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Development of Probiotic Enriched Freeze-dried Banana Snacks: Comparative Analysis between Novel Processing Techniques

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ABSTRACT

Background: The development of functional foods incorporating probiotics has gained considerable attention, particularly in minimally processed fruit-based snacks. This study aimed to formulate freeze-dried banana snacks enriched with *Bacillus coagulans* and to assess the influence of two minimal processing techniques, impregnation and sodium alginate-based edible coating on their physicochemical, nutritional, sensory and microbial properties.

Methods: Probiotic banana snacks were prepared using either impregnation or sodium alginate coating, followed by freeze-drying. Physical characterization included assessments of shape, color and dimensional uniformity. Physicochemical analyses measured pH, total soluble solids (TSS) and ascorbic acid content. Nutritional parameters such as protein, fiber and calcium content were evaluated. Sensory attributes were assessed using a structured hedonic scale. The viability of *Bacillus coagulans* was monitored over 180 days of ambient storage to determine the retention of probiotic functionality.

Result: Both the processing methods yielded physically uniform snacks, with coated samples exhibiting slightly higher weight due to biopolymer addition. Coated snacks demonstrated superior physicochemical stability, with higher pH (5.31), TSS (28.42%) and ascorbic acid (7.24 mg/100 g). Nutritional analysis revealed elevated protein (3.18 g/100 g), fiber (4.82 g/100 g) and calcium (16.59 mg/100 g) content in coated samples. Sensory evaluation indicated greater consumer acceptability for coated snacks, particularly in terms of appearance, texture and taste. Probiotic *via*bility remained above the therapeutic threshold (≥8 log CFU/g) throughout 180 days, with coated samples maintaining significantly higher *via*ble counts (8.21 log CFU/g) compared to impregnated samples (7.49 log CFU/g). These findings support the efficacy of edible coating in enhancing the functional, sensory and microbiological stability of probiotic fruit-based snacks.

Key words: *Bacillus coagulans*, Edible coating, Freeze-drying, Impregnation, Probiotic banana snacks.

INTRODUCTION

In recent years, there has been a notable shift in consumer preferences towards foods that offer health-promoting properties beyond their basic nutrition (Cammarelle *et al.,* 2025). This trend is primarily driven by increasing health awareness and a preventive approach to chronic diseases such as diabetes, cardiovascular disorders and gastrointestinal complications (Tufail *et al.,* 2025). Among the various functional foods, probiotics have gained significant attention due to their well-established benefits in enhancing gut microbiota balance, supporting immune function and exerting antimicrobial and anti-inflammatory effects (da Silva Gomes *et al.,* 2024).

The rising interest in non-dairy probiotic products is attributed to changing dietary habits, including veganism and the prevalence of lactose intolerance and dairy allergies. The development of probiotic freeze-dried banana snacks responds to today's growing demand for healthier and convenient foods. With rising issues like obesity, digestive problems and low immunity, there's a clear need for easy ways to add probiotics to everyday diets. Bananas, with their natural sweetness and soft texture make a great base especially for children and adults who may not prefer traditional supplements. Freeze-drying helps preserve nutrients and extend shelf life while reducing food waste.

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This shift has opened new possibilities for incorporating probiotics into plant-based matrices, particularly fruits and vegetables. These matrices not only fulfil the growing demand for allergen-free and sustainable foods but also serve as effective carriers due to their natural content of fiber, vitamins and bioactive compounds (Lillo-p *et al.,* 2025).

Probiotic-enriched snacks have emerged as a novel and convenient format for delivering beneficial microorganisms in alignment with modern dietary patterns. Snacks are widely consumed across all age groups, making them suitable vehicles for functional ingredients. Furthermore, transforming perishable fruits into shelf-stable probiotic snacks offers dual benefits: enhancing health value and minimizing food waste (Niro *et al.,* 2023).

Bananas are a particularly suitable fruit matrix for probiotic application, owing to their nutrient density, digestible sugars, fiber content and widespread consumer acceptance. Their naturally soft texture and pleasant flavor make them ideal for freeze-drying and compatible with probiotic addition without compromising sensory attributes (Yüksel *et al.,* 2025). Previous investigations have documented the effective incorporation of *Bacillus coagulans* into banana-derived snack products, yielding encouraging outcomes regarding microbial viability and consumer acceptability.

