



Effect of Coating and Impregnation on the Quality and Probiotic Viability of Freeze-Dried Apple Snacks

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10.18805/ajdfr.DR-2434

ABSTRACT

Background: Fruits are promising carriers for probiotic delivery; however, maintaining probiotic viability and product quality during processing and storage remains a major challenge. Apple, owing to its nutritional value and consumer acceptability, offers potential for development as a functional snack. The present study aimed to develop freeze-dried probiotic apple snacks using impregnation and coating techniques and to evaluate their quality attributes and probiotic stability.

Methods: Apple slices were prepared as control, probiotic-impregnated and probiotic-coated samples using *Bacillus coagulans*, followed by freeze drying. The developed snacks were evaluated for physical characteristics, proximate composition, mineral content, physico-chemical properties, sensory attributes and probiotic viability during storage at room temperature for 180 days. All analyses were performed in triplicate.

Result: Probiotic incorporation did not alter the physical characteristics of apple snacks. Impregnated and coated samples showed slight improvements in protein, fiber, ash and mineral content, with coated samples exhibiting higher calcium, potassium and iron levels. Physico-chemical parameters remained within acceptable limits, with good retention of ascorbic acid. Sensory evaluation indicated good overall acceptability, with coated samples receiving higher scores. Probiotic viability decreased gradually during storage however, coated samples retained higher counts, remaining above the recommended minimum level throughout 180 days.

Key words: *Bacillus coagulans*, Coating, Freeze drying, Functional foods, Probiotic apple snacks.

INTRODUCTION

In recent years, consumers have become more conscious about the link between diet and health, leading to a growing interest in functional foods that offer benefits beyond basic nutrition. Probiotic foods, known for their positive effects on gut health, immunity and overall well-being, have traditionally been delivered through dairy-based products (Abbasi *et al.*, 2025). However, issues such as lactose intolerance, milk allergies, changing dietary preferences and the increasing demand for plant-based foods have encouraged the development of non-dairy probiotic alternatives.

Fruits and vegetables are naturally rich in vitamins, minerals, dietary fiber and bioactive compounds making them attractive carriers for probiotics (Žvirdauskienė *et al.*, 2025). Among them, apple is one of the most widely consumed fruits due to its pleasant taste, year-round availability and nutritional value. Its cellular structure allows effective incorporation of probiotic cultures, while its familiar flavor supports consumer acceptance. Despite these advantages, developing probiotic fruit-based snacks is challenging, as probiotic microorganisms are sensitive to processing conditions, drying and storage, which can negatively affect their survival (Uhegwu and Anumudu, 2025). Freeze drying is recognized as an effective drying method for preserving the quality of fruits, as it minimizes heat-induced damage and helps retain nutrients such as ascorbic acid, color and texture. At the same time, ensuring the survival of probiotics during drying and subsequent

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How to cite this article: Gangakhedkar, P.S., Deshpande, H.W., Joshi, R.D., Mane, R. and Gaikwad, G. (2026). Effect of Coating and Impregnation on the Quality and Probiotic Viability of Freeze-Dried Apple Snacks. *Asian Journal of Dairy and Food Research*. **45(4)**: 577-582. doi: 10.18805/ajdfr.DR-2434.

Submitted: 30-12-2025 **Accepted:** 26-02-2026 **Online:** 12-03-2026

storage remains a key concern. Therefore, selecting a robust probiotic strain and suitable incorporation methods is essential for producing shelf-stable probiotic snacks.

Bacillus coagulans, a spore-forming probiotic, is particularly suitable for non-dairy and dried food applications

due to its high resistance to heat, oxygen and acidic environments. Techniques such as impregnation and coating have been explored to improve probiotic retention and stability in fruit matrices. Coatings can act as protective barriers, reducing moisture and oxygen exposure and helping maintain both product quality and probiotic viability.

Although these approaches show promise, limited research has focused on combining impregnation, coating and freeze drying for the development of probiotic apple snacks. Therefore, the present study aimed to develop freeze-dried probiotic apple snacks using these techniques and to evaluate their quality attributes, sensory acceptability and probiotic stability during storage, highlighting their potential as convenient and shelf-stable functional snack foods. Freeze-dried probiotic fruit snacks can serve as shelf-stable non-refrigerated functional foods suitable for commercial distribution and nutraceutical applications.

