterize the compositional and physicochemical properties of Kesar mango pulp; (ii) optimize enzymatic liquefaction of the pulp using Response Surface Methodology; (iii) optimize the synbiotic smoothie formulation using a mixture design; and (iv) characterize the physicochemical, microbiological and sensory properties of the optimized smoothie and evaluate its stability during refrigerated storage.

Materials and Methods

The study was conducted at the College of Food Processing Technology and Bio-Energy, Anand Agricultural University (AAU), Anand, Gujarat, India.

1. Raw materials

Fresh, mature Kesar mangoes (*Mangifera indica* L., cv. Kesar) were procured from the College of Horticulture, AAU, Anand. Pectinase and cellulase enzymes were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). A commercial freeze-dried direct vat set (DVS) starter-probiotic culture blend (Delvo® Yog FVV-121AB DSL; DSM), containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* as starter organisms together with the probiotic strains *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. lactis, was used for yogurt fermentation. Food-grade inulin (Pure Nutrition, Mumbai) and xanthan gum (Sattvic Foods, Panaji, Goa) were procured commercially, and sugar was sourced from the local retail market, Anand. All chemicals used for analysis were of analytical grade, and glassware conformed to Bureau of Indian Standards specifications. Sterile glass bottles (Axar Blow Plast, Anand) were used for product packaging and storage.

2. Preparation of probiotic yogurt

Standardized milk (3.0% fat, 8.5% solids-not-fat) was heat-treated at 90 °C for 5 min, cooled to 42 ± 1 °C, and inoculated with the DVS starter-probiotic blend at 0.0125% (w/v). Fermentation was carried out at 42 ± 1 °C until the pH reached 5.0 ± 0.1 (approximately 4-5 h), after which the yogurt was rapidly cooled to 5 ± 2 °C and held under refrigeration until use.

3. Preparation of Kesar mango pulp

Sorted, washed, peeled and deseeded mango slices were blended to a uniform pulp using a laboratory mixer-grinder, packed in metallized laminate pouches, and stored at -18 °C until further processing.

4. Physicochemical and biochemical analysis

Moisture, crude fat (SOCS-plus solvent extraction), crude fibre (FIBRA-plus acid-alkali digestion), crude protein (Kel-plus micro-Kjeldahl, N × 6.25) and ash content were determined following AOAC (2019) procedures; carbohydrate content was estimated by difference. Total sugars were determined by the Lane and Eynon titrimetric method (Ranganna, 2007) [33]; total phenolic content by the Folin-Ciocalteu method, expressed as mg gallic acid equivalent (GAE)/100 g or mL (Kunnika & Pranee, 2011) [20]; antioxidant activity by DPPH radical scavenging (Brand-Williams *et al*., 1995) [6]; titratable acidity by NaOH titration expressed as % citric acid (Gupta *et al.*, 2010) [17]; and total soluble solids (TSS) by digital refractometry.

5. Optimization of enzymatic liquefaction of Kesar mango pulp

Preliminary trials established that a combined pectinase-cellulase treatment gave synergistically superior juice yield, flavour and colour release compared with both enzyme alone, and this combination was selected for optimization. A four-factor Central Composite Design (CCD) under Response Surface Methodology (RSM) was employed, varying pectinase concentration (100-150 IU), cellulase concentration (2-8 IU), incubation temperature (30-45 °C) and incubation time (5-15 min) across 30 experimental runs (Design-Expert v13.0.6). Following incubation in a BOD incubator, enzymes were thermally inactivated (80 °C, 10 min) and the liquefied pulp filtered to obtain mango juice. Juice yield, viscosity (Brookfield DV-II+ Pro viscometer, spindle 63, 50 rpm, 25 ± 1 °C) and TSS were determined, and sensory quality (colour and appearance, aroma, consistency, taste, overall acceptability) was evaluated by a semi-trained panel using a 9-point hedonic scale. Juice yield and overall acceptability were modelled as quadratic responses and optimized using the desirability function.

6. Optimization of the synbiotic smoothie formulation

Based on preliminary trials, xanthan gum and inulin were fixed at 0.2% and 3%, respectively, as levels below 0.2% xanthan gum produced visible whey separation while levels above it imparted excessive viscosity. A three-component D-optimal mixture design under RSM was used to optimize Kesar mango juice (20-40 g), probiotic yogurt (50-75 g) and sugar (5-15 g) across 16 experimental runs. Mango juice and yogurt were homogenized in a blender, followed by gradual addition of sugar, xanthan gum and inulin with continuous blending; the smoothie was packaged in sterile glass bottles and stored at 5 ± 2 °C. Overall acceptability, physicochemical properties (TSS, colour by Lovibond tintometer, viscosity) and probiotic viability (selective pour-plating of L. acidophilus on Bile-MRS agar, 37 ± 2 °C, 72 h aerobic; *B. animalis* on LP-MRS agar, 37 ± 2 °C, 72 h anaerobic, expressed as log CFU/mL) were used as responses, and the formulation was optimized via the desirability function and validated experimentally.