In the present investigation, two methodologies were examined for integrating *Bacillus coagulans* into banana-based snack products: impregnation and coating. The impregnation method involves immersing banana slices in a probiotic solution to enable internal colonization. While this technique may provide homogeneous microbial distribution, it may also affect texture due to prolonged exposure to moisture (Niro *et al.,* 2023). In contrast, the coating method utilizes a probiotic-rich alginate solution cross-linked with calcium chloride, forming a protective layer around the fruit surface. This biopolymeric barrier enhances probiotic survival by minimizing oxygen exposure, desiccation and mechanical damage during processing and storage (Pop *et al.,* 2019).

Although both methods have been previously explored in various fruits and vegetables, limited studies have directly compared their performance in banana-based matrices. Furthermore, existing literature often emphasizes probiotic *via*bility, with less focus on nutritional, physicochemical and sensory outcomes (Koh *et al.,* 2022). This study aims to bridge this gap by developing freeze-dried probiotic banana snacks using both impregnation and coating methods. A comprehensive evaluation of physical, chemical, microbial and sensory attributes was conducted to determine the most effective approach for creating functional, shelf-stable snacks suitable for health-conscious consumers.

Though benefits of probiotics are well known, many people still don't get enough of them especially those who avoid dairy probiotic products due to allergies, lactose intolerance, or a vegan lifestyle. This lack can lead to issues like poor digestion, weak immunity and nutrient deficiencies which often go unnoticed. There's a clear need for easy, non-dairy, ready-to-eat probiotic options. However, most research focuses mainly on probiotic survival, with less attention to taste, nutrition or shelf life especially in fruit-based probiotic products. This study aims to fulfil this research gap by developing a healthy, accessible alternative that supports overall well-being.

MATERIALS AND METHODS

The experimental raw materials, reagents, microbial media, equipment and methodologies adopted to conduct the study are described here. The presented research work was conducted at the Department of Food Microbiology and Safety, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra during academic year 2022-25.

Raw materials

Bananas with firm texture, high sugar content and uniform ripeness were selected to ensure structural integrity and sensory appeal post-processing. Fruits were used at a mature yet non-overripe stage to limit enzymatic browning and preserve quality during freeze-drying.

Probiotic strain

Bacillus coagulans was chosen due to its spore-forming nature, offering high resilience to thermal and desiccation stresses. Its ability to survive gastrointestinal conditions and exert probiotic effects such as antimicrobial and immunomodulatory functions makes it suitable for inclusion in minimally processed, plant-based snack formulations (Chen *et al.,* 2021).

Coating and impregnation materials

Sodium alginate and calcium chloride were utilized as coating agents. Sodium alginate, a food-grade biopolymer which forms a gel matrix upon interaction with calcium ions, encapsulating probiotic cells and providing a protective barrier during processing and storage (Rodríguez *et al.,* 2016).

Probiotic incorporation techniques

Two incorporation methods impregnation and coating were employed.

Impregnation technique

Banana slices were immersed in a *Bacillus coagulans* suspension with an initial concentration of 10^9 CFU/mL. The optimal contact time was standardized at 30 minutes, allowing sufficient microbial diffusion into the fruit matrix without compromising texture or structural integrity. This method yielded a final viable count of ~10^8 CFU/g in the freeze-dried product (Niro *et al.,* 2023).

Coating technique

Banana slices were first dipped in a sodium alginate (2% w/v) solution containing *Bacillus coagulans* at 10^9 CFU/mL, followed by cross-linking in a 1% calcium chloride bath for

2 minutes. This process formed a protective gel layer encapsulating the probiotics. The optimized protocol resulted in a final *via*ble count of ~10^8 CFU/g after freeze-drying, demonstrating better probiotic retention compared to the impregnation method (Felix *et al.,* 2023).

Freeze-drying and storage conditions

All treated banana slices were subjected to freeze-drying using a programmable lyophilizer. Samples were first frozen and then dehydrated under reduced pressure to preserve cellular integrity and nutritional content. The dried snacks were stored at 4±2°C to maintain probiotic *via*bility and product stability throughout the storage period (Denkova and Denkova, 2013).