MATERIALS AND METHODS

Raw material

Fresh apples were procured from a local market in Parbhani, Maharashtra, India. Apples were selected of uniform size, maturity and absence of visible defects. The probiotic strain *Bacillus coagulans* (MCC 0554), recognized for its spore-forming ability and gastrointestinal tolerance was used. Food-grade sodium alginate and calcium chloride were purchased from Thomas Baker (Chemicals), Pvt. Ltd., Mumbai. All other chemicals used in the study were of analytical grade.

Slices preparation

Apples were washed thoroughly under running tap water to remove surface dirt and sliced into uniform discs (5 mm thick).

Probiotic culture activation

The *Bacillus coagulans* MCC 0554 strain (0.1 g) was aseptically transferred into 10 mL sterile nutrient broth and incubated at 37°C for 24 h for activation. The culture was centrifuged at 4000 rpm for 10 min and washed twice with sterile saline (0.85%). The pellet was resuspended in sterile saline to obtain approximately 10⁸ CFU/mL using McFarland standard and confirmed by plate count (AOAC International, 2021).

Impregnation of probiotics

For the impregnation method, apples slices were immersed in the probiotic suspension (10⁸ CFU/mL) at ambient conditions. The slices were allowed to soak in the suspension for 30 min with intermittent stirring every 10 minutes to ensure uniform contact and diffusion of probiotic cells into the apple matrix. The impregnated slices were drained on sterile paper towels to remove excess liquid (Huang *et al.*, 2023).

Coating with sodium alginate

For coating, apple slices were first dipped in a sodium alginate solution (2% w/v) mixed with the probiotic

suspension (10⁸ CFU/mL) and kept for 1 min to allow surface binding. The coated slices were then immersed in 0.1 M calcium chloride solution for 1 min 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 apple 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 (Ciuzyńska *et al.*, 2022).

Physical properties

Physical attributes such as slice thickness, width, weight, surface area and geometric mean diameter were measured to assess structural uniformity, coating integrity and drying performance (Sornsenee *et al.*, 2022).

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 (Rascón *et al.*, 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).

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 25 members (Meilgaard *et al.*, 2021). All samples were presented randomly and coded to minimize bias.

RESULTS AND DISCUSSION

The physical characteristics of control, impregnated and coated probiotic apple snacks are presented in Table 1. All samples showed a uniform whitish colour and round shape indicating that impregnation and coating treatments did not result in any noticeable discoloration or deformation in Fig 1. The uniform appearance reflects controlled processing conditions during pretreatment and freeze drying. The thickness of 5 mm and width of 4.8 cm remained identical for all samples, confirming uniform slicing and dimensional stability across treatments. Such consistency is desirable for standardized processing and comparable drying behavior.

A slight increase in weight was observed in impregnated and coated samples compared to the control. The weight of impregnated samples was 0.75 ± 0.01 g, while coated samples showed the highest value of 0.80 ± 0.02 g, compared to 0.72 ± 0.02 g in the control. This increase may be attributed to the incorporation of probiotic suspension during impregnation and the additional mass contributed

by the coating material in coated samples. Similar findings were reported in alginate-coated probiotic fruit snacks (Afzaal *et al.*, 2020; Huang *et al.*, 2023). The geometric mean diameter, arithmetic mean diameter and surface area remained constant across all treatments, indicating that probiotic incorporation techniques did not affect the overall geometry of the apple slices. The results suggest that impregnation and coating are suitable methods for probiotic incorporation without adversely affecting the physical characteristics of apple snack products.

The proximate composition of control, impregnated and coated probiotic apple snacks is presented in Table 2. All samples showed low moisture content, indicating effective dehydration, although impregnated and coated samples exhibited slightly higher moisture levels, possibly due to probiotic incorporation and the presence of an coating layer.

The moisture content increased slightly from 4.97% in the control to 5.27% in impregnated and 5.89% in coated samples, likely due to probiotic incorporation and the water-binding nature of the edible coating. Protein content showed

Table 1: Physical characteristics of prepared probiotic apple snacks.

