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Article

In vitro evaluation of locally isolated probiotic strains in apple and orange juice using microencapsulation process

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Abstract: Probiotic-based food items are functional foods that offer health benefits to the host. This study aimed to develop probiotic products—apple juice, orange juice, and UHT milk—by incorporating locally identified bacterial strains as mediums for probiotic consumption. The hypothesis was that microencapsulation would enhance probiotic viability during storage. Probiotic microorganisms (*Enterococcus faecium* and *Pediococcus acidilactici*) were microencapsulated using chitosan and a 2% sodium alginate solution via extrusion from July 2018 to December 2022 in the laboratory of Biotechnology and Genetic Engineering Discipline of Khulna University, Bangladesh. Both free (non-encapsulated) and microencapsulated strains were used to inoculate laboratory-prepared apple juice, orange juice, and UHT milk. These products were stored at room temperature and 4 °C for four weeks and monitored for chemical (pH) and microbiological changes. By day 28, the pH of apple juice, orange juice, and UHT milk inoculated with encapsulated probiotic organisms remained higher than in those with free probiotics. The pH of products stored at room temperature declined more than those stored at 4 °C over the 28-day period. Overall, microencapsulated probiotic bacteria outlived the free probiotic bacteria across all storage conditions. Free probiotic bacteria remained viable up to 7 days at room temperature and 14 days at 4 °C, while microencapsulated bacteria remained viable up to 14 days at room temperature and 28 days at 4 °C. Among the products, UHT milk supported the highest viability of probiotic bacteria during storage at both temperatures. Between juices, apple juice showed slightly better bacterial survival than orange juice. The results confirm that microencapsulation enhances probiotic viability during storage. These findings support the development of functional probiotic beverages from apple juice, orange juice, and UHT milk using native strains, offering practical implications for improving public health through non-dairy probiotic delivery systems.

Keywords: functional beverages; cold storage; room temperature stability; fermented products; viability testing

1. Introduction

In recent years, global consumer awareness regarding the benefits of healthy eating has significantly increased. Healthy foods are now widely recognized as those that are nutrient-dense, therapeutic, and capable of promoting overall well-being. A food is classified as functional when it contains bioactive components such as phytochemicals, dietary fiber, oligosaccharides, or probiotics (beneficial live bacteria) (Vlaicu *et al.*, 2023; Vignesh *et al.*, 2024). The preparation of functional foods typically involves several steps and modifications to the original food ingredients. One common approach is the incorporation of probiotic microorganisms, which are beneficial live bacteria that can enhance the nutritional and therapeutic value of the final product (Damián *et al.*, 2022; Obayomi *et al.*, 2024).

Probiotics represent a rapidly growing and dynamic area within the field of functional foods. The probiotic-based diets account for approximately 60–70% of all functional food products (Tripathi and Giri, 2014). Among the various types, probiotic-enriched foods are considered highly convenient and effective, meeting consumer preferences for product appearance, shape, ease of distribution, and cooling stability. These foods also offer promising potential for preserving bioactive compounds, nutrient delivery, and extended shelf life (Sharifi-Rad *et al.*, 2020). Probiotic beverages, particularly those based on fruits and dairy, are increasingly popular due to their nutritional value and palatability. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) define probiotics as live microorganisms that, when administered in adequate amounts, confer health benefits to the host (White and Hekmat, 2018). While genera such as *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* are among the most common probiotic sources, other microorganisms recognized as "generally regarded as safe" (GRAS)—including species of *Enterococcus* and *Pediococcus*—are also widely used (Khushboo *et al.*, 2023). Notably, *Pediococcus acidilactici* and *Enterococcus faecium* are lactic acid bacteria (LAB) frequently employed in probiotic formulations, either individually or in combination (Pupa *et al.*, 2022; Todorov *et al.*, 2023).

New probiotic food products are emerging in the market, including non-dairy options such as chocolate, cereals, beverages, and fruits and vegetables (Arratia-Quijada *et al.*, 2024). Apple and orange juices are widely enjoyed by people of all ages and from various socioeconomic backgrounds. These juices are not only appealing but also offer significant nutritional value, making them ideal candidates for developing probiotic fruit drinks. As such, they hold great potential for creating probiotic apple cider (Pinto *et al.*, 2022; D'Amico *et al.*, 2024).

A critical aspect of developing probiotic foods is ensuring the viability of the probiotic microorganisms. For these bacteria to provide their intended health benefits, they must survive and remain active as they pass through the stomach and small intestine (Das *et al.*, 2022). Several factors influence their survival within the gastrointestinal (GI) tract, including the specific strain used, nutrient composition, oxygen availability, pH level, moisture content, interactions with other microbes, storage duration, and temperature conditions (Mafe *et al.*, 2024; Sarita *et al.*, 2025).

Probiotic incorporation into fruit juices poses a significant challenge due to the sensitivity of beneficial bacteria to harsh environmental conditions, including storage at room temperature and the acidic environment of the gastrointestinal tract. Microencapsulation offers a promising solution by enclosing probiotic cells in protective matrices—commonly alginate coated with chitosan—to enhance their survival and functionality (Arratia-Quijada *et al.*, 2024; Vijayaram *et al.*, 2024). This study addresses the problem of limited non-dairy probiotic options for individuals with lactose intolerance or dietary preferences that exclude dairy. The central research questions are, can microencapsulation improve the survival of probiotic bacteria in fruit juices during storage and digestion? Which juice medium—apple or orange—better supports probiotic viability? It is hypothesized that microencapsulated probiotic bacteria will exhibit greater viability and stability in fruit juices compared to free (non-encapsulated) forms. Therefore, the objective of this study is to develop and evaluate probiotic-enriched apple and orange juices using microencapsulation techniques, offering a non-dairy alternative for delivering bioactive compounds to health-conscious consumers. This study highlights the broader potential of microencapsulation technology to enhance probiotic viability in functional foods, contributing to improved public health through more accessible and stable probiotic delivery systems.

2. Materials and Methods

2.1. Ethical approval

This study did not involve any animals or humans; therefore, ethical approval was not required.

2.2. Location of the study

This study was carried out from July 2018 to December 2022 in the laboratory of Biotechnology and Genetic Engineering Discipline of Khulna University (Figure 1).