7. Characterization and storage study of the optimized smoothie

The optimized synbiotic smoothie was characterized for proximate composition, total sugars, total phenolic content, antioxidant activity, titratable acidity and TSS as described above (Sections 2.4-2.6), and for microbiological quality including viable probiotic count, aerobic plate count (nutrient agar, 37 °C, 24 h), yeast and mould count (potato dextrose agar, pH 3.5, 25 ± 2 °C, 3-5 days) and coliform count (MacConkey agar, 37 °C, 48 h). For the storage study, the smoothie was stored at 5 ± 2 °C and evaluated at 3-day intervals for sensory attributes (colour and appearance, taste, aroma, consistency, overall acceptability) and probiotic viable counts throughout the storage period.

8. Statistical analysis

Design-Expert software (v13.0.6, Stat-Ease Inc.) was used for experimental design generation, regression modelling, analysis of variance (ANOVA) and optimization by the desirability function for both the CCD (enzymatic liquefaction) and the mixture design (smoothie formulation). Model adequacy was assessed via coefficient of determination (R^2), adequate precision and lack-of-fit tests, and the optimized conditions were validated experimentally against 95% prediction intervals.

Results and Discussion

1. Instruments and analytical facilities used

A range of calibrated instruments was employed to ensure analytical precision throughout the study: an analytical balance (Mettler-Toledo MS105DU) for mass measurements; a hot air oven (Nova Instruments) for moisture determination; FIBRA-plus (Pelican Equipment, Model FES 8) and SOCS-plus (Model SCS8 AS) units for crude fibre and crude fat analysis, respectively; a Kel-plus micro-Kjeldahl digestion-distillation system (Model KES 06R) for protein estimation; a muffle furnace for ashing; a UV-VIS spectrophotometer (Systonics 119) for phenolic and antioxidant assays; a digital pH meter and digital refractometer (Milwaukee MA871) for pH and TSS; a Lovibond tintometer (Model RT850i) for tristimulus colour measurement; a Brookfield viscometer (DV-II+ Pro) for rheological characterization; and an autoclave (Nova Instruments) for sterilization during microbiological work. The use of standardized, calibrated equipment across all analyses ensured that the physicochemical and microbiological data generated were comparable across raw material, in-process and finished-product stages, strengthening the internal validity of the optimization exercise.

2. Compositional and physicochemical characteristics of Kesar mango pulp

Kesar mango pulp comprised 80.25 ± 0.18% moisture, 0.52 ± 0.03% protein, 0.39 ± 0.09% fat, 0.59 ± 0.03% ash, 0.69 ± 0.03% crude fibre and 17.56 ± 0.20% carbohydrate on a wet-weight basis, with TSS of 19.21 ± 0.27 °Brix, pH 4.80 ± 0.26, titratable acidity 0.42 ± 0.05%, total sugars 17.34 ± 0.62%, antioxidant activity 11.13 ± 0.45% and total phenolic content 63.80 ± 1.70 mg GAE/100 g. The high moisture content reflected the succulent nature of ripe pulp, while carbohydrates dominated the solids fraction, consistent with the characteristic sweetness of Kesar mango. This favourable combination of sweetness, mild acidity and appreciable antioxidant/phenolic content confirmed the suitability of Kesar mango pulp as a functional base for beverage development.

Table 1: Proximate and physicochemical composition of Kesar mango pulp (mean ± SD).

Parameter	Value
Moisture (%)	80.25 ± 0.18
Protein (%)	0.52 ± 0.03
Fat (%)	0.39 ± 0.09
Ash (%)	0.59 ± 0.03
Crude fibre (%)	0.69 ± 0.03
Carbohydrate (%)	17.56 ± 0.20
TSS (°Brix)	19.21 ± 0.27
pH	4.80 ± 0.26
Titratable acidity (% citric acid)	0.42 ± 0.05
Total sugars (%)	17.34 ± 0.62
Antioxidant activity (%)	11.13 ± 0.45
Total phenolic content (mg GAE/100 g)	63.80 ± 1.70

These values are broadly consistent with reported ranges for Kesar and other Indian mango cultivars, in which moisture typically dominates fresh pulp composition and carbohydrate content governs sweetness and downstream processing behaviour (Lad *et al.,* 2017; Tasie *et al.,* 2020; Odion *et al.,* 2020) [21, 27, 43]. The measured phenolic and antioxidant levels support previous observations that mango pulp is a meaningful dietary source of polyphenolic antioxidants, including mangiferin and gallic acid derivatives, which may confer additional functional value beyond simple caloric and fibre contribution (Madalageri *et* *al.*, 2015; Maldonado-Celis *et al.*, 2019) [23, 24]. The moderate pH and titratable acidity of the raw pulp also indicated that limited additional acidification would occur upon blending with fermented yogurt, an important consideration for controlling the final pH and organoleptic balance of the smoothie.