Parameters	Values		
	Control	Impregnated	Coated
Color	Whitish	Whitish	Whitish
Shape	Round	Round	Round
Thickness (mm)	5	5	5
Width (cm)	4.8	4.8	4.8
Weight (g)	0.72 ± 0.02	0.75 ± 0.01	0.80 ± 0.02
Geometric mean diameter (cm)	4.93	4.93	4.93
Arithmetic mean diameter (cm)	4.93	4.93	4.93
Surface area (cm ²)	75.43	75.43	75.43

*Each value is a mean of three determinations.

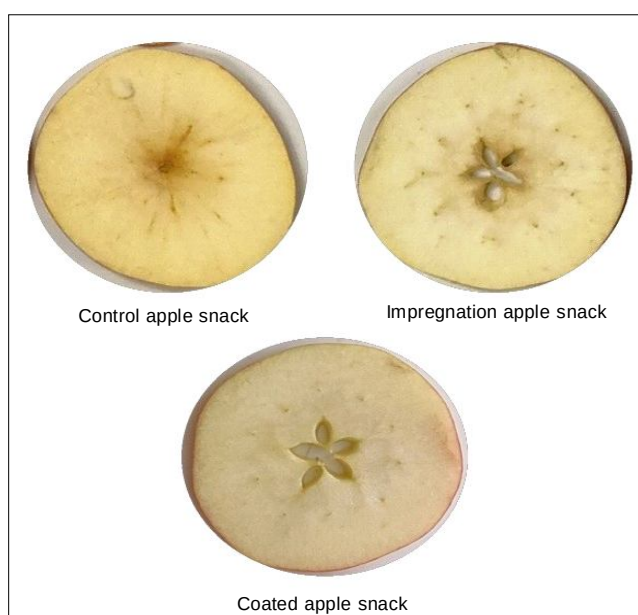


Fig 1: Control, impregnated and coated apple snacks.

a marginal increase from 2.39 ± 0.04 g to 2.57 ± 0.08 g per 100 g, while fat content increased from 1.63 ± 0.05 g to 1.79 ± 0.04 g per 100 g in coated snacks. In contrast, carbohydrate content decreased from 84.60 ± 0.29 g in the control to 82.88 ± 0.89 g per 100 g in coated samples, reflecting the relative increase in other components. Fiber and ash contents increased slightly in treated samples, with coated snacks showing the highest values. Consequently, the energy value decreased marginally from 362.63 to 357.91 kcal per 100 g.

The mineral composition of prepared probiotic apple snacks is presented in Table 3. A consistent increase in all analyzed minerals was observed in impregnated and coated samples compared to the control. Calcium content increased from 18.90 ± 0.17 mg per 100 g in the control to 19.97 ± 0.09 mg per 100 g in impregnated and 21.38 ± 0.15 mg per 100 g in coated samples, indicating improved mineral retention and contribution from coating materials.

Phosphorus also showed a slight increase from 28.63 ± 0.06 to 29.32 ± 0.08 mg per 100 g across treatments.

Sodium content increased from 1.94 ± 0.02 mg per 100 g in the control to 2.58 ± 0.09 mg per 100 g in coated samples, while potassium, the major mineral in apple snacks, increased from 576.40 to 602.89 mg per 100 g, suggesting minimal mineral loss during processing. Iron content increased from 0.39 ± 0.04 to 0.57 ± 0.06 mg per 100 g, which may enhance the nutritional value of the product. Magnesium increased from 7.65 ± 0.17 to 8.04 ± 0.18 mg per 100 g, while trace minerals such as copper and zinc increased from 0.21 ± 0.03 to 0.33 ± 0.06 mg per 100 g and from 0.19 ± 0.03 to 0.29 ± 0.04 mg per 100 g, respectively. Overall, the results indicate that probiotic incorporation through impregnation and edible coating improved mineral retention, with coated samples showing the highest mineral content. This trend is consistent with similar studies that report increased mineral retention in

Table 2: Proximate composition of prepared probiotic apple snacks.