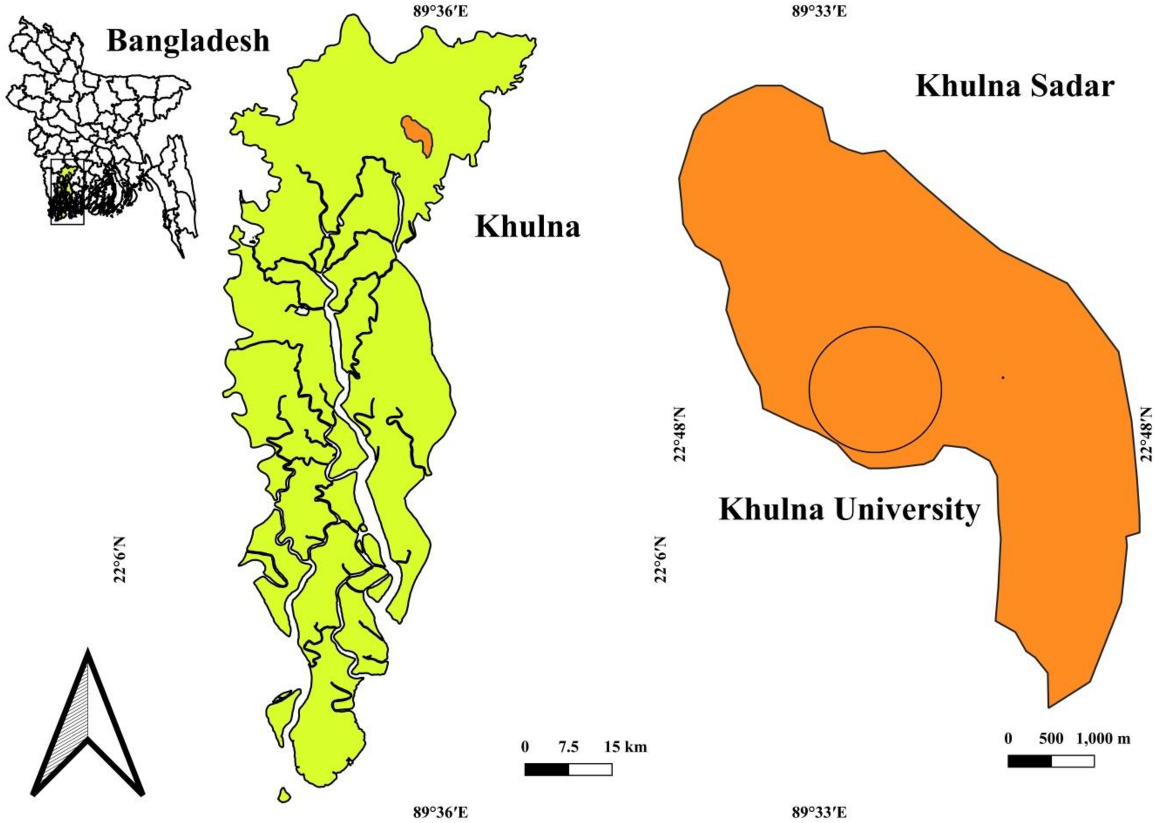

Figure 1. Map of the study site, Khulna Agricultural University.

2.3. Preparation of probiotic culture

A modified version of the protocol by Krasaekoopt and Kitsawad (2010) was followed to activate the bacterial cultures using the streak plate technique. Probiotic bacteria were first inoculated into 10 ml of de Man, Rogosa, and Sharpe (MRS) broth (Merck KGaA, Darmstadt, Germany) and incubated overnight at 37 °C under anaerobic conditions. The cultures were then transferred into 95 ml of fresh MRS broth and incubated under the same conditions. This procedure was applied to mixed cultures of *Enterococcus faecium* (accession nos. MK640926 and MK640929) and *Pediococcus acidilactici* (accession nos. MK640932 and MK640924) as described by Jalil *et al.* (2019).

2.4. Microencapsulation of probiotic bacteria

Microencapsulation was carried out using the extrusion technique following the method described by Castro-Rosas *et al.* (2021).

2.5. Preparation of fruit juice and UHT milk

Fresh apples and oranges were procured from a local supermarket in Khulna, Bangladesh, and stored at 4 °C until use. The fruits were thoroughly washed with running tap water followed by rinsing with distilled water. Juice extraction from both apple and orange was performed following the methods outlined by Castro-Rosas *et al.* (2021).

2.6. Application of probiotics in fruit juice and UHT milk

Aseptically, 10 grams of microencapsulated probiotic beads or 10 ml of free-cell suspension containing mixed probiotic strains (*Pediococcus acidilactici* and *Enterococcus faecium*) were added to 100 ml of sterilized fruit juices (apple and orange) and UHT milk, following the procedures described by Castro-Rosas *et al.* (2021) and Krasaekoopt and Kitsawad (2010).

2.7. Microbiological and chemical analysis of the product

The microbiological and chemical analysis of the product included a viable cell count conducted over a 4-week shelf life. For non-encapsulated cells (free cells), viable cell counts were obtained using sterile peptone water and serial dilution up to a 10^{-6} dilution, following the methodology outlined by Vinderola and Reinheimer

(2000) with slight modifications. For encapsulated cells, the depolymerization procedure recommended by Koh *et al.* (2022) was used to soften the capsules and facilitate the release of the cells into the solution, allowing for the determination of the viable count of the encapsulated probiotics. The pH of the probiotic juice was measured on day 0 and weekly for 4 weeks using a pH meter (Hanna Instruments, USA).

2.8. Statistical analysis

The data were compiled using Excel 2016, and statistical analysis was performed using GraphPad Prism 8.0.1 software. A one-way ANOVA was conducted to assess the differences in pH and viable microbial counts between the treatments. Post-hoc comparisons were made using the Tukey test to determine significant differences ($P<0.05$) between the means of the different groups.