Physical properties

Physical attributes including color, thickness (cm), width (cm), weight (g), geometric mean diameter, arithmetic mean diameter and surface area (cm^2) were measured to evaluate product uniformity and structural integrity. These parameters also provided insight into coating performance and drying efficiency (Sornsenee *et al.,* 2022).

Chemical properties

Chemical characteristics such as pH, titratable acidity, total soluble solids (°Brix) and sugar content were analyzed to assess flavor profile, microbial compatibility and shelf-life potential. These parameters are critical to both probiotic stability and consumer acceptability (Dias *et al.,* 2023).

Proximate composition

Nutritional analysis followed AOAC standard methods to quantify moisture, ash, protein, fat, crude fiber and total carbohydrates. This assessment enabled evaluation of the nutritional impact of each processing method and supported labeling accuracy and health claims (Rascón and Bonilla, 2018).

Mineral analysis

Selected minerals were quantified using atomic absorption spectrophotometry (AAS). Samples were dry-ashed at 500-550°C, digested in nitric-perchloric acid and filtered prior to analysis. Mineral profiling was used to support the functional food status of the snack (Seth *et al.,* 2025).

Sensory evaluation

A semi-trained panel (n = 20-30) conducted sensory evaluation using a 9-point hedonic scale, assessing appearance, color, texture, flavor, taste and overall acceptability. The panel included students and faculty familiar with food product evaluation (Boisteanu *et al.,* 2025).

Microbiological analysis

The *via*bility of *Bacillus coagulans* was determined by serial dilution and spread plating on a selective medium for spore-formers. Results were expressed as log CFU/g. A count ≥8 log CFU/g was considered adequate for delivering probiotic benefits (Lakshith, 2022).

RESULTS AND DISCUSSION

The physical attributes of probiotic banana snacks prepared by control, impregnation and coating methods demonstrated minimal variation in appearance and dimensions (Table 1). Both samples maintained a uniform yellowish color and round shape, with consistent thickness 5 mm and width 3.2 cm, suggesting that the freeze-drying process did not adversely affect structural integrity (Fig 1). Coated samples exhibited a marginally higher weight 1.02±0.03 g compared to impregnated 0.97±0.01 g and control 0.93±0.01 g samples, likely due to the presence of the biopolymer matrix formed by sodium alginate and calcium chloride. The geometric and arithmetic mean diameters 1.72 and 2.30 cm, respectively and surface area 9.33 cm^2 remained constant across treatments, indicating the physical enhancement was specific to mass gain without altering dimensional parameters. These observations align with Niro *et al.* (2023) and Felix *et al.* (2021), who reported that coatings can improve product weight and appearance without dimensional distortion.

The physicochemical profiles of the snacks varied significantly among treatments (Table 2). Coated samples exhibited the highest pH 5.31±0.16, indicating a buffering effect likely due to the alginate matrix, in contrast to lower pH values in the control 4.54±0.11 and impregnated 4.43±0.12 samples. Total soluble solids (TSS) were highest in coated samples 24.42±0.20, potentially due to solute

Table 1: Physical characteristics of prepared probiotic banana snacks.

Parameters	Values		
	Control	Impregnated	Coated
Color	Yellowish	Yellowish	Yellowish
Shape	Round	Round	Round
Thickness (cm)	5 mm	5 mm	5 mm
Width (cm)	3.2	3.2	3.2
Weight (g)	0.93±0.01	0.97±0.01	1.02±0.03
Geometric mean diameter (cm)	1.72	1.72	1.72
Arithmetic mean diameter (cm)	2.30	2.30	2.30
Surface area (cm^2)	9.33	9.33	9.33

*Each value is a mean of three determinations.

entrapment and interactions with the coating material. In contrast, the lowest TSS was noted in impregnated snacks 19.76±0.11, likely due to sugar dilution or microbial metabolism during soaking. Total acidity did not differ significantly across treatments, indicating mild fermentation by *Bacillus coagulans*. The ascorbic acid content was best preserved in coated snacks 7.24±0.40, while the impregnated samples showed notable reduction 6.76±0.15, likely due to oxidative degradation during the impregnation process. These results support findings by Felix *et al.* (2021) and Dias *et al.* (2023) on nutrient retention in coated products.