3. Optimization of enzymatic liquefaction of Kesar mango pulp

Preliminary single-enzyme trials confirmed that pectinase or cellulase alone produced incomplete cell-wall disruption and inferior sensory outcomes, whereas their combined application synergistically degraded pectin and cellulose components of the pulp matrix, improving juice release, flavour and colour extraction - an outcome consistent with reports of combined pectinase-cellulase treatments enhancing juice recovery in other fruit systems (Sharma *et al.*, 2017; Ozyilmaz & Gunay, 2023; Adelakun *et al.*, 2020) [1, 29, 38]. The complete four-factor CCD comprising 30 runs, used to optimize this combined treatment, is presented in Table 2.

Table 2. Central Composite Design matrix for optimization of enzymatic liquefaction of Kesar mango pulp

Run	Pectinase (IU)	Cellulase (IU)	Temp. (°C)	Time (min)
1	137.5	3.5	33.75	12.5
2	125.0	5.0	37.50	15.0
3	125.0	5.0	30.00	10.0
4	100.0	5.0	37.50	10.0
5	137.5	6.5	33.75	7.5
6	112.5	6.5	33.75	12.5
7	112.5	3.5	33.75	7.5
8	137.5	6.5	33.75	12.5
9	112.5	3.5	41.25	7.5
10	125.0	5.0	37.50	5.0
11	125.0	8.0	37.50	10.0
12	137.5	3.5	41.25	7.5
13	137.5	6.5	41.25	12.5
14	125.0	5.0	37.50	10.0
15	125.0	5.0	37.50	10.0
16	112.5	6.5	41.25	7.5
17	125.0	5.0	45.00	10.0
18	112.5	6.5	41.25	12.5
19	125.0	5.0	37.50	10.0
20	112.5	3.5	33.75	12.5
21	137.5	6.5	41.25	7.5
22	125.0	2.0	37.50	10.0
23	112.5	6.5	33.75	7.5
24	112.5	3.5	41.25	12.5
25	125.0	5.0	37.50	10.0
26	137.5	3.5	41.25	12.5
27	125.0	5.0	37.50	10.0
28	137.5	3.5	33.75	7.5
29	125.0	5.0	37.50	10.0
30	150.0	5.0	37.50	10.0

Across these 30 runs, juice yield ranged from 78.26 to 86.58% and overall acceptability from 6.41 to 7.91 on the 9-point hedonic scale. The fitted quadratic models for both responses were highly significant ($p < 0.0001$) with satisfactory coefficients of determination ($R^2 = 0.9324$ for juice yield; $R^2 = 0.9402$ for overall acceptability), adequate precision values of 17.40 and 13.79 respectively (both comfortably exceeding the desirable threshold of 4, which indicates an adequate signal-to-noise ratio for navigating the design space), and non-significant lack of fit, confirming reliable predictive capability of both models. Pectinase concentration and incubation temperature were identified as the most influential factors for both responses, consistent with their central role in pectic-substance hydrolysis and thermal activation of enzymatic action during cell-wall disruption and juice release. This agrees with earlier reports that pectinase dose and reaction temperature are typically the dominant variables governing yield and clarity in enzyme-assisted fruit juice extraction (Sreenath *et al.*, 1995; Sakhale *et al.*, 2016) [37, 41], while excessively high cellulase levels or prolonged incubation can promote undesirable browning or flavour deterioration, underscoring the value of a bounded, statistically optimized process window rather than maximal enzyme dosing.

Numerical optimization via the desirability function identified an optimum treatment of 112.50 IU pectinase, 6.50 IU cellulase, 41.25 °C and 11.05 min, predicting a juice yield of 83.49% and acceptability of 7.91 (overall desirability = 0.857). Experimental validation under these conditions gave a mean juice yield of 83.71% and acceptability of 7.85, both within their respective 95% prediction intervals, confirming the robustness and predictive adequacy of the optimized enzymatic liquefaction process and its suitability for direct application in smoothie formulation without a separate clarification step.

4. Optimization of the synbiotic smoothie formulation

Preliminary screening trials established that xanthan gum levels below 0.2% resulted in visible sedimentation and whey separation, whereas levels above 0.2% imparted excessive viscosity and a gummy mouthfeel; 0.2% xanthan gum and 3% inulin were therefore fixed as the stabilizing and prebiotic components, consistent with reported optimal ranges for hydrocolloid stabilization of fruit-dairy beverages (Elderwy *et al.*, 2025; Asase & Glukhareva, 2024) [4, 10] and for prebiotic inulin fortification without adverse sensory impact (Shoaib *et al.*, 2016; Teferra, 2021) [39, 44]. The remaining three components - mango juice, yogurt and sugar - were varied according to the D-optimal mixture design shown in Table 3.

