RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.106

ScienceAsia 50 (6): 2024: ID 2024106: **1–8**

Development of probiotic-enriched dog snacks for improved canine digestive health

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Received 10 Sep 2023, Accepted 13 Jun 2024
Available online 30 Nov 2024

ABSTRACT: This study aimed to develop a probiotic carrier for dogs in the form of dried pork liver and bacon slices. *Lactobacillus* sp. Pom1 and *Limosilactobacillus (Li.) fermentum* Pom5 were impregnated into the slices, which were then dried using convectional drying at 45 °C, vacuum-sealed, and stored at 4 °C for 8 days. The optimal drying time of 12 h at 45 °C resulted in the highest survival of probiotics, exceeding recommended standards for shelf life. The viability of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in dried liver and bacon slices during a 28-day storage period was evaluated in 3 different treatments: coating with probiotics in peptone water (coated), coating with sodium alginate (coated-SA), and coating with sodium alginate and glycerol (coated-SA-G). The study demonstrated that the slices coated with SA-G exhibited significantly higher viable cell counts for both *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5, compared to the SA-coated and coated slices. Additionally, coating the slices with SA-G not only effectively protected the probiotics from simulated GI digestion but also significantly improved their survival and viability during a 28-day storage at 4 °C. Bacteriological analysis demonstrated the absence of yeast, fungi, and *Salmonella*, ensuring product safety during storage. Scanning electron microscopy confirmed successful probiotic incorporation onto the slices with SA-G coating providing additional protection and adherence. In conclusion, SA-G-coated dried liver and bacon slices enriched with *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 offer a stable and effective probiotic delivery system for dogs, presenting possibilities for the development of functional pet food products to enhance canine health and well-being.

KEYWORDS: dog snack, pork liver, bacon, probiotics

INTRODUCTION

At present, there are millions of domesticated animals worldwide, which has led to the expansion of the pet food industry, with a strong focus on meeting the complete nutritional needs of pets. Pets do not have the freedom to choose their own food and may be given food that closely resembles human food based on their owners' preferences [1]. In addition to this, owners also tend to purchase dry pet food because it has a long shelf life and adequate nutrients and is inexpensive [2]. Similarly, dog treats are also popular among pet owners, often given as a reward when the dog follows commands. Unlike the numerous studies focusing on probiotics from human and livestock sources, there is limited research on probiotic supplements specifically designed for pets, including dogs. Our work addresses this gap by isolating and studying probiotics from the digestive tract of dogs, contributing valuable insights to the development of probiotic supplements tailored for canine health.

Probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts [3]. Probiotics, like lactic acid bacteria (LAB), enhance pet health by balancing gut bacteria [3–5]. Selecting safe, identifiable strains that compete in the intestine is crucial. Drying methods, like convective drying (CD), extend product shelf life and prevent microbial growth [8]. To boost probiotic survival in pets, encapsulation with hydrocolloids like starch or alginate is common, improving their viability in the intestines [9–12]. In a previous study, potential probiotic strains were isolated from canine feces and characterized for their probiotic properties [13]. Based on our earlier findings, we selected *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 due to their extended viability under laboratory conditions. Furthermore, our previous research successfully produced probiotics using microencapsulation with sodium alginate (SA) and sodium alginate-goat milk (SAGM) matrices, providing an alternative carrier for probiotics for dogs [14, 15]. The present study aims to comprehensively evaluate the safety aspects of these selected probiotic bacterial strains while developing probiotic-based products for dogs. The liver and bacon slices were chosen as carriers, and the CD method was applied to incorporate

the probiotics. To enhance the viability and protection of the probiotic cells, a coating process involving a mixture of probiotics, alginate, and alginate-glycerol hydrogel was utilized. The results obtained from this research will contribute to the development of commercial dog treats enriched with beneficial probiotics, thereby promoting the overall health and well-being of dogs. These probiotic treats have the potential to provide significant health benefits to canine companions, making them a valuable addition to the pet food market.

MATERIAL AND METHODS

Bacterial strains and culture media

Lactobacillus sp. Pom1 and *Li. fermentum* Pom5, isolated from dog feces and used in this study [13], were cultured on de Man, Rogosa, and Sharpe (MRS) agar plates or in MRS broth (HiMedia, Mumbai, India), and then microaerophilically incubated at 37 °C for 48 h.

