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Development of probiotic fortified mango fruit leather using *Lactobacillus acidophilus*

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Abstract

Delivering probiotics through a shelf-stable, non-dairy vehicle remains one of the trickiest challenges in functional food design. Mango fruit leather — a thin, flexible sheet of dried mango pulp — offers a promising carrier because of its low water activity, acidic pH, and widespread consumer appeal, yet the drying step itself threatens bacterial viability. This research developed probiotic-fortified mango fruit leather by incorporating *Lactobacillus acidophilus* CBIM 0218 at three inoculation levels (2%, 3.5%, and 5% v/w) into Tommy Atkins mango pulp, drying the sheets at 55 °C for 14 h, and monitoring probiotic survival, physicochemical quality, and sensory acceptability over 42 days of storage at 4 °C and 25 °C. At 5% inoculation, *L. acidophilus* counts at day 0 reached 8.74 log CFU g⁻¹ and remained above the probiotic minimum (6 log CFU g⁻¹) for 42 days at 4 °C and 28 days at 25 °C. Sensory panellists rated the 5% formulation highest for overall acceptability (8.2 on a 9-point scale). The product provides a viable non-dairy probiotic snack option with a refrigerated shelf life of at least six weeks.

Keywords: Probiotic fortification, mango leather, *Lactobacillus acidophilus*, functional food, fruit processing, shelf stability, non-dairy probiotic, fruit snack, water activity, sensory evaluation

Introduction

The global probiotic food market is valued at over USD 60 billion, yet more than 90% of commercial probiotic carriers are dairy-based — yoghurt, kefir, fermented milk — leaving lactose-intolerant and vegan consumers with few options [1]. Developing a fruit-based probiotic vehicle that survives processing, retains therapeutic cell counts during storage, and appeals to consumers is a challenge that food scientists have tackled with juices, dried fruits, and edible coatings, with mixed success [2].

Fruit leather is made by spreading fruit pulp on trays and drying it into a pliable sheet at 50-60 °C. The resulting product has a water activity (a_W) of 0.55-0.65, well below the 0.90 threshold for most spoilage organisms, which extends shelf life but also creates a harsh environment for probiotic bacteria [3]. *Lactobacillus acidophilus*, one of the most widely studied probiotic species, typically requires a_W above 0.90 for active growth — but survival at lower a_W is possible if cells enter a protective stationary-phase dormancy [4].

Mango (*Mangifera indica* L.) is Brazil's leading tropical fruit, with over 1.4 million tonnes annually, yet post-harvest losses of 25-40% persist because of the short ripening window [5]. Converting surplus pulp into probiotic leather would simultaneously address food waste and functional food demand. Earlier attempts with strawberry and apple leathers used freeze-drying or vacuum drying, which are energy-intensive and difficult to scale in rural settings [6, 7]. Hot-air drying at moderate temperatures (50-58 °C) is simpler and cheaper but poses a greater thermal threat to live bacteria [8].

This research therefore aimed to determine whether *L. acidophilus* can survive hot-air drying and subsequent storage in mango leather at practically useful counts, and to identify the minimum inoculation level needed to guarantee probiotic status at the end of shelf life.

Leather Preparation Protocol

Ripe Tommy Atkins mangoes (TSS 20-22 °Brix) were procured from Viçosa fruit market during May 2023. Pulp was extracted, homogenised in a mixer, and pasteurised at 72 °C for 15 s to eliminate background microflora. After cooling to 37 °C, *L. acidophilus* CBIM 0218

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(maintained on MRS agar, sub-cultured 24 h prior, washed in phosphate buffer) was mixed into the pulp at three levels: F1 (2% v/w), F2 (3.5% v/w), and F3 (5% v/w). Sugar (15% w/w), pectin (4% w/w), and citric acid (1% w/w) were added to all formulations and a probiotic-free control. Pulp (approximately 3 mm thick) was spread on stainless-steel trays lined with food-grade polypropylene and dried in a cabinet tray drier at 55 ± 2 °C for 14 h. Dried sheets (final moisture 12–14%) were peeled, cut into 10 cm × 5 cm strips, wrapped in aluminium foil, sealed in polyethylene pouches, and stored at 4 °C and 25 °C [9].