Significant differences were observed in the proximate composition (Table 3). Moisture content was highest in coated samples 5.15±0.07%, suggesting superior water retention due to alginate's hygroscopic properties. Protein 3.18±0.07 g and fat 1.54±0.07 g contents were also highest in coated samples, potentially indicating enhanced microbial biomass and coating component contribution. Carbohydrate levels declined with probiotic incorporation, with coated samples showing the lowest content 82.58±0.21 g, possibly due to microbial metabolism. Fiber and ash contents increased notably in coated samples 4.82±0.21 g and 2.73±0.11 g, respectively, consistent with structural reinforcement *via* biopolymer crosslinking. The energy content declined marginally from control 369.41 kcal/100 g to coated 356.90 kcal/100 g, reflecting nutrient redistribution. Similar enhancements in nutritional profile have been reported in coated functional snacks by Praveena *et al.* (2024).

The mineral content of the probiotic banana snacks (Table 4), improved noticeably with both the impregnation and coating methods, especially in the coated samples. Calcium levels saw a slight rise from 32.45 mg/100 g in the control to 33.89 mg/100 g in the coated snacks, likely due to the use of calcium chloride in the coating process. A similar pattern was seen with phosphorus, which increased from 27.41 mg/100 g in the control to 30.62 mg/100 g in the coated variant suggesting that the coating helped preserve or even boost the mineral content. Sodium content also went up slightly, likely because of the alginate used for coating, which naturally contains sodium. Potassium, a key mineral in bananas, remained well-preserved across all samples, with the highest level 503.37 mg/100 g found in the coated snack, showing that the drying and probiotic addition methods had little negative impact on this



Fig 1: Probiotic control, impregnated and coated banana snacks.

Table 2: Effect of freeze drying on physico-chemical properties of prepared probiotic banana snacks.

Parameters	Values		
	Control	Impregnated	Coated
pH	4.54±0.11	4.43±0.12	5.31±0.16
TSS (%)	22.60±0.20	19.76±0.11	24.42±0.20
Total acidity (%)	0.66±0.04	0.68±0.03	0.71±0.03
Ascrobic acid (mg/100 g)	7.63±0.25	6.76±0.15	7.24±0.40

*Each value is a mean of three determinations.

Table 3: Proximate composition of prepared probiotic banana snacks.

Parameters	Values		
	Control	Impregnated	Coated
Moisture (%)	3.66±0.36	4.41±0.10	5.15±0.07
Protein (g)	2.50±0.06	2.96±0.05	3.18±0.07
Fat (g)	1.29±0.04	1.38±0.35	1.54±0.07
Carbohydrates (g)	86.95±0.12	84.87±0.22	82.58±0.21
Fiber (g)	3.76±0.02	4.17±0.08	4.82±0.21
Ash (g)	1.83±0.09	2.19±0.05	2.73±0.11
Energy (kcal/100 g)	369.41	363.74	356.90

*Each value is a mean of three determinations.

Table 4: Mineral composition of prepared probiotic banana snacks.

Parameters	Values (mg/100 g)		
	Control	Impregnated	Coated
Calcium	32.45±0.23	33.29±0.18	33.89±0.28
Phosphors	27.41±0.35	28.32±0.17	30.62±0.57
Sodium	24.46±0.25	24.99±0.09	26.11±0.07
Potassium	489.58±2.56	492.63±2.48	503.37±2.15
Iron	0.48±0.03	0.51±0.03	0.63±0.02
Magnesium	29.95±0.12	31.41±0.15	34.70±0.11
Copper	0.86±0.02	0.89±0.03	0.95±0.02
Zinc	0.35±0.02	0.43±0.01	0.51±0.02

*Each value is a mean of three determinations.

Table 5: Sensory evaluation of prepared probiotic banana snacks.

Sample	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
Control	8.43±0.15	8.23±0.15	8.16±0.25	8.10±0.10	8.46±0.15	8.28
BT1	8.33±0.25	7.86±0.20	8,03±0.25	7.90±0.10	8.33±0.15	8.09
BT2	8.56±0.15	8.46±0.15	7.93±0.15	8.26±0.20	8.53±0.20	8.35

Table 6: The viability of probiotic *Bacillus coagulans* in the probiotic banana snacks during storage at room temperature.