3. Results and Discussion

3.1. Values of pH during storage of probiotic products

After four weeks of storage at room temperature, the average pH of apple juice, orange juice, and UHT milk inoculated with free probiotic bacteria decreased from 3.87 ± 0.00 to 2.79 ± 0.11, 3.74 ± 0.00 to 2.66 ± 0.10, and 6.61 ± 0.00 to 5.58 ± 0.18, respectively. In comparison, the control samples without probiotics showed a smaller decline in pH, reaching 3.67 ± 0.04 for apple juice, 3.56 ± 0.03 for orange juice, and 6.40 ± 0.02 for UHT milk after the same storage period (Figure 2). After four weeks of storage at room temperature, the pH of apple juice, orange juice, and UHT milk inoculated with free probiotic bacteria decreased significantly, with the probiotic samples showing a greater decline compared to the control samples. This suggests that the probiotic bacteria actively fermented the sugars in the products, leading to a more pronounced acidification over time (Fenster *et al.*, 2019).

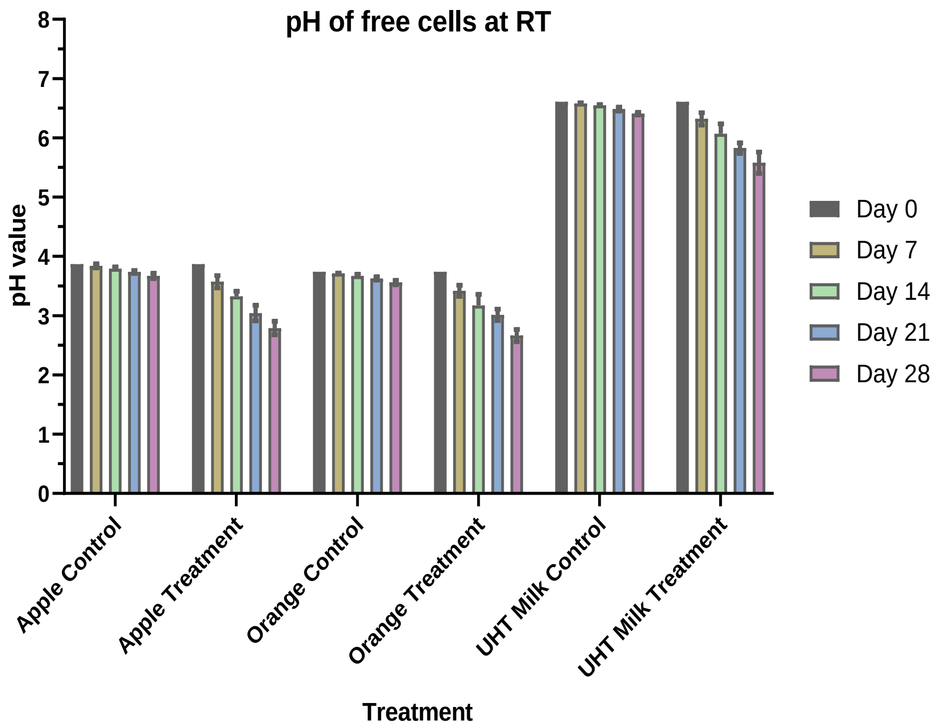

Figure 2. The pH values of probiotic products containing free bacteria over a period of 28 days of storage at room temperature. Data are expressed as the mean ± SD of 3 replicates.

When apple juice, orange juice, and UHT milk containing microencapsulated probiotic bacteria were stored at room temperature for four weeks, their average pH decreased from 3.87 ± 0.00 to 3.13 ± 0.11, 3.74 ± 0.00 to 3.07 ± 0.10, and 6.61 ± 0.00 to 5.88 ± 0.09, respectively. In contrast, the final pH values of the control samples without probiotic bacteria were 3.67 ± 0.04 for apple juice, 3.56 ± 0.03 for orange juice, and 6.40 ± 0.02 for UHT milk (Figure 3). After four weeks of storage at room temperature, the pH of apple juice, orange juice, and UHT milk containing microencapsulated probiotic bacteria showed a noticeable decrease, although it was less pronounced compared to the samples inoculated with free probiotic bacteria. This indicates that microencapsulation helped maintain the stability of the probiotics, reducing their impact on the pH change and suggesting a more controlled fermentation process in these products (Shoaei *et al.*, 2022).

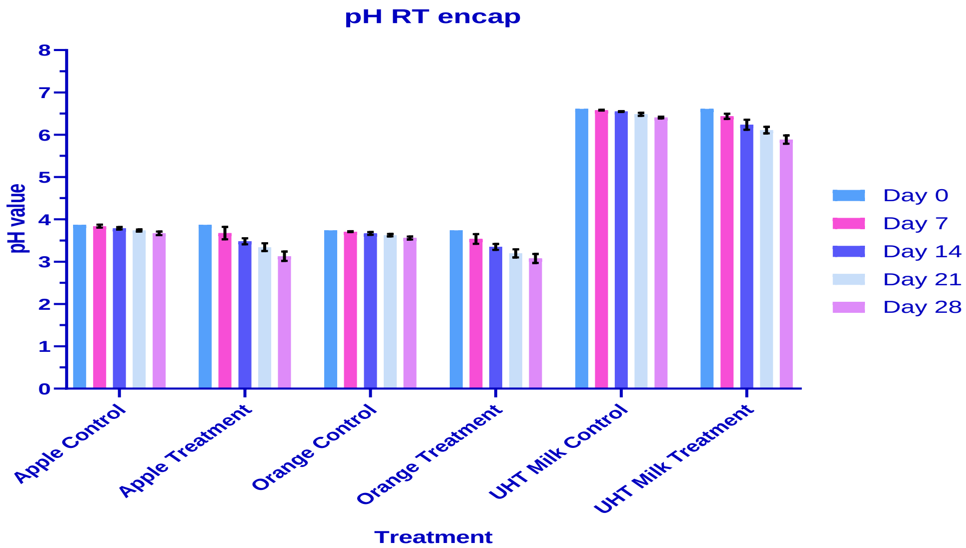

Figure 3. The pH values of probiotic products containing encapsulated bacteria over a period of 28 days of storage at room temperature. Data are expressed as the mean ± SD of 3 replicates.