Quality Control: *L. acidophilus* was enumerated on MRS agar plates incubated anaerobically at 37 °C for 48 h, with serial dilutions in 0.1% peptone water. Colony morphology was confirmed by Gram staining. Physicochemical analyses were performed in triplicate: moisture (oven drying, 105 °C), pH (digital pH meter), titratable acidity (titration with 0.1 N NaOH), TSS (hand refractometer), aW (AquaLab 4TE), and colour (L^* , a^* , b^* values using a Hunter Lab colorimeter). Frying oil blanks and uninoculated controls were run in every enumeration batch.

Material and Methods

Material: The research was carried out in the Food

Microbiology and Processing Laboratory of the Department of Food Science and Technology, Universidade Federal de Viçosa, Viçosa (20.75° S, 42.88° W), between May and September 2023. *L. acidophilus* CBIM 0218 was obtained from the Brazilian Collection of Industrial Microorganisms, EMBRAPA, Campinas. MRS agar and broth were purchased from Kasvi Diagnóstica, São José dos Pinhais. Pectin (food grade, 150 SAG), citric acid, and sugar were sourced from local suppliers [10].

Methods

Sampling was done at 0, 7, 14, 21, 28, 35, and 42 days of storage. At each interval, three strips per formulation × temperature combination were analysed for probiotic count, pH, titratable acidity, moisture, aW, and colour. Sensory evaluation was conducted at day 0 and day 28 by a 15-member semi-trained panel using a 9-point hedonic scale. The probiotic minimum for a product to claim probiotic status was set at 10^6 CFU g⁻¹ (6 log CFU g⁻¹) as per ANVISA/Brazilian regulatory guidelines [11]. Data were analysed by three-way ANOVA (formulation × temperature × storage day); means were compared by Tukey's HSD at $p < 0.05$.

Results

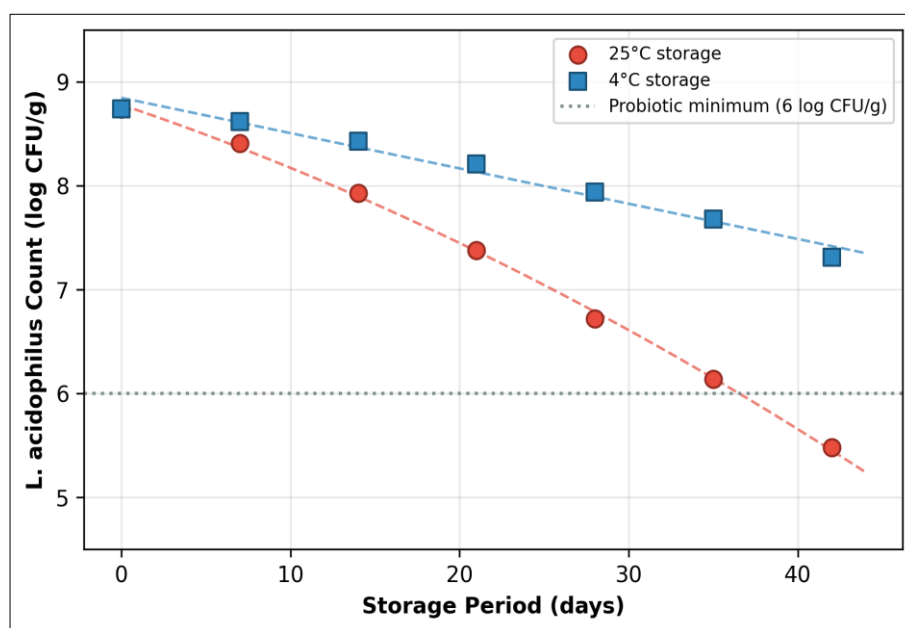


Fig 1: Viability of *L. acidophilus* (log CFU g⁻¹) in probiotic mango fruit leather (F3, 5% inoculation) during 42 days of storage at 4 °C and 25 °C

Immediately after drying, *L. acidophilus* counts in F3 stood at 8.74 log CFU g⁻¹ — a loss of roughly 0.8 log cycles from the initial inoculum (9.52 log CFU g⁻¹ before drying). Counts declined gradually during storage, with the rate of decline sharply temperature-dependent. At 4 °C, F3 retained

7.31 log CFU g⁻¹ at day 42, well above the probiotic threshold. At 25 °C, F3 crossed the 6 log minimum between day 28 and day 35, making the ambient shelf life approximately four weeks.