Adhesion ability of probiotics to Caco-2 cells

The methodology for determining the adhesion ability of the probiotics on Caco-2 cells followed the approach described by Foongsawat et al [13]. The adhesion rate was calculated using the following formula.

$$\text{Adhesion rate } (\%) = (N/N_0) \times 100,$$

where N is the number of colony-forming unit (cfu) of probiotic cells after adhesion to the Caco-2 cell line (4 h), and N_0 is the number of cfu of the initially inoculated probiotic cells [13].

Safety evaluation of isolates

Blood hemolysis test

The safety of selected isolates was assessed on Columbia agar with 5% sheep blood (HiMedia) [16]. *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 were streaked in triplicate. After 48 h at 37 °C, hemolysis was categorized as alpha (partial, green zone), beta (complete, clear zone), or gamma (no change).

Antibiotic sensitivity testing

The antibiotic susceptibility of the LAB strains was assessed using the MIC Test Strip (Liofilchem® MTSTM, Roseto degli Abruzzi, Italy). LAB cultures (5×10^5 cfu/ml) were spread on Mueller–Hinton agar plates (HiMedia). MIC Test Strips with ampicillin, chloramphenicol, doxycycline, gentamicin, and tetracycline were placed on each plate. After approximately 24 h at 37 °C, the minimum inhibitory concentration (MIC) for each antibiotic was determined by examining the zones of bacterial growth inhibition and their intersection with the test strip's concentration mark. Susceptibility or resistance followed the cutoff values set by the European Food Safety Authority (EFSA) in 2012 [17].

Biogenic amine production analysis

To investigate the production of biogenic amines, a decarboxylase base medium supplemented with 2% histidine, lysine, ornithine, and tyrosine (Sigma-Aldrich, St. Louis, MO, USA) was employed. The base medium was incorporated into MRS broth at a concentration of 0.25% (w/v). After incubation for 4 days at 37 °C, a positive reaction was indicated by a color change to purple in the broth [18].

Preparation of dog snack impregnated with probiotic bacteria

Preparation of probiotics

Lactobacillus sp. Pom1 and *Li. fermentum* Pom5 were cultured in MRS broth at 37 °C for 48 h. The cells were then harvested by centrifugation at 10,000×g for 15 min at 4 °C, washed twice with peptone water, and resuspended in peptone water to a concentration of 10^9–10^{10} cfu/ml.

Sample preparation

Fresh pork liver and bacon were purchased from a local market and stored at 0 °C for 24 h before use. They were washed, wiped with a paper towel, and sliced into 5 × 5 cm^2 pieces. The bacon and liver pieces were blanched in boiling water for 10 s and 2 min, respectively, to reduce bacterial contamination on the surface (this time was proved to be effective for reduction). They were then placed in a sterile peptone water containing the probiotic strains prepared as previously described and shaken at 150 rpm for 20 min. The pieces were then dried in a biosafety cabinet class 2 at room temperature for 20 min to allow the probiotic bacteria to adhere to the bacon and liver pieces and to evaporate excess water vapor. The bacon and liver pieces impregnated in peptone water without probiotics were prepared simultaneously as the control [19].

Convective drying (CD) of bacon and liver pieces coated with probiotics

The bacon and liver pieces coated with probiotic bacteria, as prepared earlier, were subjected to the CD method for drying. The CD process involved heating the bacon and liver pieces coated with probiotics at 45 °C for 6, 12, and 18 h in a hot air oven (Thermo Fisher Scientific Inc., Massachusetts, USA). Afterwards, the product samples were stored in vacuum-sealed bags and kept at 4 °C for 8 days. The surviving probiotic bacteria were counted, and the total number of bacteria in the product was determined as cfu/g of sample [19].

Coating of probiotic bacteria in dried bacon and liver using alginate

To prepare the alginate-coated probiotic bacteria, a 2% (w/v) solution of sodium alginate (HiMedia) solution

was prepared and sterilized by autoclaving at 121 °C for 20 min. This solution was then combined with the probiotic bacterial cells that were prepared as outlined above at a 4:1 ratio (sodium alginate solution to bacterial cells). Pieces of liver and bacon were coated with this mixture, shaken gently for 20 min, and placed in a 0.5 M calcium chloride solution for calcium alginate film formation. After 20 min, the samples underwent CD and were vacuum-sealed for storage at 4 °C and 25 °C for 4 weeks to assess shelf life [20].