All products exhibited a decreasing trend in pH during storage. After four weeks at 4 °C, the mean pH values of apple juice, orange juice, and UHT milk containing free probiotic bacteria declined from 3.87 ± 0.00 to 3.43 ± 0.08, 3.74 ± 0.00 to 3.34 ± 0.07, and 6.61 ± 0.00 to 6.24 ± 0.03, respectively. In comparison, the pH values of the control samples without probiotics dropped to 3.75 ± 0.06 for apple juice, 3.66 ± 0.02 for orange juice, and 6.54 ± 0.03 for UHT milk (Figure 4). After four weeks of storage at low temperature, the pH of the probiotic-inoculated apple juice, orange juice, and UHT milk decreased, though it remained higher than the pH of the control samples without probiotics. This suggests that while the probiotics continued to influence the pH, the cooler storage temperature may have slowed down the acidification process, allowing the probiotics to remain more stable than at room temperature (Mokhtari *et al.*, 2019).

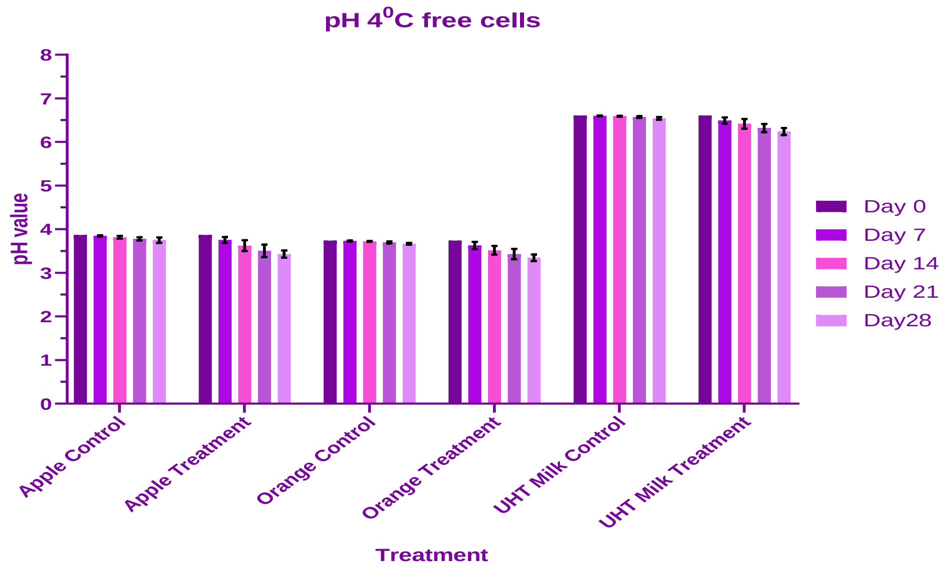

Figure 4. The pH values of probiotic products containing free bacteria over a period of 28 days of storage at 4 °C. Data are expressed as the mean ± SD of 3 replicates.

All treated probiotic products exhibited a reduction in pH. After four weeks of storage at 4 °C, the average pH of apple juice, orange juice, and UHT milk containing microencapsulated probiotic bacteria decreased from 3.87 ± 0.00 to 3.72 ± 0.10, 3.74 ± 0.00 to 3.61 ± 0.08, and 6.61 ± 0.00 to 6.49 ± 0.06, respectively. In contrast, the pH values of the control samples without probiotic bacteria were 3.75 ± 0.06 for apple juice, 3.66 ± 0.02 for orange juice, and 6.54 ± 0.03 for UHT milk at the end of the storage period (Figure 5). The pH of all probiotic-treated products decreased over four weeks of storage at low temperature, with those containing microencapsulated probiotics showing a smaller reduction compared to the free probiotic bacteria. This indicates that microencapsulation helped maintain a more stable pH, potentially preserving the viability and activity of the probiotics in these products during refrigeration (D'Amico *et al.*, 2025).

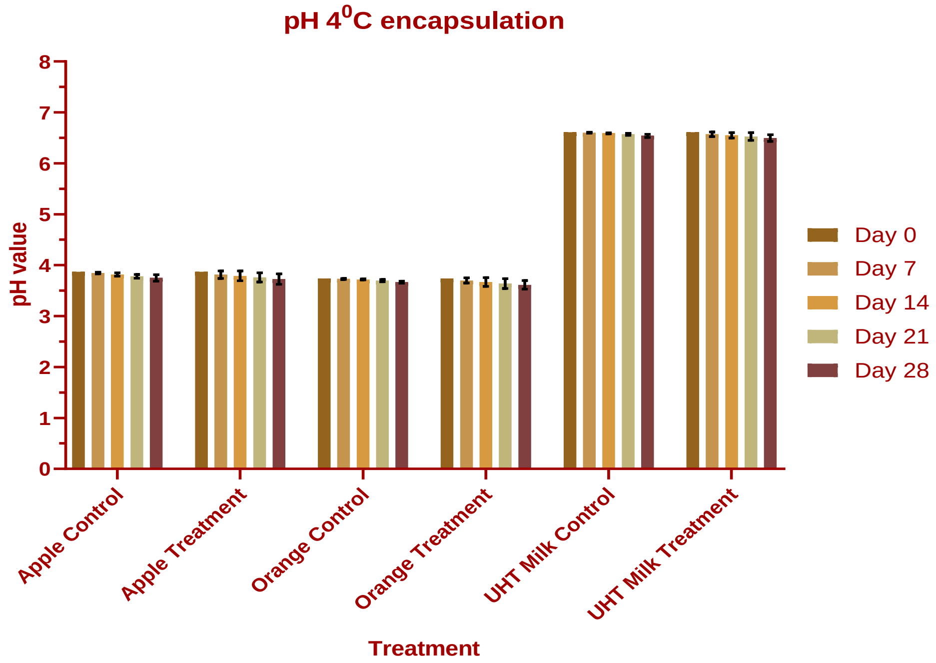

Figure 5. The pH values of probiotic products containing encapsulated bacteria over a period of 28 days of storage at 4 °C. Data are expressed as the mean ± SD of 3 replicates.

