Development and Evaluation of Probiotic Carrot Snacks using Impregnation and Coating Techniques

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ABSTRACT

Background: Probiotic snack foods are gaining popularity due to their benefits, including improving gut health and boosting immunity. Carrot based probiotic snacks, incorporating *Bacillus coagulans*, provide an innovative way to combine the nutritional and functional benefits of both probiotics and vegetables This investigation aimed to assess the influence of two different methodologies for probiotic integration, specifically impregnation and coating, on the physicochemical, sensory and microbial attributes of freeze-dried probiotic carrot snacks throughout the storage period.

Methods: Fresh carrots were processed by slicing into uniform pieces and subjected to either an impregnation or coating technique for incorporating *Bacillus coagulans*. The impregnation process involved immersing the carrot slices in a probiotic suspension under vacuum, while the coating method involved dipping the slices in a probiotic-enriched alginate solution followed by gel formation in a calcium chloride bath. The samples that were carefully prepared were subsequently subjected to freeze-drying and maintained at ambient temperature for a period of up to 180 days. The physical, chemical, proximate, mineral, sensory and microbial characteristics of the snacks were assessed at different storage intervals.

Result: The results showed that both impregnation and coating methods resulted in a significant reduction in moisture content, with the coating method maintaining slightly higher mineral content and sensory acceptability. The viability of *Bacillus coagulans* was better preserved in the coated samples, with higher probiotic counts maintained throughout the storage period compared to the impregnated samples. Sensory evaluation indicated that both methods were acceptable, with the coated samples receiving slightly higher ratings for flavor and overall acceptability.

Key words: *Bacillus coagulans*, Coating, Freeze-drying, Functional foods, Impregnation, Probiotic carrot snacks.

INTRODUCTION

The integration of probiotics into plant-based food matrices has attracted considerable scholarly interest, driven by an escalating consumer appetite for functional foods that provide health advantages extending beyond fundamental nutritional requirements. Historically, dairy products have constituted the primary medium for probiotic administration; nevertheless, the increase in lactose intolerance, milk protein sensitivities and the transition to vegan dietary practices has catalyzed a burgeoning interest in non-dairy substitutes. Within this context, fruits and vegetables present promising matrices due to their rich composition of dietary fiber, inherent antioxidants, vitamins and phytochemicals, which can synergistically augment probiotic efficacy and enhance consumer health outcomes (Bustos *et al.,* 2023; Koh *et al.,* 2022).

Carrot (*Daucus carota* L.) constitutes one of the most significant seasonal root vegetables within the Apiaceae (Umbelliferae) family, cultivated globally. This vegetable is characterized by its cost-effectiveness and exceptional nutritional value. China is the largest producing country of carrot in the world (Arora *et al.,* 2019). It is significant in nutritional and technological relevance in the advancement of probiotic products. It is distinguished by its elevated concentrations of β-carotene, polyphenols and dietary fiber, which contribute to its prebiotic properties and appropriateness for probiotic integration (Boev *et al.,* 2024).

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The innovation of minimally processed carrot-based snacks augmented with probiotics represents a novel strategy to

deliver health-promoting microorganisms in a convenient, shelf-stable and consumer-accessible format.

Bacillus coagulans, a spore-forming, lactic acid-producing microorganism, has exhibited remarkable advantages in functional food applications due to its resilience, thermal stability and capacity to survive gastrointestinal transit. In contrast to conventional lactic acid bacteria, *Bacillus coagulans* can withstand severe processing environments, rendering it an optimal candidate for incorporation into thermally processed and dehydrated snack products (Kruk *et al.,* 2024; Prapa *et al.,* 2023). The utilization of bacterial spore formers, specifically Bacillus spp., as probiotics may confer benefits for human health. The capacity to generate spores bestows probiotics with enhanced resilience against technological stresses encountered during production and storage phases, as well as increased resistance to gastric (pH, digestive enzymes) and intestinal environmental conditions (Haldar *et al.,* 2019).

Advanced techniques such as impregnation and edible coating with biopolymers have been explored to ensure probiotic viability during processing and storage. The impregnation technique facilitates the infusion of probiotics into the internal matrix of the food substrate, thereby enhancing microbial retention and functional efficacy. Meanwhile, sodium alginate-based edible coatings serve as protective barriers that reduce oxygen permeability and moisture loss, while simultaneously safeguarding probiotic cells against external stressors (Shigematsu *et al.,* 2018; Koh *et al.,* 2022). These strategies not only aid in maintaining high viable cell counts but also contribute to preserving the sensory and nutritional qualities of the final product.