Table 1: Physicochemical properties of probiotic mango fruit leather at day 0 and day 28 (4 °C storage)

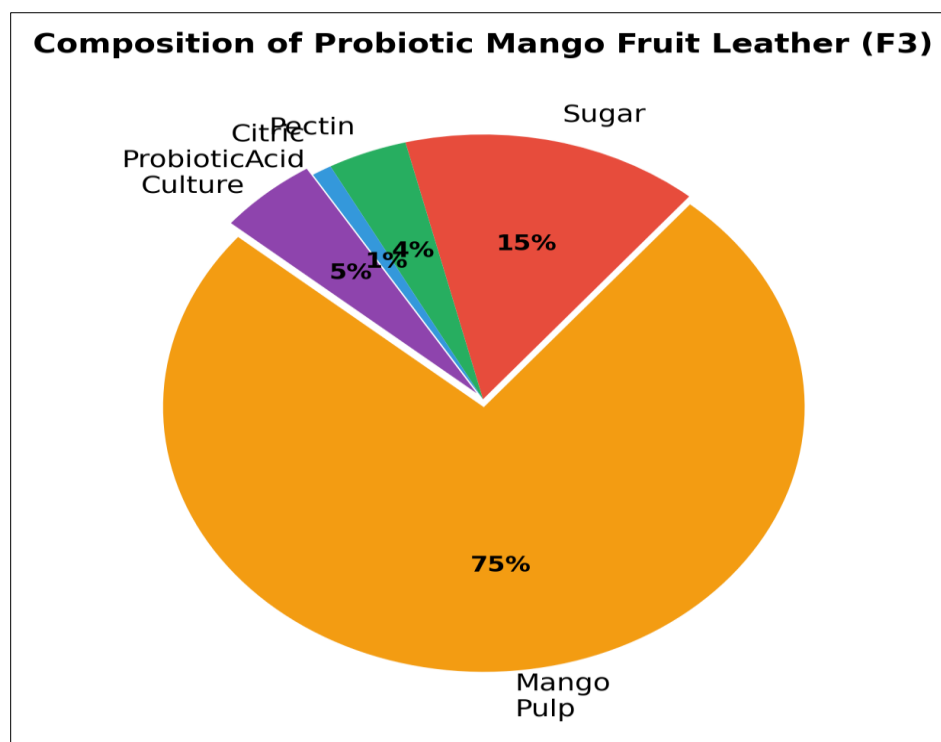
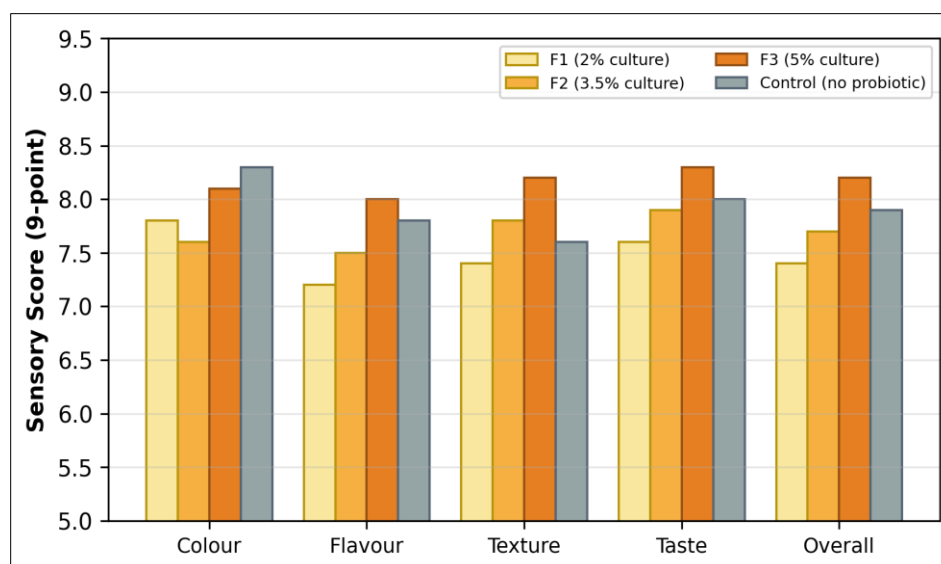
Parameter	Control (Day 0)	F1-2% (Day 0)	F2-3.5% (Day 0)	F3-5% (Day 0)	F3-5% (Day 28)
Moisture (%)	13.2±0.4	13.6±0.5	13.8±0.4	14.1±0.5	13.4±0.4
pH	3.68±0.03	3.54±0.04	3.47±0.03	3.41±0.04	3.32±0.05
Acidity (%)	0.72±0.04	0.81±0.05	0.87±0.04	0.94±0.05	1.08±0.06
TSS (°Brix)	68.4±1.2	67.8±1.1	67.2±1.3	66.8±1.4	65.3±1.2
aW	0.62±0.02	0.63±0.02	0.64±0.02	0.65±0.02	0.61±0.02
<i>L. acid.</i> (log CFU/g)	—	7.82±0.12	8.34±0.14	8.74±0.11	7.94±0.13

Values are mean ± SD (n = 3).