On 28th day of storage at room temperature, the mean pH values of products containing microencapsulated and free probiotic bacteria differed significantly ($P < 0.05$) (Figure 6). Apple juice with free probiotic bacteria showed a significantly lower mean pH of 2.79 ± 0.11 compared to 3.13 ± 0.11 in juice with microencapsulated probiotics. Similarly, the average pH of orange juice with free probiotic bacteria was 2.68 ± 0.11, which was significantly lower ($P < 0.05$) than that of orange juice containing microencapsulated probiotics, which had a mean pH of 3.05 ± 0.10.

After 28 days of storage at room temperature, the mean pH of UHT milk containing free probiotic bacteria was 5.54 ± 0.15, which was significantly lower ($P < 0.05$) than the pH of milk with microencapsulated probiotics, measured at 5.88 ± 0.09. Across all tested products, those with encapsulated probiotics maintained higher pH values at the end of the four-week period compared to their free-cell counterparts, indicating that microencapsulation provides a more stable environment for probiotic survival. The products containing microencapsulated probiotics maintained significantly higher pH values compared to those with free probiotics after four weeks of storage at room temperature, indicating better stability. This suggests that microencapsulation effectively preserves the probiotics, likely by offering enhanced protection against environmental factors that contribute to pH reduction (Vivek *et al.*, 2023).

Similarly, after 28 days of refrigerated storage at 4 °C, there were significant differences ($P < 0.05$) in the mean pH values between samples containing free and microencapsulated probiotics (Figure 7). Apple juice with free probiotics had a mean pH of 3.44 ± 0.08, significantly lower than the 3.71 ± 0.10 observed in samples with encapsulated bacteria. Orange juice containing free probiotics reached a pH of 3.34 ± 0.07, while juice with encapsulated probiotics maintained a significantly higher pH of 3.61 ± 0.08 ($P < 0.05$). Under refrigerated conditions, the samples with microencapsulated probiotics retained significantly higher pH levels than those with free probiotics, reflecting greater stability. These findings reinforce the protective effect of microencapsulation in maintaining probiotic integrity during cold storage (Taghrir *et al.*, 2024).

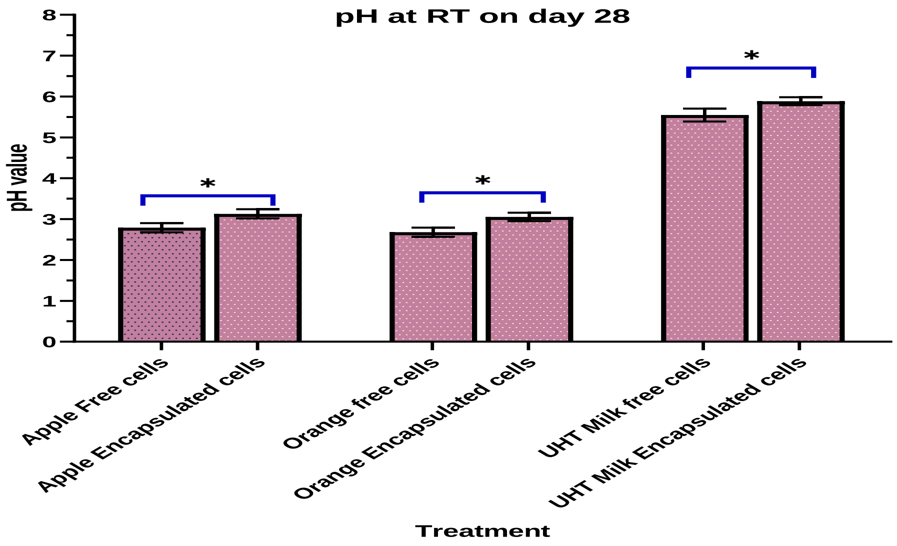

Figure 6. The pH values of probiotic products containing free and encapsulated bacteria on 28 days of storage at room temperature. Data are expressed as the mean ± SD of 3 replicates. Significant differences between the free bacteria and encapsulated bacteria indicate ****P* **< 0.05.

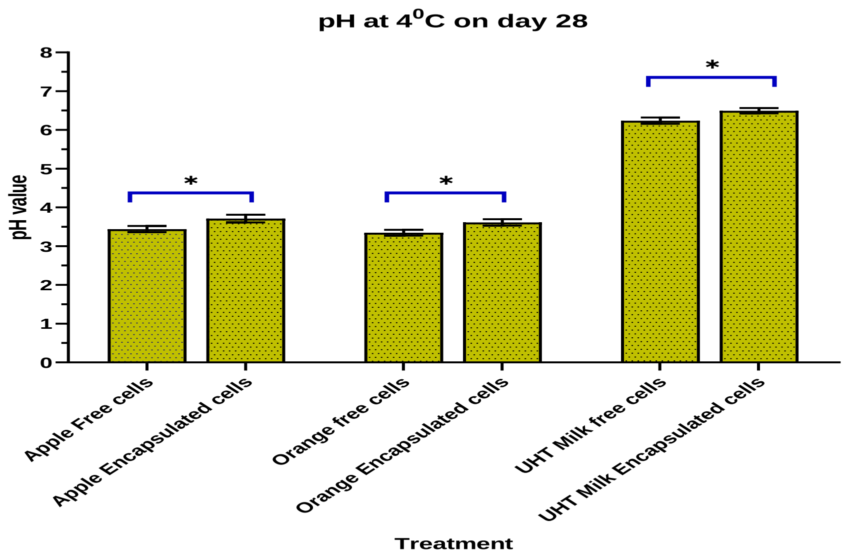

Figure 7. The pH values of probiotic products containing free and encapsulated bacteria on 28 days of storage at 4 °C. Data are expressed as the mean ± SD of 3 replicates. Significant differences between the free bacteria and encapsulated bacteria indicate ****P* **< 0.05.

After 28 days of storage at 4 °C, the average pH of UHT milk inoculated with free probiotic bacteria was 6.24 ± 0.08, which was significantly lower ($P < 0.05$) than the pH of UHT milk containing microencapsulated probiotic bacteria, which measured 6.49 ± 0.06. Across all products, samples containing encapsulated probiotic strains consistently maintained higher pH levels than those with free cells after four weeks of cold storage, indicating enhanced environmental stability for probiotics through microencapsulation. These findings align with previous research, where fruit juices such as apple, orange, sapodilla, grape, and watermelon inoculated with both free and microencapsulated bacteria exhibited a general decline in pH over time, with encapsulated strains maintaining significantly higher pH values throughout the storage period (Perricone *et al.*, 2015; Thakur and Joshi, 2017).