For the alginate coating with glycerol, we initially prepared a 2% sodium alginate solution and a 2% glycerol solution separately. Subsequently, these solutions were combined and mixed, and the resulting mixture underwent sterilization. The final composition was established by mixing these solutions with probiotic bacterial cells at a 4:1 ratio (sodium alginate-glycerol solution to bacterial cells). The processed mixture was then subjected to the procedures outlined above.

Microbiological analysis

Bacterial count

Five grams of bacon and liver pieces were placed into a stomacher bag, followed by the addition of 45 ml of peptone water. The mixture was homogenized for 2 min using a stomacher (Seaward, West Sussex, England). Next, a 10-fold dilution of the homogenized sample was prepared with peptone water and plated onto MRS agar for LAB or Plate count agar (PCA) (HiMedia) for total aerobic bacteria. The plates were then incubated at 37 °C for 48 h under microaerophilic conditions for LAB and aerobic conditions for total aerobic bacteria. Finally, bacterial colonies were counted and recorded as cfu/g.

Yeast and mold count

To enumerate the number of yeast and mold present in the sample, a homogenized sample was uniformly spread onto Sabouraud dextrose agar (SDA) (HiMedia) supplemented with 0.05 g/l of chloramphenicol. The plates were then incubated at 25 °C for 5–7 days. The colonies were then counted and recorded as cfu/g.

Salmonella count

To detect *Salmonella* according to Standard PN-EN ISO 6579:2003, 5 g of liver or dry bacon were mixed with 25 ml of buffered peptone water (BPW) (HiMedia) and incubated at 35 °C for 24 h. Then, the sample was transferred to Rappaport-Vassiliadis (RV) broth (HiMedia) and incubated at 42 °C for 24 h. Subsequently, it was streaked onto xylose lysine deoxycholate (XLD) agar (HiMedia) and incubated at 35 °C for 24 h. The presence of *Salmonella* was confirmed by the appearance of characteristic colonies on the agar plates.

Physical analysis: pH, water activity (Aw), and moisture content

Five grams of liver and bacon were used to measure the water activity with a water activity meter (Charpa Techcenter Co. Ltd., Bangkok, Thailand). For pH measurement, the liver and bacon samples were homogenized by mixing them with 45 ml of distilled water using a stomacher. The pH of the homogenized mixture was then measured using a pH-meter (Denver Instrument, Bohemia, New York, USA). To determine the moisture content, 5 g of the sample were weighed and then dried at 105 °C for 24 h. The dried sample was allowed to cool in a desiccator before calculating the moisture content using the following formula:

$$\text{Moisture Content (\%)} = \frac{W_i - W_f}{W_f} \times 100$$

where W_i is the initial weight and W_f is the final weight of the sample after drying.

Survival of probiotic bacteria in dried liver and bacon slices in the gastrointestinal system

The survival of bacteria in dried liver and bacon slices within the gastrointestinal system was assessed by subjecting them to artificial gastric juice (AGJ) and artificial intestinal juice (AIJ), and the enumeration of viable cells was carried out as follows:

AGJ was prepared by dissolving 0.3 g of pepsin (1,000 U/mg) (HiMedia) and 0.5 g of NaCl in 100 ml of solution at pH 2 [21]. Five grams of probiotic bacteria-coated liver or bacon were mixed with 45 ml of sterilized AGJ and shaken at 100 rpm at 37 °C for 180 min. The surviving probiotic bacteria were then enumerated following the previously described method.

AIJ was prepared by dissolving 0.3 g of bile salt (HiMedia) and 0.5 g of NaCl in 100 ml of solution at pH 8 [21]. Five grams of probiotic bacteria-coated liver or bacon were mixed with 45 ml of sterilized AIJ and shaken at 100 rpm at 37 °C for 240 min. The surviving probiotic bacteria were enumerated using the method described earlier.

Cell adhesion analysis by Scanning Electron Microscope (SEM)

Samples of liver or bacon coated with probiotics were cut and immersed in 70%, 80%, 90%, 95% ethanol twice for 30 min each, and 100% ethanol twice for 30 min each. The samples were then dried at the critical point and mounted onto a stub. Next, they were coated with gold and observed using an SEM (JSM-IT300, JEOL Ltd., Tokyo, Japan) for surface adhesion analysis.