Table 3: D-optimal mixture design matrix for optimization of mango juice, yogurt and sugar proportions in the synbiotic Kesar mango smoothie.

Run	Mango juice (%)	Yogurt (%)	Sugar (%)
1	20.0	74.5	5.5
2	27.6	67.4	5.0
3	27.4	62.6	10.0
4	28.5	56.5	15.0
5	33.9	56.1	10.0
6	20.0	65.5	14.5
7	22.1	68.8	9.1
8	39.0	56.0	5.0
9	27.4	62.6	10.0
10	33.9	51.1	15.0
11	27.4	62.6	10.0
12	33.9	61.1	5.0
13	20.0	65.5	14.5
14	40.0	50.7	9.3
15	40.0	50.7	9.3
16	33.9	51.1	15.0

Across these 16 formulations, overall acceptability ranged from 7.31 to 8.41 on the 9-point hedonic scale. The fitted quadratic mixture model was highly significant ($F = 26.81$, $p < 0.0001$; $R^2 = 0.9306$; adequate precision = 17.45) with non-significant lack of fit, indicating excellent agreement between predicted and observed responses. All binary interaction terms among mango juice, yogurt and sugar significantly influenced acceptability, the yogurt-sugar interaction (BC) exerting the most pronounced effect - reflecting the well-documented synergy between dairy creaminess and moderate sweetness in consumer perception of fermented beverages (Mehta *et al.*, 2017; Raut *et al.*, 2015) [25, 34]. Increasing the mango juice proportion progressively increased TSS and colour intensity (a* and b* values) but reduced viscosity, lightness (L*) and the viable counts of both L. acidophilus and B. animalis, reflecting the diluting effect of reduced yogurt content on the probiotic carrier phase and the comparatively lower buffering capacity of the fruit fraction; notably, probiotic counts across all 16 formulations remained comfortably above the therapeutic minimum of 6-7 log CFU/mL recommended by FAO/WHO (2001) and subsequent probiotic guidance documents (Kechagia *et al.*, 2013) [19], indicating that the yogurt-to-juice ratio evaluated in this design did not compromise functional adequacy even at the lower yogurt extremes.

The optimum formulation identified by the desirability function comprised 40 g mango juice, 50 g yogurt and 10 g sugar, with a predicted acceptability of 8.35 (overall desirability = 0.946). Experimental validation gave an observed acceptability of 8.33, closely matching the predicted value and lying within the 95% prediction interval (8.15-8.55), confirming the reliability of the optimized formulation and its readiness for scale-up trials.

5. Physicochemical, nutritional and microbiological characteristics of the optimized smoothie

The freshly prepared optimized smoothie comprised 75.95 ± 0.24% moisture, 1.47 ± 0.07% protein, 1.78 ± 0.05% fat, 0.67 ± 0.03% ash, 0.98 ± 0.05% crude fibre and 19.15 ± 0.28% carbohydrate, with TSS of 24.50 ± 0.20 °Brix, pH 4.46 ± 0.03, titratable acidity 0.81 ± 0.02%, total sugars 18.81 ± 0.38%, antioxidant activity 47.90 ± 1.45%, total phenolic content 240.60 ± 5.20 mg GAE/100 mL, and viscosity of 1090.31 ± 6.10 cP. The product exhibited a characteristic yellow-orange colour (L* 56.61 ± 0.07, a* 6.32 ± 0.03, b* 40.13 ± 0.11). The marked increase in phenolic content and antioxidant activity relative to raw mango pulp reflected the combined contribution of the yogurt matrix and concentration of soluble solids during formulation. Microbiologically, the smoothie carried viable *L. acidophilus* and *B. animalis* counts of 8.20 ± 0.03 and 8.01 ± 0.03 log CFU/mL, respectively - well above the recommended therapeutic minimum of 6-7 log CFU/mL - while yeast, mould and coliforms remained undetected, confirming hygienic preparation and product safety.

Table 4. Physicochemical, nutritional and microbiological characteristics of the optimized synbiotic Kesar mango smoothie (mean ± SD).