In some studies, it was observed that the pH of both peach synthetic jam and peach jam inoculated with probiotic microorganisms gradually declined during storage at 5 °C and 25 °C, with the lowest pH values recorded after 30 days at 25 °C (Randazzo *et al.*, 2013; Prieto-Santiago *et al.*, 2024). The pH of fruit juices and UHT milk decreased during storage at both room temperature and 4 °C (Daszkiewicz *et al.*, 2024). This could be attributed to the varying acidifying capacities of the strains used in the products, as well as the buffering capacities of these substrates.

3.2. Viability study during storage of probiotic products

On day 14 of storage at room temperature, the average probiotic count in apple juice, orange juice, and UHT milk with free probiotic bacteria decreased from their initial counts of 9.74 ± 0.00 log cfu/ml to 2.57 ± 0.28 log cfu/ml, 2.45 ± 0.31 log cfu/ml, and 4.77 ± 0.20 log cfu/ml, respectively. By day 7, the mean counts had dropped to 5.53 ± 0.34 log cfu/ml, 5.31 ± 0.50 log cfu/ml, and 6.59 ± 0.31 log cfu/ml. On day 21, orange and apple juices showed no viable probiotic bacteria, while UHT milk with free probiotic bacteria exhibited lower viability at 3.83 ± 0.49 log cfu/ml (Figure 8). The viability of free probiotic bacteria declined rapidly in all products stored at ambient temperature, with fruit juices losing all detectable probiotic cells before the end of the third week. These results highlight the poor thermal stability of free probiotics in acidic and nutrient-limited environments (Taghrir *et al.*, 2024).

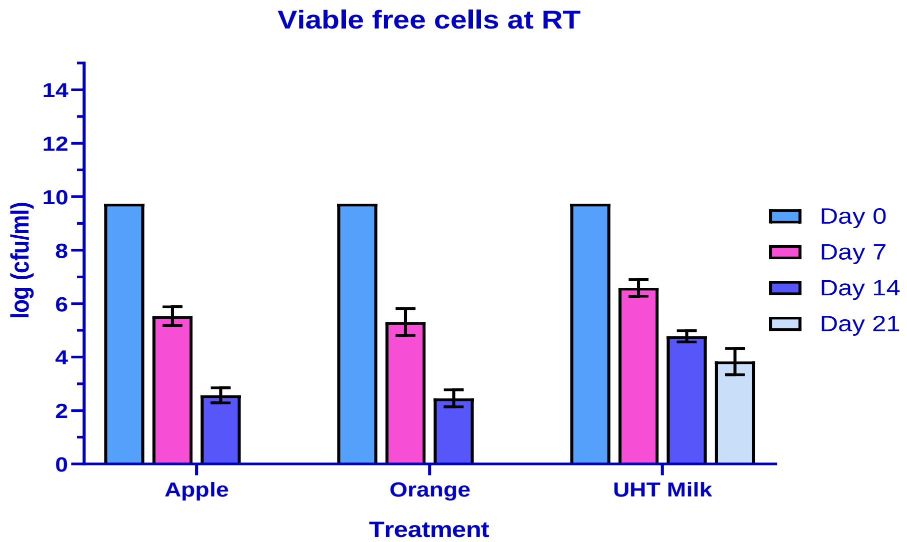

Figure 8. Viability of free bacteria in probiotic products stored at room temperature over a period of 28 days. Data are expressed as the mean ± SD of 3 replicates.

Over the course of four weeks at room temperature, the average count of microencapsulated probiotic bacteria in apple juice, orange juice, and UHT milk decreased from 9.74 ± 0.00 log cfu/ml to 2.45 ± 0.26 log cfu/ml, 2.14 ± 0.44 log cfu/ml, and 3.50 ± 0.55 log cfu/ml, respectively. On day 14 of room temperature storage, the mean viable count in apple juice, orange juice, and UHT milk with microencapsulated probiotic bacteria was 6.46 ± 0.33 log cfu/ml, 6.14 ± 0.36 log cfu/ml, and 7.44 ± 0.49 log cfu/ml, respectively (Figure 9). Microencapsulated probiotic bacteria demonstrated improved survival in all tested beverages during storage at ambient temperature, retaining higher viability over time compared to free cells. This suggests that microencapsulation offers protective benefits against environmental stress, particularly in acidic and low-nutrient conditions (Rodrigues *et al.*, 2020).

After four weeks of storage at 4 °C, the average microbial count in apple juice, orange juice, and UHT milk with free probiotic bacteria decreased from 9.74 ± 0.00 log cfu/ml to 3.36 ± 0.47 log cfu/ml, 3.25 ± 0.60 log cfu/ml, and 4.32 ± 0.60 log cfu/ml, respectively. On day 14 of storage at 4 °C, the mean microbial count dropped to 7.24 ± 0.48 log cfu/ml, 7.16 ± 0.44 log cfu/ml, and 7.80 ± 0.23 log cfu/ml (Figure 10). Free probiotic bacteria showed moderate survival under refrigeration, with a gradual decline in viable counts throughout the storage period. The lower temperature helped slow down bacterial loss but was not sufficient to maintain optimal viability over time (Nag and Das, 2013).