Statistical analysis

Statistical analysis was performed using the Statistical Packages for the Social Science (SPSS) version 26 software. One-way analysis of variance (ANOVA) was used to analyze the variation. The mean values of each group were compared using the least significant difference (LSD) test (Fisher's least significant difference) with a 95% confidence level.

RESULTS AND DISCUSSION

Probiotic characterization

Lactobacillus sp. Pom1 and *Li. fermentum* Pom5 were identified and recognized as potential probiotics due to their specific properties, as reported by Foongsawat et al [13]. Moreover, both strains demonstrated effective adhesion to Caco-2 cells with adherence rates of 84.25±1.85% for *Li. fermentum* Pom5 and 87.63±2.70% for *Lactobacillus* sp. Pom1. *In vitro* safety evaluation revealed that both strains were sensitive to ampicillin, chloramphenicol, gentamicin, and erythromycin. One of the prerequisites for identifying particular strains as potential probiotics is the absence of acquired and transferable antibiotic resistance [22]. Hence, it is imperative to thoroughly screen microbes for antibiotic resistance genes before considering them for use as probiotics. Additionally, they exhibited gamma-hemolysis (no hemolysis) and lacked the ability to produce biogenic amines, which can be toxic to animals when present in high amounts. Based on these findings, both LAB strains are considered safe for canine health.

Impact of drying time on the viability of probiotics in liver and bacon slices

As shown in Fig. 1, the highest number of surviving bacteria was observed at the 6–12 h drying for both liver and bacon slices. This trend remained consistent over the 8-day storage period. The viability of both strains exceeded the recommended amount of 6 log cfu/g which exceeds the FDA recommended standards for probiotic effectiveness in providing health benefits [23] when dried for 6 and 12 h. However, the number of surviving bacteria declined when the slices were dried for 18 h. After 8 days, the water activity of liver slices was 0.93±0.05, 0.82±0.02, and 0.72±0.01 following 6, 12, and 18 h, respectively, of drying. For bacon slices, the water activity was 0.90±0.01, 0.72±0.03, and 0.70±0.06, respectively. Consequently, the optimal drying time for the liver and bacon slices was determined to be 12 h. This duration proved to be highly effective in reducing the availability of water, while simultaneously ensuring a high level of probiotic survivability. Reduced water activity helps prevent spoilage and foodborne illnesses caused by microbial contamination, significantly extending the product shelf life. Typically, the minimum water activity required for bacterial growth falls within the range of 1.00 to 0.87 for bacteria, 0.91 to 0.87 for many yeasts, and 0.87 to 0.80 for most foodborne molds [24].

Viability of probiotics in dried liver and bacon slices during 28 days of storage

The survival of probiotics is challenged not only during drying processes but also during storage. Various factors such as residual moisture content, atmospheric oxygen level, exposure to light, relative humidity, and storage temperature significantly influence the viability of probiotics [25–27]. To address this challenge, microencapsulation was incorporated in this study to extend the viability of probiotic cells during storage.

For this purpose, alginate was used as the encapsulation material. The alginate coating acts as a physical barrier, protecting probiotics from oxidative reactions, low pH, and bile salts, thereby extending their shelf life, enabling controlled release, and enhancing survival during gastrointestinal transit [28]. In this study, the addition of glycerol to the alginate solution was intended to enhance the mechanical properties and stability of the coating material. Alginate alone forms a gel-like structure when exposed to divalent cations such as calcium ions (Ca^{2+}), but pure alginate coatings can be brittle and prone to cracking, especially during the drying process. The incorporation of glycerol acted as a plasticizer, increasing the flexibility and reducing the brittleness of the coating material. Additionally, glycerol improved the moisture retention properties of the coating, which is essential for protecting the probiotic bacteria during drying or storage processes [29]. The study investigated the viability of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in dried liver and bacon slices during a 28-day storage period at 4 °C, as shown in Fig. 2. The results demonstrated that the slices coated with SA-G exhibited significantly higher viable cell counts for both *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 compared to the SA-coated and coated slices ($p < 0.05$). Similar results were obtained at room temperature, where SA-G-coated slices exhibited higher viable cell counts (Table S1). This outcome suggests that the SA-G coating method effectively enhanced the survival and viability of the probiotic bacteria on the slices. Importantly, all treatments maintained cell viability above the recommended threshold of 6 log cfu/g even after 28 days of storage. These findings align with previous research highlighting the protective effects of alginate coatings against issues such as water migration [30] and oxidation reactions [31] during storage.