Parameter	Value
Moisture (%)	75.95 ± 0.24
Protein (%)	1.47 ± 0.07
Fat (%)	1.78 ± 0.05
Ash (%)	0.67 ± 0.03
Crude fibre (%)	0.98 ± 0.05
Carbohydrate (%)	19.15 ± 0.28
TSS (°Brix)	24.50 ± 0.20
pH	4.46 ± 0.03
Titratable acidity (% citric acid)	0.81 ± 0.02
Total sugars (%)	18.81 ± 0.38
Antioxidant activity (%)	47.90 ± 1.45
Total phenolic content (mg GAE/100 mL)	240.60 ± 5.20
Viscosity (cP)	1090.31 ± 6.10
L. acidophilus (log CFU/mL)	8.20 ± 0.03
B. animalis (log CFU/mL)	8.01 ± 0.03

The nutritional profile of the optimized smoothie compares favourably with previously reported fruit-yogurt and probiotic smoothie systems, in which protein and fat contributions from the dairy phase combine with fruit-derived carbohydrates, fibre and phenolics to yield a nutritionally balanced beverage (Gallina *et al.*, 2019; Gallina & Barbosa, 2022; Costa *et al.*, 2015) [7, 14, 15]. The substantially higher total phenolic content and antioxidant activity of the finished smoothie relative to raw mango pulp is consistent with reports that fermentation and blending with dairy proteins can increase extractable phenolic activity and free-radical scavenging capacity in fruit-dairy matrices (Ejtahed *et al.*, 2012; Vij *et al.*, 2011) [9, 45], although matrix concentration effects arising from the addition of sugar and stabilizer solids likely also contributed. The viscosity value obtained (1090.31 cP) falls within the range typically reported for stabilized fruit-yogurt smoothies containing xanthan gum, reflecting adequate body and pourability without excessive thickening (Nowicka *et al.*, 2016; Costa *et al.*, 2015) [7, 26].

6. Storage stability of the synbiotic smoothie

During 27 days of refrigerated storage (5 ± 2 °C), with evaluations conducted at 3-day intervals, all sensory attributes - colour and appearance, aroma, taste, consistency and overall acceptability - declined gradually and significantly ($p \leq 0.05$) with increasing storage period. The decline was modest up to day 12 but became more pronounced thereafter, with taste and aroma most strongly affected owing to progressive lactic-acid accumulation and consequent souring, a pattern widely reported for refrigerated probiotic fruit-dairy beverages in which continued metabolic activity of viable starter and probiotic organisms drives post-acidification even at chill temperatures (Oğuz *et al.*, 2023; Dan *et al.*, 2023) [5, 8]. Colour and appearance and consistency attributes were comparatively more stable, consistent with the antioxidant protection afforded by mango phenolics against pigment degradation and with the stabilizing action of xanthan gum against phase separation over the storage period (Zakariás *et al.*, 2024; Picouet *et al.*, 2016) [30, 47].

Table 5: Overall sensory acceptability and probiotic viability of the synbiotic Kesar mango smoothie during refrigerated storage (5 ± 2 °C).

Storage Period (Days)	Overall Acceptability	*Lactobacillus acidophilus* (log CFU/mL)	*Bifidobacterium animalis* (log CFU/mL)
0	8.33 ± 0.09	8.20 ± 0.03	8.01 ± 0.03
3	8.22 ± 0.09	8.15 ± 0.04	7.96 ± 0.04
6	8.00 ± 0.10	8.08 ± 0.04	7.90 ± 0.04
9	7.72 ± 0.10	7.98 ± 0.04	7.80 ± 0.05
12	7.38 ± 0.10	7.86 ± 0.05	7.68 ± 0.05
15	7.00 ± 0.11	7.72 ± 0.05	7.52 ± 0.05
18	6.62 ± 0.11	7.52 ± 0.06	7.34 ± 0.06
21	6.24 ± 0.12	7.30 ± 0.06	7.12 ± 0.06
24	5.90 ± 0.12	7.02 ± 0.07	6.88 ± 0.07
27	5.62 ± 0.13	6.78 ± 0.07	6.62 ± 0.07

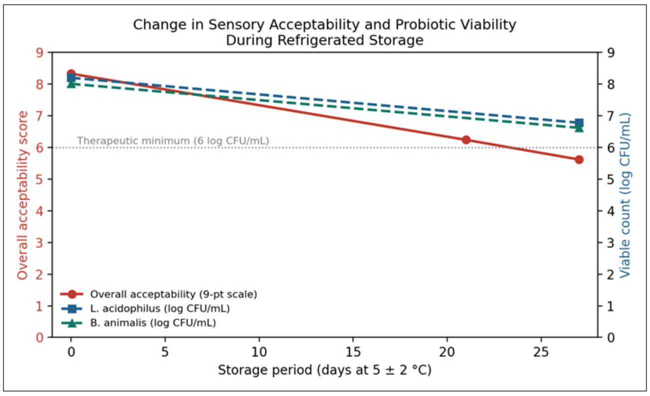

Fig 1: Change in overall sensory acceptability and probiotic viability of the synbiotic Kesar mango smoothie during 27 days of refrigerated storage (5 ± 2 °C).