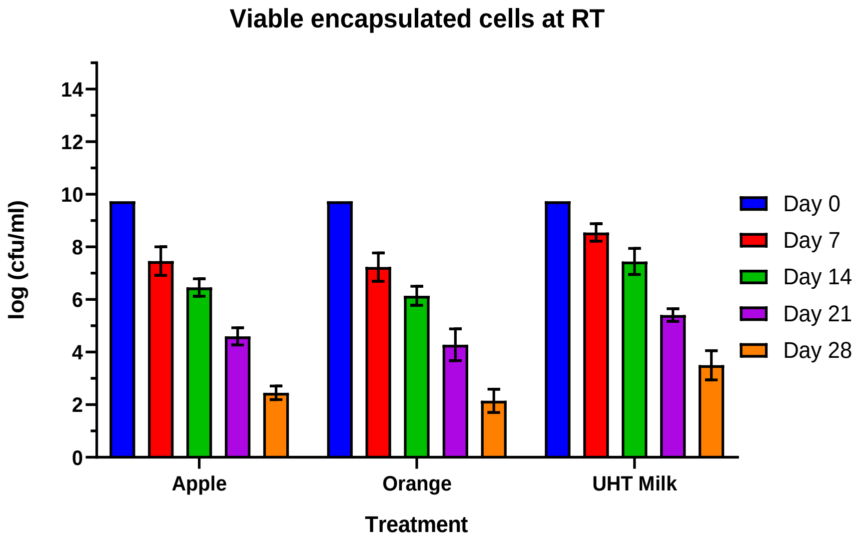

Figure 9. Viability of encapsulated bacteria in probiotic products stored at room temperature over a period of 28 days. Data are expressed as the mean ± SD of 3 replicates.

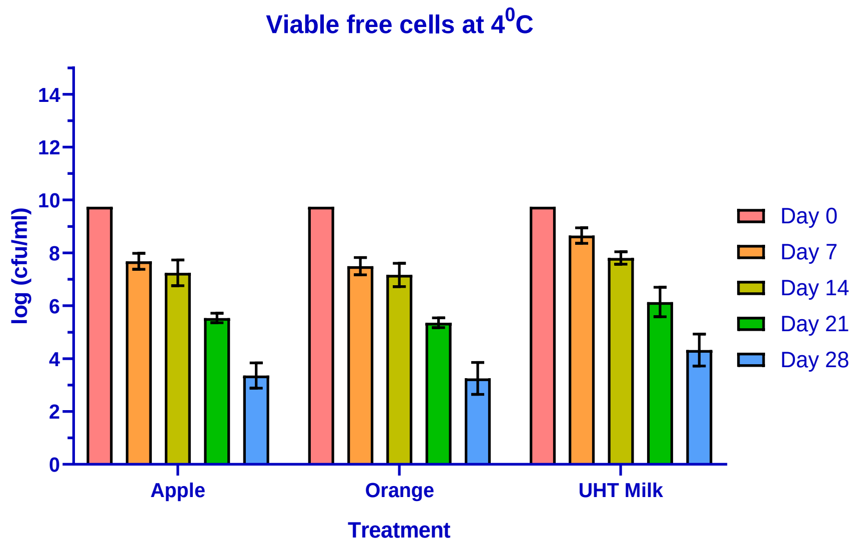

Figure 10. Viability of free bacteria in probiotic products stored at 4 °C over a period of 28 days. Data are expressed as the mean ± SD of 3 replicates.

As the storage duration increased, the viable count decreased in all probiotic-treated products. In apple juice, orange juice, and UHT milk containing microencapsulated probiotic bacteria, the average microbial count decreased from 9.74 ± 0.00 to 7.56 ± 0.32, 7.27 ± 0.31, and 7.74 ± 0.16, respectively, after four weeks of storage at 4 °C (Figure 11). Microencapsulated probiotic bacteria maintained high viability during cold storage, with only a slight reduction in microbial counts over time. This suggests that encapsulation offers protective benefits that enhance probiotic survival in refrigerated functional beverages (Dianawati *et al.*, 2016).

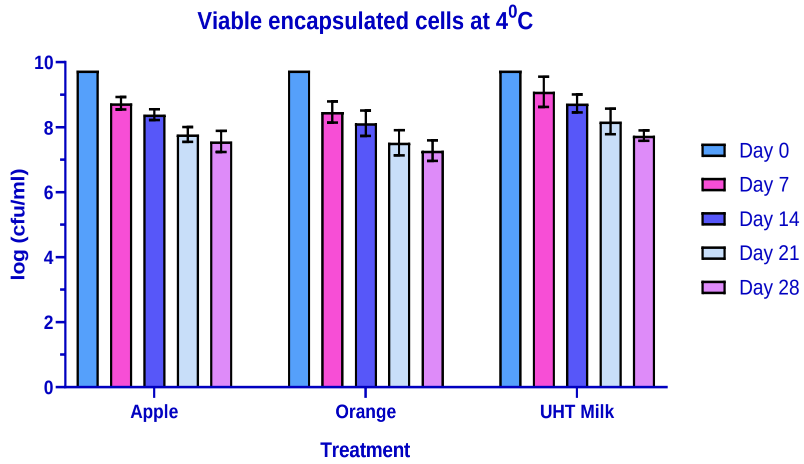

Figure 11. Viability of encapsulated bacteria in probiotic products stored at 4 °C over a period of 28 days. Data are expressed as the mean ± SD of 3 replicates.

The mean microbial count of free and microencapsulated probiotic bacteria in each product on day 14 at room temperature showed a significant difference (P<0.001) (Figure 12). In apple juice, the mean viable count with free probiotic bacteria was 2.57 ± 0.28 log cfu/ml on day 14 at room temperature, which was significantly lower (P<0.001) than the apple juice with microencapsulated probiotic bacteria, which had a mean viable count of 6.46 ± 0.33 log cfu/ml. Similarly, on day 14, the average viable count of orange juice containing free probiotic bacteria was 2.45 ± 0.31 log cfu/ml, while the mean viable count of orange juice containing microencapsulated probiotic bacteria was significantly higher (P<0.001) at 6.14 ± 0.36 log cfu/ml.
For UHT milk, the mean viable count with free probiotic bacteria was 4.77 ± 0.20 log cfu/ml on day 14 at room temperature. In contrast, UHT milk with microencapsulated probiotic bacteria had a mean viable count of 7.44 ± 0.49 log cfu/ml on the same day, which was substantially higher (P<0.001). All products containing microencapsulated probiotic bacteria showed a higher viable microbial count at the end of the four-week room temperature storage period compared to those with free probiotic bacteria. This suggests that microencapsulation provides a more stable environment for probiotic bacteria. Microencapsulated probiotics demonstrated significantly better survival than free probiotics during ambient storage, as shown by consistently higher microbial counts across all products. This indicates that encapsulation enhances probiotic stability and viability, making it a more effective delivery method for maintaining functional properties in non-refrigerated conditions (Vivek *et al.*, 2023).