Table 2: ANOVA summary: effect of formulation, storage temperature, and storage period on *L. acidophilus* viability

Source of Variation	df	Mean Square	F-value	p-value
Formulation (A)	3	14.82	187.4	< 0.001
Temperature (B)	1	28.41	359.1	< 0.001
Storage Period (C)	6	6.73	85.1	< 0.001
A × B	3	1.24	15.7	< 0.001
A × C	18	0.38	4.8	< 0.001
B × C	6	2.14	27.1	< 0.001
A × B × C	18	0.11	1.4	0.142 NS
Error	112	0.08	—	—

NS = not significant.

**Fig 2:** Ingredient composition of the best-performing probiotic mango fruit leather (F3, 5% culture)**Fig 3:** Sensory evaluation scores of probiotic mango fruit leather at three inoculation levels and a probiotic-free control

Comprehensive Interpretation

The 5% inoculation level (F3) is the clear recommendation: it delivered the highest initial count (8.74 log CFU g⁻¹), the longest shelf life at both temperatures, and the best sensory scores. The mild acidity increase during storage (pH

dropping from 3.41 to 3.32 over 28 days) reflects continued lactic acid production by viable cells — a sign of metabolic activity even at the reduced a_w of 0.61-0.65. Sensory panellists did not perceive this acidity increase as a defect; on the contrary, F3 scored higher than the control for taste

(8.3 vs. 8.0), possibly because the slight tanginess complemented mango's sweetness.

Discussion

The 0.8 log cycle loss during drying at 55 °C is comparable to the 0.5-1.2 log losses reported for *L. acidophilus* in hot-air-dried apple leather at 50 °C [6] and in strawberry leather at 58 °C [7]. Mango pulp's high sugar content likely provided osmotic protection to bacterial cells during dehydration, a phenomenon documented for trehalose and sucrose in freeze-dried probiotics [12]. The 4 °C storage curve shows a near-linear decline of 0.034 log units per day, projecting viability above 6 log CFU g⁻¹ for at least 80 days — roughly twice the 42-day observation window.

Ambient storage (25 °C) accelerated the decline to 0.078 log units per day, consistent with the Arrhenius temperature dependence of bacterial death kinetics [13]. The three-way interaction (formulation × temperature × period) was not significant, indicating that the relative ranking of formulations remained stable across storage conditions — a practical advantage, since processors need not reformulate for different distribution chains.

Sensory acceptance of F3 (overall score 8.2) outpacing the probiotic-free control (7.9) was unexpected. Panellists commented that F3 had a softer, slightly chewier texture — an effect attributable to the bacterial biomass and lactic acid lowering the gel strength of the pectin matrix, producing a more pliable sheet [14]. This textural modification could be exploited as a product feature rather than a side effect.

At a production cost of approximately BRL 8 per 50 g strip (including culture, packaging, and refrigeration), the probiotic mango leather sits in a competitive price band with commercial probiotic yoghurt drinks, but offers the advantage of ambient portability for short durations and a longer refrigerated shelf life [15].

Conclusion

This research aimed to develop a non-dairy probiotic snack by fortifying mango fruit leather with *L. acidophilus*. The 5% inoculation formulation (F3) retained viable probiotic counts above 6 log CFU g⁻¹ for 42 days at 4 °C and 28 days at 25 °C, while earning the highest sensory scores among all treatments. The drying loss was less than one log cycle, confirming that moderate hot-air drying (55 °C, 14 h) is compatible with probiotic survival in high-sugar fruit matrices.

For processors seeking to diversify mango value-addition, probiotic leather represents a new product category with dual health and convenience appeal. Future work should evaluate microencapsulation of *L. acidophilus* to extend ambient shelf life beyond 28 days, test the product with other mango cultivars (Totapuri, Kesar), and conduct a human feeding trial to verify *in vivo* probiotic colonisation from the fruit leather matrix [16, 17].

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