Overall acceptability fell from 8.33 on day 0 to 6.24 by day 21 and further to 5.62 by day 27, with the product remaining within a generally acceptable sensory range (score ≥ 6, corresponding to "like slightly" or better on the hedonic scale) up to approximately day 21 of storage. Viable counts of *L. acidophilus* and *B. animalis* decreased steadily throughout storage, from 8.20 and 8.01 log CFU/mL on day 0 to 6.78 and 6.62 log CFU/mL, respectively, on day 27, yet remained above the minimum therapeutic threshold of 6 log CFU/mL throughout the entire storage period, indicating that the smoothie retained probiotic functionality even at the end of the study. *B. animalis* exhibited a marginally greater decline than *L. acidophilus*, consistent with its comparatively higher acid sensitivity and lower tolerance of the mildly acidic, oxygenated conditions typical of refrigerated fruit-yogurt matrices (Xie *et al.*, 2024; Andreasen *et al.*, 2010) [2, 46]. Yeast, mould and coliform organisms remained undetected throughout the storage period, confirming that the product remained microbiologically safe and hygienically stable under refrigerated conditions, and that the combination of pasteurized milk base, hygienic pulp handling and cold-chain storage effectively controlled spoilage-organism proliferation despite the fruit- and sugar-rich composition of the beverage (Boyte *et al.*, 2023; Falah *et al.*, 2021) [5, 12].

Taken together, the sensory and microbiological data indicate that the shelf life of the synbiotic Kesar mango smoothie is ultimately governed by sensory rather than safety limits: probiotic viability and microbiological safety were sustained well beyond the point at which sensory acceptability fell below a commercially desirable threshold. This is a common finding for refrigerated probiotic fruit-dairy beverages (Luciano *et al.*, 2018[22]; Škegro *et al.*, 2021) and suggests that, from a functional-food perspective, the practical shelf life of approximately three weeks reported here is a conservative, sensory-driven estimate rather than a probiotic-viability-limited one.

Limitations and Future Scope

Several limitations of the present study should be acknowledged. The sensory panel, while adequately trained for discriminative evaluation, was semi-trained rather than a fully trained descriptive panel, and consumer-level hedonic testing with a larger, demographically diverse panel would strengthen claims of commercial acceptability. The storage study was restricted to a single refrigerated temperature (5 ± 2 °C) and to glass-bottle packaging; evaluation across a wider temperature range, and comparison with alternative packaging formats (e.g., PET, retort pouches, aseptic cartons), would clarify the robustness of the three-week shelf-life estimate under real distribution conditions. Nutrient retention and probiotic viability under simulated gastrointestinal digestion were not assessed in this study and represent an important direction for confirming the physiological relevance of the reported viable counts. Finally, while the RSM models demonstrated strong statistical adequacy within the studied design space, pilot- or industrial-scale validation, together with a formal cost-benefit and shelf-life economic analysis, would be required before commercial translation. Future work should also explore partial or complete replacement of added sugar with natural sweeteners or additional inulin to enhance the functional positioning of the product, in line with recent trends toward reduced-sugar functional mango beverages (Saentaweesuk *et al.*, 2025) [36], and should examine alternative or additional probiotic strains and encapsulation strategies to further extend viable shelf life.

Conclusion

This study demonstrated the feasibility of converting Kesar mango pulp into a nutritionally enriched, sensorially acceptable and microbiologically safe synbiotic beverage through a systematic, RSM-based approach spanning enzymatic liquefaction and formulation optimization. Kesar mango pulp exhibited a favourable proximate and phytochemical profile suited to functional beverage development. Combined pectinase-cellulase liquefaction, optimized by Central Composite Design, enhanced juice yield to as high as 86.58% while preserving sensory quality, with pectinase concentration and incubation temperature identified as the dominant process variables. Mixture Response Surface Methodology successfully optimized the proportions of mango juice, yogurt and sugar, yielding a formulation (40 g mango juice, 50 g yogurt, 10 g sugar) with high acceptability (8.35) and robust probiotic viability, validated by close agreement between predicted and experimental responses. The resulting synbiotic smoothie was nutritionally rich, exhibited desirable physicochemical and colour properties, and carried probiotic counts exceeding 8 log CFU/mL with excellent microbiological safety. Under refrigerated storage, the product retained acceptable sensory quality for approximately three weeks and sustained probiotic viability above the therapeutic minimum throughout the 27-day storage period, while remaining free of yeast, mould and coliform contamination. The developed synbiotic Kesar mango smoothie therefore represents a commercially promising, value-added, health-oriented dairy-fruit beverage with an indicative refrigerated shelf life of about three weeks, offering a pathway for diversifying Kesar mango utilization and expanding the functional beverage segment of the Indian dairy-fruit market.