Evaluation of total aerobic counts, yeast, mold, and *Salmonella*

To assess the bacteriological quality of the dried meat products, measurements were conducted for the total

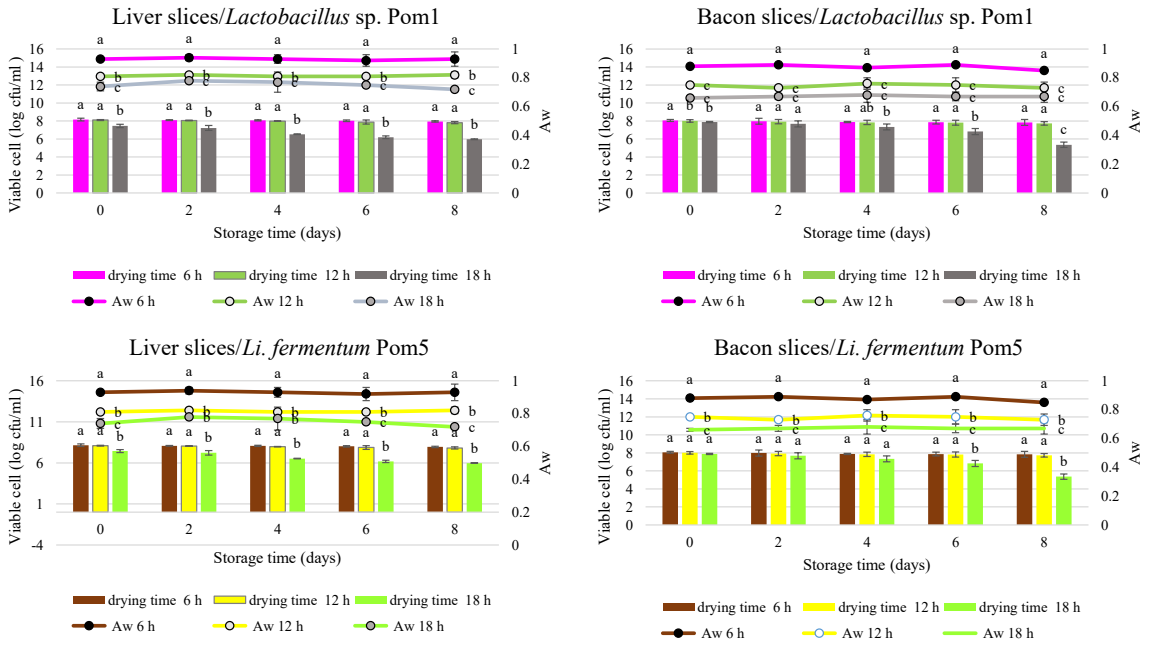

Fig. 1 Effect of drying time on the viability and Aw of impregnated *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in dried liver and bacon slices. The bar graph illustrates the viable cell count (in log cfu/g), while the line graph depicts the Aw value. Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different letters indicate statistically significant differences at each condition of the experiment with a significance level of $p \leqslant 0.05$.