Furthermore, freeze-drying has emerged as a preferred preservation method for probiotic-enriched snacks due to its ability to retain cellular viability and minimize degradation of heat-sensitive bioactive compounds. Pre-treatments such as osmotic dehydration may further improve texture and reduce structural damage during drying (Lyu *et al.,* 2022).

In light of these considerations, the present study aims to develop minimally processed carrot snacks incorporating *Bacillus coagulans* using sodium alginate coating and impregnation techniques, followed by freeze-drying. The investigation focuses on assessing probiotic viability, physicochemical properties and sensory acceptability, thereby establishing carrots as a viable plant-based carrier for probiotic delivery in functional snack formulations.

MATERIALS AND METHODS

Raw material

Fresh carrots (*Daucus carota*) were sourced from a local market in Parbhani, Maharashtra, India. Only carrots of uniform size and maturity, free from visible defects, were selected for the study. The probiotic strain *Bacillus coagulans* (MCC 0554), known for its spore-forming capability and resilience in the gastrointestinal environment, was used in the research. Food-grade sodium alginate and calcium chloride were procured from thomas baker (Chemicals) Pvt. Ltd., Mumbai. All other chemicals used were of analytical grade. This research was conducted in the department of food microbiology and safety and allied departments of the college of food technology at Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, during the academic years 2022–2024. Additionally, part of the analytical work was carried out at the doctoral school of animal sciences, university of debrecen, hungary, as part of a semester exchange program in the academic year 2024-2025.

Sample preparation

Carrots were washed thoroughly under running tap water to remove surface dirt, peeled using a stainless-steel peeler and sliced into uniform discs (2-3 mm thick and 2.5 cm diameter). The slices were blanched in hot water at 90±2°C for 2 minutes to inactivate endogenous enzymes and reduce surface microbial load (Lu *et al.,* 2019). The blanched slices were immediately cooled in ice-cold water to halt further cooking and then drained using sterile muslin cloth.

Probiotic culture activation

The *Bacillus coagulans* MCC 0554 strain was rehydrated in nutrient broth and incubated at 37±1°C for 24 hours. The culture was centrifuged at 4000 rpm for 10 minutes and the pellet was resuspended in sterile saline solution to achieve a viable count of approximately 10^8 CFU/mL, confirmed through standard plate count technique (AOAC International, 2021).

Impregnation of probiotics

For the impregnation method, carrot slices were immersed in the probiotic suspension (10^8 CFU/mL) at ambient conditions. The slices were allowed to soak in the suspension for 1 hour with intermittent stirring every 10 minutes to ensure uniform contact and diffusion of probiotic cells into the carrot matrix. The impregnated slices were drained on sterile paper towels to remove excess liquid (Huang *et al.,* 2023).

Coating with sodium alginate

For coating, carrot slices were first dipped in a sodium alginate solution (2% w/v) mixed with the probiotic suspension (10^8 CFU/mL) and kept for 10 minutes to allow surface binding. The coated slices were then immersed in 0.1 M calcium chloride solution for 10 minutes to induce gelation and encapsulate the probiotics on the surface in the form of calcium alginate coating (Wang *et al.,* 2022). The slices were removed, air-drained and prepared for drying.

Freeze drying

All treated carrot slices (control, impregnated and coated) were frozen at -40°C for 12 hours. Freeze drying was carried out in a laboratory-scale lyophilizer (Labconco, USA) operated at -50°C and 0.2 mbar for 24-36 hours, until a constant dry weight was achieved. This method ensured

minimal thermal damage and maximum probiotic viability (Ciurzyñska *et al.,* 2022).

Physical properties

Physical attributes such as slice thickness, width, weight, color (L*, a*, b* values), surface area, geometric mean diameter and shrinkage ratio were measured to assess structural uniformity, coating integrity and drying performance.

Chemical properties

Chemical characteristics including pH, titratable acidity, total soluble solids (°Brix) and sugar content were analyzed to evaluate flavor profile, microbial compatibility and shelf-life potential. These parameters are critical to both probiotic stability and consumer acceptability (Ranganna, 2021).

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 labelling accuracy and health claims.

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.

Microbiological analysis

Viable probiotic counts (CFU/g) were determined using the standard plate count method. Serial dilutions were prepared in 0.85% sterile saline solution, plated on nutrient agar and incubated at 37°C for 24-48 hours. Colonies were counted and expressed as log CFU/g of dry sample (Prapa *et al.,* 2023).

Sensory evaluation

Sensory attributes such as appearance, aroma, taste, texture and overall acceptability were evaluated using a 9-point hedonic scale by a semi-trained panel of 20 members (Meilgaard *et al.,* 2021). All samples were presented randomly and coded to minimize bias.