Storage time (days)	Log 8 CFU/g	
	Impregnated	Coated
0	9.07±0.03	9.32±0.03
30	9.01±0.05	9.21±0.02
60	8.83±0.04	9.09±0.01
90	8.58±0.03	8.91±0.02
120	8.29±0.04	8.78±0.02
150	8.01±0.05	8.36±0.01
180	7.49±0.01	8.21±0.02

*Each value is a mean of three determinations.

essential nutrient. Trace minerals such as iron, magnesium, copper and zinc also improved across treatments. For example, iron increased from 0.48 mg/100 g in the control to 0.63 mg/100 g in the coated sample and magnesium rose from 29.95 mg/100 g to 34.70 mg/100 g. Copper and zinc followed a similar trend, with the coated samples consistently showing the highest values. These boosts in mineral levels may be due to the protective nature of the coating, which helps prevent nutrient loss and may even contribute additional minerals from the coating ingredients themselves. Overall, the coating method not only helped maintain the snacks' original nutrients but also enhanced their overall mineral profile, making them a more nutritious and functional choice. These results highlight the advantage of coating techniques in fortifying snacks with essential micronutrients, consistent with observations by Musa *et al.* (2025).

Sensory were carried out and scores were noted (Table 5). The scores revealed that coated banana snacks (BT2) received the highest ratings for appearance 8.56±0.15, color 8.46±0.15, texture 8.26±0.20 and taste 8.53±0.20, achieving the highest overall acceptability 8.35. This superior performance is likely due to improved mouthfeel, color stability and flavor retention offered by the edible coating. Control samples also performed well, particularly in flavor and taste, benefiting from the intrinsic qualities of fresh banana. While impregnated samples (BT1) maintained acceptable scores, slightly lower values for texture and color were noted, possibly due to textural softening during soaking. These findings confirm consumer preference for coated formulations, as supported by previous studies Deshpande *et al.* (2024).

Table 6 shows viability of *Bacillus coagulans* in probiotic banana snacks over 180 days of storage at room temperature using two methods: impregnation and coating. At 180th day, the coated snacks had slightly higher probiotic levels *i.e.*, 9.32 log CFU/g compared to the impregnated snacks 9.07 log CFU/g, suggesting that the coating method provided better initial protection. As time passed, both techniques experienced a gradual drop in probiotic counts, which is normal due to factors like air exposure and storage conditions. However, the coated snacks consistently maintained higher probiotic levels. By the end of six months, the coated samples still had 8.21 log CFU/g, while the impregnated ones dropped to 7.49 log CFU/g. Both values are above the minimum level needed to provide health benefits, but the coated snacks clearly offered better long-term stability. This is likely because the alginate coating formed a protective barrier that helped shield the probiotics from damage. Overall, the coating method proved more effective in keeping the probiotics alive during storage, making it a better choice for creating shelf-stable, healthy banana snacks. These results support the protective role of hydrocolloid-based coatings in probiotic stabilization, as observed in related studies by Kuo *et al.* (2022) and Niro *et al.* (2023).

CONCLUSION

This study highlights the successful development of functional probiotic banana snacks using two incorporation techniques impregnation and coating with the coating method demonstrating superior outcomes. Coated samples showed improved structural integrity, enhanced retention of essential minerals such as calcium, magnesium and potassium and higher sensory acceptability. Most importantly, the alginate-based coating effectively preserved the viability of *Bacillus coagulans* over 180 days of storage at room temperature, maintaining probiotic levels above the therapeutic threshold (≥6 log CFU/g). These findings underscore the potential of edible hydrocolloid coatings to enhance the nutritional, functional and shelf-life characteristics of fruit-based probiotic snacks, while catering to the growing demand for plant-based, clean-label and non-dairy probiotic products.

For future studies, it is recommended to explore the application of other encapsulating materials or composite coatings that may further improve probiotic stability and nutritional outcomes. Additionally, incorporating prebiotic components into the formulation could create synbiotic snacks, offering enhanced gut health benefits. Investigating consumer acceptance across different age groups, storage under varied climatic conditions and scale-up feasibility for commercial production will also be valuable in validating the industrial potential of such functional snack innovations.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this research paper.

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