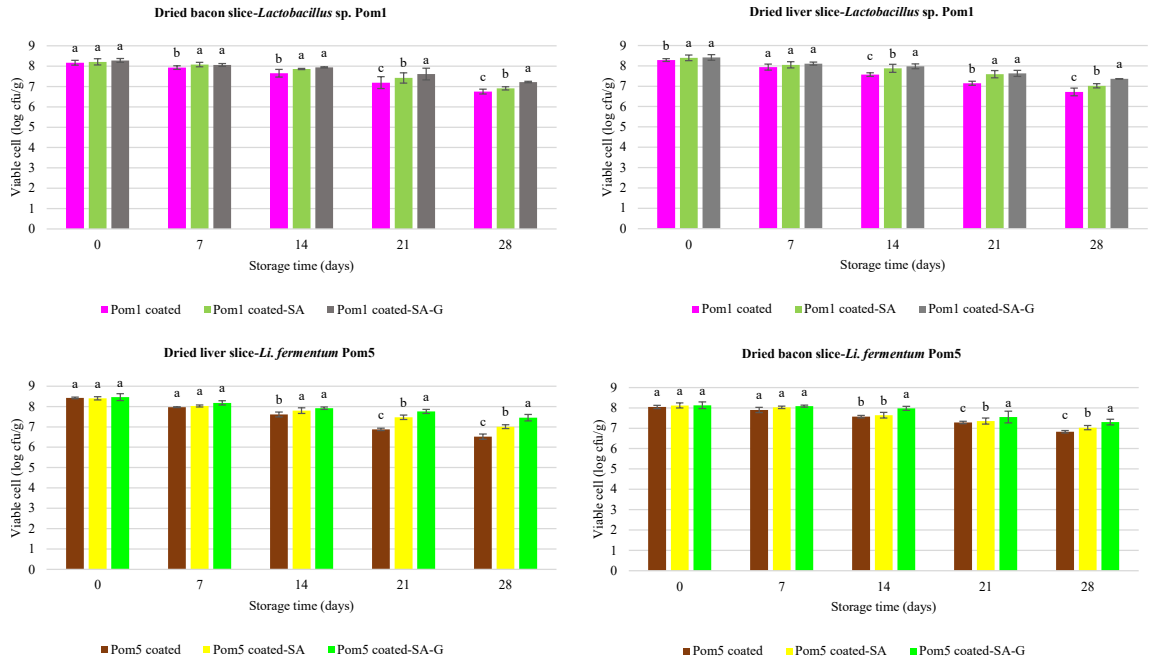

Fig. 2 Viability of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in dried liver and bacon slices with various coatings (coated, alginate-coated (coated-SA), and alginate glycerol-coated (coated SA-G)) during the 28-day storage at 4 °C. Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different letters indicate statistically significant differences at each condition of the experiment with a significance level of $p \leqslant 0.05$.

aerobic count, yeast, mold, and *Salmonella*. For dried liver and bacon slices impregnated with *Lactobacillus* sp. Pom1, the total aerobic counts ranged from 3.45 to 3.67 log cfu/g after 28 days of storage at 4 °C. Similarly, for *Li. fermentum* Pom5, the total aerobic counts ranged from 2.88 to 3.02 log cfu/g after the same storage period (Table S2). At room temperature, the aerobic counts ranged from 3.20 to 3.81 log cfu/g after 28 days of storage (data not shown). It is important to note that the recommended standards for total aerobic counts can vary depending on specific regulations or guidelines in different regions or industries. For instance, according to Kukier et al [32] concerning the microbiological quality of livestock feed, the total aerobic microbial count (TAMC) should not exceed 10^6 cfu/g.

Furthermore, the microbiological analysis revealed no detection of yeast, fungi, or *Salmonella* in any of the samples after the 28-day storage period at both 4 °C and room temperature. This finding suggests that the dried meat products were free from these potential contaminants and maintained their bacteriological quality throughout the storage period.

Physical analysis of dried liver and bacon slices during 28 days of storage

Aw, pH, and moisture content are important parameters that indicate microbial and physicochemical changes in low-moisture food systems [33]. The Aw values of liver slices, whether coated or uncoated with the 2 probiotics, remained stable throughout the 28 days, ranging from 0.80 to 0.82. In bacon slices, the Aw values ranged from 0.72 to 0.75 (Table S3). The absence of yeast, fungi, and *Salmonella* indicates that the Aw values were sufficiently low to prevent microbial growth. Regarding pH, the pH values fluctuated during the 28-day period, ranging from 5.98 to 6.84 in liver slices and from 5.98 to 7.22 in bacon slices. These values fall within the optimal pH range for the growth of lactobacilli (pH 5.0–6.5). It is worth noting that the survival of probiotics during storage is significantly influenced by the pH of the products [34]. For example, lactobacilli can grow and survive in fermented products with pH values ranging from 3.7 to 4.3 [35]. The moisture content also varied, with liver slices ranging from 20.02% to 23.67% under all conditions. The moisture content values obtained in this study, ranging from 20.02% to 23.67%, fell within the typical moisture content range (20% to 40%) conducive to the growth and viability of lactobacilli. However, the moisture content of bacon slices, ranging from 11.50% to 16.08%, was below the typical range. Despite this, certain strains were found to survive, potentially influenced by the protective nature of the lipid content in bacon, which may shield these strains. Additionally, other internal and external factors of the food could contribute to their survival during the 28 days of storage.