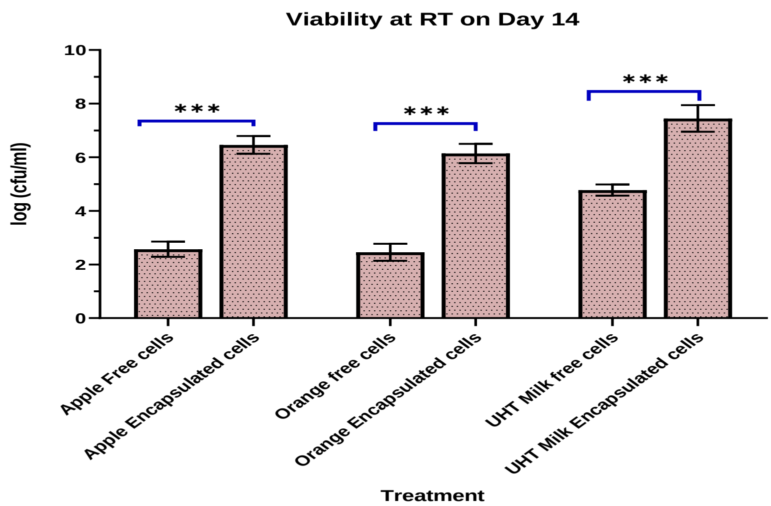

Figure 12. Viability of free and encapsulated bacteria in probiotic products stored at room temperature on day 14. Data are expressed as the mean ± SD of 3 replicates. Significant differences between the free bacteria and encapsulated bacteria indicate ******P* **< 0.001.

The mean viable microbial count of free and microencapsulated probiotic bacteria in all products on day 28 at 4 °C showed a significant difference (*P*<0.001) (Figure 13). For example, on day 28 at 4 °C, the mean viable microbial count of apple juice with free probiotic bacteria was 3.36 ± 0.47, which was significantly lower (*P*<0.001) compared to apple juice with microencapsulated probiotic bacteria, which had a mean viable count of 7.56 ± 0.32. The average microbial count of orange juice containing free probiotic bacteria was 3.25 ± 0.60 on day 28 of storage at 4 °C, while the mean count of orange juice containing microencapsulated probiotic bacteria was significantly higher (*P*<0.001) at 7.27 ± 0.31. Additionally, the mean viable count of UHT milk inoculated with free probiotic microbes was 4.32 ± 0.60 on day 28 of storage at 4 °C. The mean viable count of UHT milk containing free probiotic bacteria on day 28 at 4 °C was significantly lower (*P*<0.001) compared to UHT milk with microencapsulated probiotic bacteria, which had a mean count of 7.74 ± 0.16. After a four-week storage period at 4 °C, all products containing encapsulated probiotic bacteria exhibited a higher viable microbial count than those treated with free probiotic bacteria. This suggests that the habitat of probiotic bacteria is more stable when they are microencapsulated.

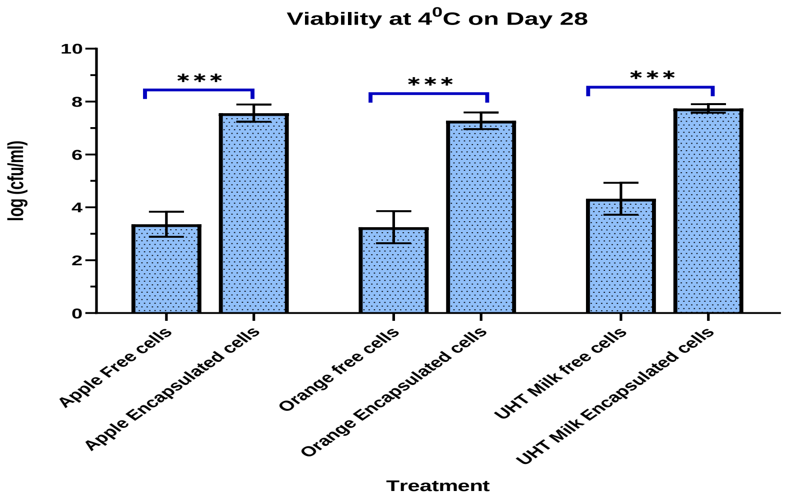

Figure 13. Viability of free and encapsulated bacteria in probiotic products stored at 4 °C on day 28. Data are expressed as the mean ± SD of 3 replicates. Significant differences between the free bacteria and encapsulated bacteria indicate ******P* **< 0.001.

After 48 hours of fermentation and storage at 4 °C, lactobacilli proliferation was found to be higher in apple juice compared to grape and orange juices (Mousavi *et al.*, 2011; Espirito-Santo *et al.*, 2015). Orange juice exhibited the highest probiotic viability during fermentation and storage, while grape juice showed the lowest viability throughout a 30-day storage period (White and Hekmat, 2018). This variation could be attributed to differences in the types of fruit juices and the bacterial strains used. In both UHT yogurt and conventionally treated milk, microencapsulated cells survived longer than free cells (Krasaekoopt *et al.*, 2006; Ribeiro *et al.*, 2014).

4. Conclusions

The pH of probiotic apple juice, orange juice, and UHT milk, along with the viability of locally isolated *Pediococcus acidilactici* and *Enterococcus faecium* strains, decreased over time. Microencapsulated bacteria exhibited greater stability in the fruit juices and UHT milk compared to free bacteria, both at room temperature and at 4°C. Further extensive studies on animal and human models are necessary to assess the safety and efficacy of these products. Probiotic products may offer potential health benefits to the host.

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Data availability

All relevant data are within the manuscript.

Conflict of interest
None to declare.

Authors' contribution
Md. Abdul Jalil: conceptualization, planning, design, laboratory work of the study, data analysis and manuscript writing; Farzana Fiza Rahima: bacterial culture, data assemble in excel; Anirudha Paul Shirshaw and Nahida Akter: purchased fruits, prepared fruit juice in laboratory and assistance in manuscript writing; Md. Taslim Hossain: conceptualization and edited manuscript; S. M. Khaledur Rahman: microencapsulation process and reviewed the manuscript writing. All authors have read and approved the final manuscript.

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