References

1. Adelakun OE, Oke MO, Akande EA, Adebiyi-Olabode AO. Extraction of mango juice with pectinase influences quality. Asian Food Science Journal,2020:16(3):43-52.
2. Andreasen AS, Larsen N, Pedersen-Skovsgaard T, Berg RM, Møller K, Svendsen KD, et al. Effect of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. British Journal of Nutrition,2010:104(12):1831-1838.
3. AOAC. Official methods of analysis. Association of Official Analytical Chemists, Washington, D.C., USA, 2019.
4. Asase RV, Glukhareva TV. Production and application of xanthan gum-prospects in the dairy and plant-based milk food industry: a review. Food Science and Biotechnology,2024:33(4):749-767.
5. Boyte ME, Benkowski A, Pane M, Shehata HR. Probiotic and postbiotic analytical methods: a perspective of available enumeration techniques. Frontiers in Microbiology,2023:14:1304621.
6. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology,1995:28(1):25-30.
7. Costa MP, Frasao BS, Silva ACO, Freitas MQ, Franco RM, Conte-Junior CA. Cupuassu (*Theobroma grandiflorum*) pulp, probiotic, and prebiotic: Influence on color, apparent viscosity, and texture of goat milk yogurts. Journal of Dairy Science,2015:98(9):5995-6003.
8. Dan T, Hu H, Tian J, He B, Tai J, He Y. Influence of different ratios of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* on fermentation characteristics of yogurt. Molecules,2023:28(5):2123.
9. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition,2012:28(5):539-543.
10. Elderwy Y, Kalita R, Hamouda ME, Chaudhary P, Elfaruk MS, Don PU, et al. Influence of guar gum and xanthan gum on the rheological behavior, texture and microstructure of probiotic low-fat yogurt. Processes,2025:13(10):3301.
11. Essa MM, Bishir M, Bhat A, Chidambaram SB, Al-Balushi B, Hamdan H, et al. Functional foods and their impact on health. Journal of Food Science and Technology,2023:60(3):820-834.
12. Falah F, Vasiee A, Yazdi FT, Behbahani BA. Preparation and functional properties of synbiotic yogurt fermented with *Lactobacillus brevis* PML1 derived from a fermented cereal-dairy product. BioMed Research International,2021:2021:1057531.
13. FAO, WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Food and Agriculture Organization of the United Nations, & World Health Organization, 2001.
14. Gallina DA, Barbosa PDPM. Viability of probiotics, physicochemical and microbiological characterization of beverage (smoothie) with symbiotic yogurt and berries pulp. Research, Society and Development,2022:11(3):19511325975.
15. Gallina DA, Barbosa PDPM, Ormenese RDCSC, Garcia ADO. Development and characterization of probiotic fermented smoothie beverage. Revista Ciência Agronômica,2019:50:378-386.
16. Gupta AK, Gurjar PS, Beer K, Pongener A, Ravi SC, Singh S, et al. A review on valorization of different byproducts of mango (*Mangifera indica* L.) for functional food and human health. Food Bioscience,2022:48:101783.
17. Gupta S, Cox S, Abu-Ghannam N. Process optimization for the development of a functional beverage based on lactic acid fermentation of oats. Biochemical Engineering Journal,2010:52(2-3):199-204.
18. IMARC Group. India functional beverages market: Growth, trends and forecast 2025-2033. https://www.imarcgroup.com/india-functional-beverages-market, 2025.
19. Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, et al. Health benefits of probiotics: A review. International Scholarly Research Notices,2013:2013:481651.
20. Kunnika S, Pranee A. Influence of enzyme treatment on bioactive compounds and colour stability of betacyanin in flesh and peel of red dragon fruit *Hylocereus polyrhizus* (Weber) Britton and Rose. International Food Research Journal,2011:18(4):1437-1448.
21. Lad P, Khodke SK, Salunkhe RV. Studies on physico-chemical properties of different varieties of mango. Agricultural Update,2017:12:2352-2360.
22. Luciano WA, Matte TC, Portela IA, de Medeiros LL, dos Santos Lima M, Maciel JF, et al. Effects of *Lactobacillus acidophilus* LA-3 on physicochemical and sensory parameters of açaí and mango based smoothies and its survival following simulated gastrointestinal conditions. Food Research International,2018:114:159-168.
23. Madalageri D, Bharati PC, Orsat V, Raghavan V, Kage U. Antioxidant activity in pulp and peel of three mango varieties. Journal of Horticultural Sciences,2015:10(2):199-209.
24. Maldonado-Celis ME, Yahia EM, Bedoya R, Landázuri P, Loango N, Aguillón J, et al. Chemical composition of mango (*Mangifera indica* L.) fruit: Nutritional and phytochemical compounds. Frontiers in Plant Science,2019:10:1073.
25. Mehta D, Kumar MS, Sabikhi L. Development of high protein, high fibre smoothie as a grab-and-go breakfast option using response surface methodology. Journal of Food Science and Technology,2017:54(12):3859-3866.
26. Nowicka P, Wojdyło A, Teleszko M, Samoticha J. Sensory attributes and changes of physicochemical