RESULTS AND DISCUSSION

The physical attributes of probiotic carrot snacks prepared using control, probiotic impregnation and sodium alginate coating methods (Table 1) showed consistent morphological integrity across all samples. All treatments retained a round shape and reddish colour (Fig 1) indicating that the probiotic incorporation techniques did not adversely impact the visual appeal of the carrots. The thickness of 5 mm and the width of 2.3 cm were standardized for all samples to ensure uniform drying and analysis. A considerable weight gain was seen in the impregnated samples at 0.69±0.01 g and the coated samples at 0.72±0.01 g, relative to the control at 0.66±0.01 g, related to the absorption of the probiotic solution and the creation of the alginate gel matrix, respectively. This higher weight gain is related to the absorption of probiotic suspensions during vacuum impregnation and the incorporation of the alginate-calcium gel matrix in the coating process. Shigematsu *et al.* (2018) revealed related findings, revealing that minimally processed

Table 1: Physical characteristics of prepared probiotic carrot snacks.

Parameters	Values		
	Control	Impregnated	Coated
Color	Reddish	Reddish	Reddish
Shape	Round	Round	Round
Thickness (mm)	5	5	5
Width (cm)	2.3	2.3	2.3
Weight (g)	0.66±0.01	0.69±0.01	0.72±0.01
Geometric mean diameter (cm)	1.38	1.38	1.38
Arithmetic mean diameter (cm)	1.70	1.70	1.70
Surface area (cm^2)	6.07	6.07	6.07

*Each value is a mean of three determinations.

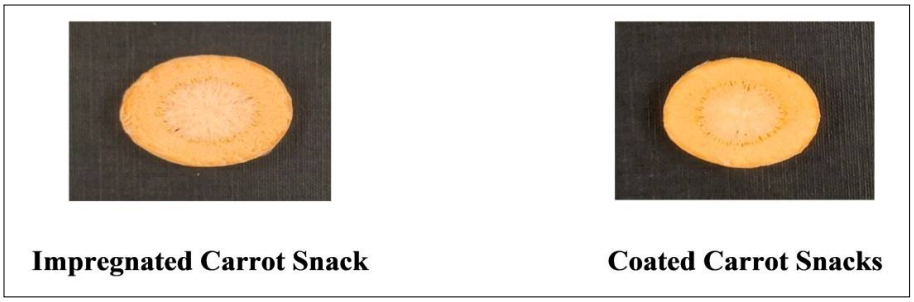


Fig 1: Probiotic impregnated and coated carrot snacks.

carrots with edible coatings have more moisture content than uncoated samples, indicating that coatings may promote water retention and lead to weight enhancement. Furthermore, the geometric mean diameter 1.38 cm, arithmetic mean diameter 1.70 cm and surface area 6.07 cm^2 remained similar across all treatments, demonstrating that the probiotic inclusion methods did not affect the physical dimensions of the carrot slices. These findings correlate with the results of Freire *et al.* (2024), who demonstrated that incorporating probiotics into carrot-based products did not adversely alter their physical attributes.

The physico-chemical analysis of freeze-dried probiotic carrot snacks revealed (Table 2) that both impregnation and coating methods had minimal but notable effects on quality parameters when compared to the control. The pH values showed slight variations, with the control 5.46±0.25 remaining marginally higher than the impregnated 5.26±0.40 and coated 5.40±0.26 samples, indicating a minor acidification likely due to the presence of probiotics, consistent with findings by Coşkun *et al.* (2024), who also reported pH reductions in probiotic-enriched fruit and vegetable matrices. Total soluble solids (TSS) were relatively stable across samples, with a slight increase observed in the coated snack 7.53±0.35%, possibly due to the contribution of the alginate matrix, an effect similarly reported by Freire Le *et al.* (2024) in coated vegetable-based foods. Total titratable acidity arose in the impregnated 0.78±0.03% and coated 0.81±0.04% samples compared to the control 0.69±0.11%, showing slight fermentation activity from *Bacillus coagulans*, in keeping with observations by Shaikh *et al.* (2019) in probiotic carrot products. Notably, β-carotene retention was maximum in the coated sample, 33.07±0.08 mg/100 g, just above the control, 32.92±0.24 mg/100 g, but the impregnated sample, 29.73±0.17 mg/100 gm showed an average reduction. This indicates that the alginate coating may offer protective effects against oxidative degradation of sensitive compounds during freeze-drying, as also demonstrated by Jyothsna *et al.* (2024) in coated plant-based snacks. These findings collectively support the effectiveness of coating methods in maintaining the chemical and nutritional integrity of functional carrot-based snacks.