Parameters	Values		
	Control	Impregnated	Coated
Moisture (%)	4.97 ± 0.11	5.27 ± 0.23	5.89 ± 0.07
Protein (g)	2.39 ± 0.04	2.45 ± 0.05	2.57 ± 0.08
Fat (g)	1.63 ± 0.05	1.71 ± 0.02	1.79 ± 0.04
Carbohydrates (g)	84.60 ± 0.29	83.92 ± 0.57	82.88 ± 0.89
Fiber (g)	3.52 ± 0.18	3.63 ± 0.18	3.71 ± 0.29
Ash (g)	2.89 ± 0.06	3.02 ± 0.23	3.15 ± 0.18
Energy (kcal/100 g)	362.63	360.87	357.91

*Each value is a mean of three determinations.

Table 3: Mineral composition of prepared probiotic apple snacks.

Parameters	Values (mg/100 g)		
	Control	Impregnated	Coated
Calcium	18.90 ± 0.17	19.97 ± 0.09	21.38 ± 0.15
Phosphors	28.63 ± 0.06	29.01 ± 0.07	29.32 ± 0.08
Sodium	1.94 ± 0.02	2.12 ± 0.04	2.58 ± 0.09
Potassium	576.40 ± 2.59	589.97 ± 2.76	602.89 ± 2.92
Iron	0.39 ± 0.04	0.48 ± 0.04	0.57 ± 0.06
Magnesium	7.65 ± 0.17	7.82 ± 0.16	8.04 ± 0.18
Copper	0.21 ± 0.03	0.28 ± 0.02	0.33 ± 0.06
Zinc	0.19 ± 0.03	0.23 ± 0.02	0.29 ± 0.04

*Each value is a mean of three determinations.

Table 4: Effect of freeze drying on physico-chemical properties of prepared probiotic apple snacks.

Parameters	Values		
	Control	Impregnated	Coated
pH	4.10 ± 0.20	4.02 ± 0.30	3.96 ± 0.15
TSS (%)	15.50 ± 0.20	15.69 ± 0.35	15.88 ± 0.30
Total acidity (%)	3.18 ± 0.43	3.43 ± 0.25	3.67 ± 0.32
Ascorbic acid (mg/100 g)	7.88 ± 0.19	7.76 ± 0.25	7.64 ± 0.38

*Each value is a mean of three determinations.

foods processed with edible coatings and encapsulation technologies by Deshpande *et al.* (2024).

Freeze drying had a measurable influence on the physico-chemical properties of the probiotic apple snacks, as shown in Table 4. The pH of the control sample was 4.10 ± 0.20 , which slightly decreased to 4.02 ± 0.30 in impregnated and further to 3.96 ± 0.15 in coated samples. This gradual reduction in pH was accompanied by an increase in total acidity from 3.18% in the control to 3.43% in impregnated and 3.67% in coated snacks. These changes indicate the contribution of probiotic incorporation and coating materials, which slightly enhanced the acidic nature of the product and may help improve microbial stability during storage.

Total soluble solids increased marginally from 15.50% in the control to 15.69% in impregnated and 15.88% in coated samples, suggesting better retention of soluble components due to the protective effect of the coating during freeze drying. Ascorbic acid content showed a small decline from 7.88 ± 0.19 mg per 100 g in the control to 7.76 ± 0.25 and 7.64 ± 0.38 mg per 100 g in impregnated and coated

samples, respectively. This reduction is expected during processing and drying; however, the overall retention indicates that freeze drying effectively preserved this heat-sensitive nutrient.

Sensory evaluation results in Fig 2 showed that all samples were well accepted by panelists. The overall acceptability score was 8.06 for the control, 7.90 for the impregnated sample and highest for the coated sample at 8.18. The coated snacks received better scores for appearance, colour and texture, which may be attributed to improved surface integrity and mouthfeel provided by the edible coating. Importantly, probiotic incorporation did not negatively affect taste or flavour.

The viability of *Bacillus coagulans* during storage is presented in Table 5. Initial probiotic counts were high, at 9.17 ± 0.03 log CFU per g in impregnated and 9.29 ± 0.04 log CFU per g in coated samples. Although a gradual decline was observed over 180 days, coated samples consistently retained higher counts. After six months of storage, impregnated samples showed 7.68 ± 0.04 log CFU per g, while coated samples maintained 8.19 ± 0.04 log CFU per g. In both cases, probiotic levels remained above the recommended minimum of 7 log CFU per g, confirming good storage stability. The superior survival in coated samples highlights the protective role of the edible coating against environmental stress. In their earlier studies, Afzaal *et al.* (2020) and Gangakhedkar *et al.* (2025) 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.