properties during storage of smoothies prepared from selected fruit. LWT - Food Science and Technology,2016:71:102-109.
27. Odion EE, Nwaokobia K, Ogboru RO, Olorode EM, Gbolagade OT. Evaluation of some physico-chemical properties of mango (*Mangifera indica* L.) pulp in south-south, Nigeria. Ilorin Journal of Science,2020:7(2):236-248.
28. Oğuz Ş, Andiç S, Badak N, Demir T. Determination of probiotic viability in yoghurts produced with acid adapted *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 11863 during refrigerated storage. Turkish Journal of Agriculture-Food Science and Technology,2023:11(12):2426-2434.
29. Ozyilmaz G, Gunay E. Clarification of apple, grape and pear juices by co-immobilized amylase, pectinase and cellulase. Food Chemistry,2023:398:133900.
30. Picouet PA, Hurtado A, Jofré A, Bañon S, Ros JM, Guàrdia MD, et al. Effects of thermal and high-pressure treatments on the microbiological, nutritional and sensory quality of a multi-fruit smoothie. Food and Bioprocess Technology,2016:9(7):1219-1232.
31. Rakholiya K, Kaneria M, Chanda S. Physicochemical and phytochemical analysis of different parts of Indian Kesar mango-a unique variety from Saurashtra region of Gujarat. Pharmacognosy Journal,2016:8(5):502-506.
32. Raman M, Ambalam P, Doble M. Probiotics, prebiotics, and fibers in nutritive and functional beverages. In Nutrients in Beverages. Academic Press,2019:12:315-367.
33. Ranganna S. Handbook of analysis and quality control for fruit and vegetable products (2nd ed.). Tata McGraw-Hill Publications, 2007.
34. Raut V, Sawant P, Sawant D, Ingole AS. Studies on preparation of mango yoghurt drink. Asian Journal of Dairy & Food Research, 2015.
35. Ribeiro LDO, Santos JGCD, Gomes FDS, Cabral LMC, Sá DDGCF, Matta VMD, et al. Sensory evaluation and antioxidant capacity as quality parameters in the development of a banana, strawberry and juçara smoothie. Food Science and Technology,2017:38(4):653-660.
36. Saentaweesuk S, Chaikham P, Jirasatid S. Reduced-calorie mango juice: Quality evaluation of stevia-sweetened and inulin-enriched functional beverage. Food Chemistry Advances, 2025, 101097.
37. Sakhale BK, Pawar VN, Gaikwad SS. Studies on effect of enzymatic liquefaction on quality characteristics of Kesar mango pulp. International Food Research Journal,2016:23(2):860-865.
38. Sharma HP, Patel H, Sugandha. Enzymatic added extraction and clarification of fruit juices - A review. Critical Reviews in Food Science and Nutrition,2017:57(6):1215-1227.
39. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, et al. Inulin: Properties, health benefits and food applications. Carbohydrate Polymers,2016:147:444-454.
40. Škegro M, Putnik P, Bursać Kovačević D, Kovač AP, Salkić L, Čanak I, et al. Chemometric comparison of high-pressure processing and thermal pasteurization: The nutritive, sensory, and microbial quality of smoothies. Foods,2021:10(6):1167.
41. Sreenath HK, Sudarshana Krishna KR, Santhanam K. Enzymatic liquefaction of some varieties of mango pulp. LWT - Food Science and Technology,1995:28(2):196-200.
42. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nature Reviews Gastroenterology & Hepatology,2020:17(11):687-701.
43. Tasie M, Altemımı A, Alı R, Takeoka G. Study of physicochemical properties and antioxidant content of mango (*Mangifera indica* L.) fruit. Eurasian Journal of Food Science and Technology,2020:4(2):91-104.
44. Teferra TF. Possible actions of inulin as prebiotic polysaccharide: A review. Food Frontiers,2021:2(4):407-416.
45. Vij S, Hati S, Yadav D. Biofunctionality of probiotic soy yoghurt. Food and Nutrition Sciences,2011:2(5):502-509.
46. Xie Z, Kim C, Miller MJ, Jin YS. Effects of 2'-fucosyllactose on the viability of starter cultures and *Bifidobacterium* strains of human origin in yogurt during refrigerated storage. Journal of Food Science,2024:89(5):2546-2556.
47. Zakariás F, Hidas KI, Kovacs Z, Bázár G, Taczman-Brückner A, Dalmadi I, et al. Shelf life and organoleptic attributes of multifruit smoothies treated by combined mild preservation technologies. Applied Sciences,2024:14(23):11223.