The proximate composition of the prepared probiotic carrot snacks (Table 3) revealed some significant variations among the control, impregnated and coated samples, particularly in moisture, protein, fat, fibre, ash content and energy. The moisture content was observed to increase with the incorporation of the probiotic strain, with the coated snacks showing the highest value, 5.34±0.07%. This increase may be attributed to the protective barrier formed by the coating and impregnation, which could retain more moisture during processing. Similarly, protein, fat and fibre contents were significantly greater in the coated snacks 3.47±0.20 g protein, 1.14±0.07 g fat and 5.86±0.16 g fibre, possibly due to the retention of nutrients inside the gel matrix of the alginate coating, which could help limit nutrient leaching during drying. The ash content also followed a similar trend, with the coated samples exhibiting the highest value, 4.49±0.18 g, which could indicate enhanced retention of minerals due to the coating process. On the other hand, carbohydrates were marginally lower in the coated samples, 79.58±0.22 g, which may be attributed to slight changes in the carbohydrate structure during the encapsulation and drying processes. Overall, these findings suggest that both the impregnation and coating methods significantly affect the nutritional profile of probiotic carrot snacks. Similar results were observed in studies where coating and impregnation were used to encapsulate probiotics, showing enhanced retention of bioactive compounds and nutritional content by Barrera (2016). The energy values 351.56 kcal for the control, 348.26 kcal for the impregnated and 342.46 kcal for the coated were relatively consistent across the samples, suggesting that the impact of these techniques on energy content was minimal.

The mineral composition analysis of the probiotic carrot snacks (Table 4) indicates an increase in the mineral content, particularly calcium, magnesium, copper and zinc,

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

Parameters	Values		
	Control	Impregnated	Coated
ph	5.46±0.25	5.26±0.40	5.40±0.26
TSS (%)	7.50±0.20	7.36±0.30	7.53±0.35
Total acidity (%)	0.69±0.11	0.78±0.03	0.81±0.04
β-carotene (mg/100 g)	32.92±0.24	29.73±0.17	33.07±0.08

*Each value is a mean of three determinations.

Table 3: Proximate composition of prepared probiotic carrot snacks.

Parameters	Values		
	Control	Impregnated	Coated
Moisture (%)	4.59±0.32	5.05±0.67	5.34±0.07
Protein (g)	2.45±0.27	2.91±0.32	3.47±0.0.20
Fat (g)	0.88±0.06	0.98±0.04	1.14±0.07
Carbohydrates (g)	83.46±0.26	81.95±0.24	79.58±0.22
Fiber (g)	4.85±0.06	5.29±0.0.11	5.86±0.16
Ash (g)	3.60.±0.16	3.78±0.0.07	4.49±0.18
Energy (kcal/100 g)	351.56	348.26	342.46

*Each value is a mean of three determinations.

Table 4: Mineral composition of prepared probiotic carrot snacks.

Parameters	Values (mg/100 g)		
	Control	Impregnated	Coated
Calcium	26.53±0.12	27.37±0.19	30.93±0.17
Phosphors	43.65±0.21	44.97±0.12	47.31±0.20
Sodium	15.76±0.15	17.56±0.07	20.53±0.12
Potassium	258.73±2.64	261.15±2.36	265.71±2.32
Iron	0.59±0.03	0.61±0.04	0.66±0.02
Magnesium	11.48±0.16	13.52±0.18	16.54±0.11
Copper	0.28±0.02	0.33±0.02	0.37±0.02
Zinc	0.42±0.04	0.45±0.02	0.53±0.03

*Each value is a mean of three determinations.

Table 5: Sensory evaluation of prepared probiotic carrot snacks.

Sample	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability
Control	8.63±0.15	8.46±0.30	8.23±0.15	8.40±0.10	8.43±0.15	8.43
CT1	8.26±0.25	8.33±0.15	8.13±0.25	8.06±0.20	8.26±0.15	8.21
CT2	8.66±0.20	8.73±0.25	8.23±0.15	8.50±0.10	8.33±0.15	8.49

*Each value is a mean of three determinations.

CT1 - Impregnated Carrot Snacks.

CT2 - Coated Carrot Snacks.