Overall, these results demonstrate that edible coating combined with freeze drying effectively preserves product quality, enhances sensory appeal and improves long-term probiotic viability in apple-based snack products.

Table 5: The viability of probiotic *B. coagulans* in the probiotic apple snacks during storage at room temperature.

Storage time (days)	Log 8 CFU/g	
	Impregnated	Coated
0	9.17 ± 0.03	9.29 ± 0.04
30	9.05 ± 0.08	9.16 ± 0.02
60	8.87 ± 0.04	9.04 ± 0.02
90	8.66 ± 0.06	8.84 ± 0.04
120	8.31 ± 0.04	8.56 ± 0.05
150	8.02 ± 0.04	8.27 ± 0.03
180	7.68 ± 0.04	8.19 ± 0.04

*Each value is a mean of three determinations.

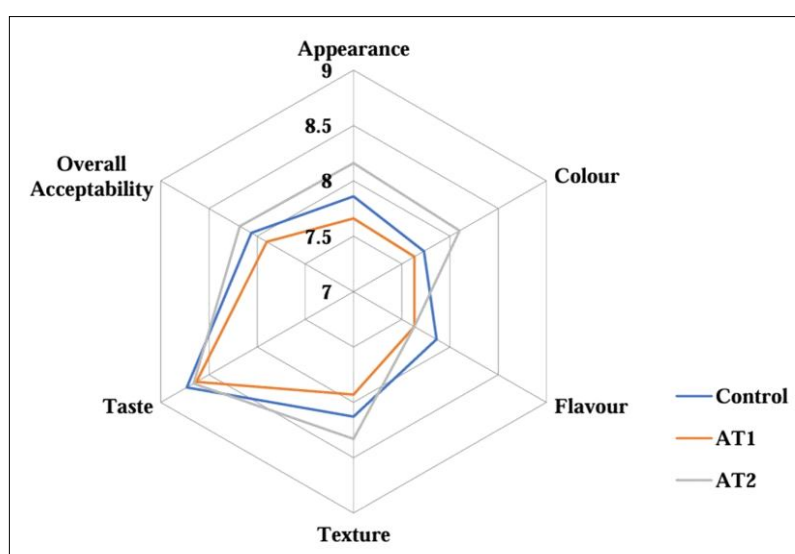


Fig 2: Sensory analysis of probiotic apple snacks.

CONCLUSION

The present study demonstrated the successful development of freeze-dried probiotic apple snacks using impregnation and coating techniques without compromising product quality. The physical characteristics of the snacks remained uniform across all treatments, indicating good dimensional stability and suitability for standardized processing. Probiotic incorporation resulted in minor but favorable changes in proximate composition, with slight increases in protein, fiber, ash and mineral content, particularly in coated samples, thereby enhancing the nutritional value of the product.

The physico-chemical properties of the developed snacks remained within acceptable limits, with only marginal changes in pH, total soluble solids, acidity and ascorbic acid content confirming the effectiveness of freeze drying in preserving quality attributes. Sensory evaluation revealed good overall acceptability of all samples with coated snacks receiving comparatively higher scores for appearance, texture and overall acceptability, indicating improved consumer appeal. The probiotic viability study showed that *Bacillus coagulans* remained stable during 180 days of storage at room temperature, with coated samples exhibiting better survival compared to impregnated samples. Importantly, probiotic counts remained above the recommended minimum level for functional foods throughout storage.

Overall, the results confirm that coating combined with freeze drying is an effective approach for producing shelf-stable, nutritionally enriched and sensory acceptable probiotic apple snacks highlighting their potential for functional food applications and commercial exploitation.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the College of Food Technology, VNMKV, Parbhani, for providing research facilities and support. I also extend our sincere thanks to the Doctoral School of Animal Sciences, University of Debrecen, Hungary, for academic and research assistance during the semester exchange program under the Stipendium Hungaricum Scholarship.

Conflict of interest

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

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