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

Storage time (days)	Log 8 CFU/g	
	Impregnated	Coated
0	9.20±0.03	9.25±0.03
30	9.07±0.02	9.11±0.04
60	8.94±0.03	9.02±0.04
90	8.71±0.02	8.78±0.03
120	8.34±0.04	8.41±0.03
150	8.08±0.02	8.21±0.02
180	7.82±0.06	8.14±0.03

*Each value is a mean of three determinations.

when the impregnation and coating methods were applied. With zinc rising from 0.42±0.04 mg/100 g control to 0.53±0.03 mg/100 g coated, magnesium rising from 11.48±0.16 mg/100 g control to 16.54±0.11 mg/100 g coated and calcium rising from 26.53±0.12 mg/100 g control to 30.93±0.17 mg/100 g coated, the coated snacks had the highest mineral levels. The encapsulation process, which may improve the retention of minerals within the food matrix, is responsible for these increases. Comparing the impregnated and coated samples to the control, additional minerals like sodium, potassium, phosphorus and iron also displayed an increasing trend. The impregnation process likely facilitated a better incorporation of the probiotic culture, which could have also influenced the retention of these minerals. In contrast, the control group showed the lowest values for all minerals, indicating that the probiotic incorporation and coating methods effectively contributed to the enrichment of the snacks. The higher mineral contents in the coated snacks suggest that the encapsulation technique may play a significant role in preserving and potentially enhancing the bioavailability of these essential micronutrients. This trend is consistent with similar studies that report increased mineral retention in foods processed with edible coatings and encapsulation technologies by Deshpande *et al.* (2024) and Coşkun *et al.* (2024).

The sensory evaluation (Table 5) results reveal significant insights into the quality attributes of the probiotic carrot snacks, as influenced by the coating methods. The control sample, which likely represents the uncoated or untreated carrot snack, showed relatively high scores across all sensory parameters, with overall acceptability rated at 8.43. However, both CT1 (impregnated) and CT2 (coated) samples showed slight variations in sensory performance. For example, CT1 displayed slightly lower scores for appearance 8.26, flavour 8.13 and texture 8.06 compared to the control, indicating that the impregnation method might have affected the perceived sensory quality. In contrast, the CT2 (coated) sample exhibited the highest scores, with appearance 8.66, colour 8.73 and overall acceptability 8.49, showing the best results. These findings suggest that the coating method, potentially through the retention of moisture and preservation of texture, enhanced the sensory appeal of the probiotic carrot snacks. The increase in overall acceptability in the CT2 sample can be attributed to the better integration of the probiotic culture, which did not negatively affect the sensory qualities and might have contributed to a more desirable flavour and texture. The results align with previous studies of Alwis *et al.* (2016), indicating that coating methods often improve both the physical and sensory attributes of functional foods, particularly in terms of appearance, texture and overall acceptability.

Over 180 days at room temperature, the survivability of *Bacillus coagulans* in both coated and impregnated probiotic carrot snacks (Table 6) was tested. Both samples displayed high initial viability (day 0), with the coated snacks having a log count of 9.25±0.03 CFU/g and the impregnated snacks displaying a log count of 9.20±0.03 CFU/g. The viability of both techniques steadily diminished over time, with the impregnated snacks demonstrating a larger rate of deterioration than the coated snacks. The coated snacks maintained a higher log count of 8.14±0.03 CFU/g by 180 days, whereas the impregnated snacks exhibited a log count of 7.82±0.06 CFU/g. This means that the probiotic bacteria were better protected during storage by the coating approach, likely as a result of the physical barrier the coating generated, which may have covered the bacterial cells from environmental stressors. In their earlier studies, Afzaal *et al.* (2020) and Chaudhari *et al.* (2022) noticed similar patterns, suggesting that probiotics durability during storage was boosted by encapsulation or coating. These results demonstrate how critical the coating process is to retaining the viability of probiotics in functional foods.

CONCLUSION

The results derived from the current investigation underscore the influence of various processing techniques,

including impregnation and coating, on the physical, chemical, microbial and sensory attributes of probiotic carrot snacks. The coating technique was determined to confer superior preservation of the viability of *Bacillus coagulans* during the storage duration, as indicated by the elevated probiotic counts after the 180 days in comparison to the impregnation technique. The physico-chemical assessment revealed negligible differences between the two methodologies; however, the coating technique exhibited a marginal benefit in terms of mineral retention and sensory acceptability. Notably, both methodologies assured that the probiotic snacks sustained favorable characteristics such as flavor, texture and appearance throughout the entire storage duration. These findings imply that coating may represent a more efficacious strategy for enhancing the shelf-life and stability of probiotic microorganisms in functional snack products. Future research could further explore the long-term implications of various coating substances and their mechanisms in preserving probiotic viability, along with the broader applicability of these techniques across diverse fruit and vegetable matrices.

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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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