Survival of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in dried liver and bacon slices with various coatings within the gastrointestinal system

The intestine is the primary site of action for probiotics, and various targeted delivery systems have been developed to help probiotics reach their intended locations and produce beneficial effects. To evaluate the effect of coating formulation on the survival of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5, dried liver and bacon slices with and without alginate coating were subjected to AGJ and AIJ. The viability of probiotics in AGJ at pH 2 for 180 min was depicted in Fig. S1. It was observed that the dried liver and bacon slices, when coated with SA-G, exhibited a significant protective effect on the viability of the 2 probiotic strains, as compared to the SA coating and the coating without alginate. The latter demonstrated less protection after being exposed to AGJ for 180 min ($p < 0.05$). Furthermore, the viability of probiotics in the dried liver and bacon slices coated with SA-G and SA remained consistently higher than 6 log cfu/g after 180 min.

Fig. S2 presents the viability of probiotics in AIJ at pH 8 for a duration of 4 h. Similar to the observations in AGJ, it was found that the dried liver and bacon slices, when coated with SA-G, exhibited the highest viability of the 2 probiotic strains, surpassing the viability observed with SA coating and the coating without alginate. Notably, regardless of the coating condition, the viability of probiotics in the dried liver and bacon slices remained consistently higher than 6 log cfu/g even after 240 min in the AIJ environment.

SEM micrographs of *Li. fermentum* Pom5 in dried liver and bacon slices

SEM micromorphology of probiotic-enriched on liver and bacon slices was performed to analyze the surface structure of the dried liver (Fig. 3) and dried bacon (Fig. 4) and determine the presence and attachment of *Li. fermentum* Pom5 to those tissues. The SEM results revealed that *Li. fermentum* Pom5 cells were effectively incorporated onto the surface of dried liver and bacon slices through CD method prior to storage. Interestingly, in the presence of SA (Fig. 3B and Fig. 4B) and SA-G coatings (Fig. 3C and Fig. 4C), the films seemed to enhance cell attachment, resulting in a less clear observation of the cell shape compared to the non-alginate-coated samples. This finding suggests that alginate plays a protective role in maintaining the integrity of the attached cells, particularly when combined with glycerol in the SA-G coating. The flexible and elastic properties provided by glycerol improved the adherence of the coating, while its moisture-retaining ability prevented excessive drying, creating a favorable environment for cell survival. Furthermore,

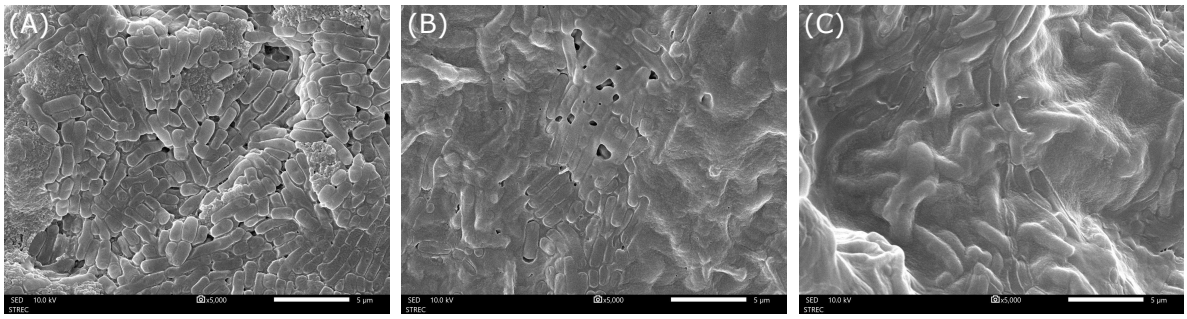

Fig. 3 SEM micrographs showing the location and attachment of *Li. fermentum* Pom5 on dried liver slices. The micrographs (5000×) depict the following conditions: (A) Coated slices, (B) SA-coated slices, and (C) SA-G-coated slices. These images provide insights into the interaction and distribution of *Li. fermentum* Pom5 on the different coatings applied to the dried liver slices.

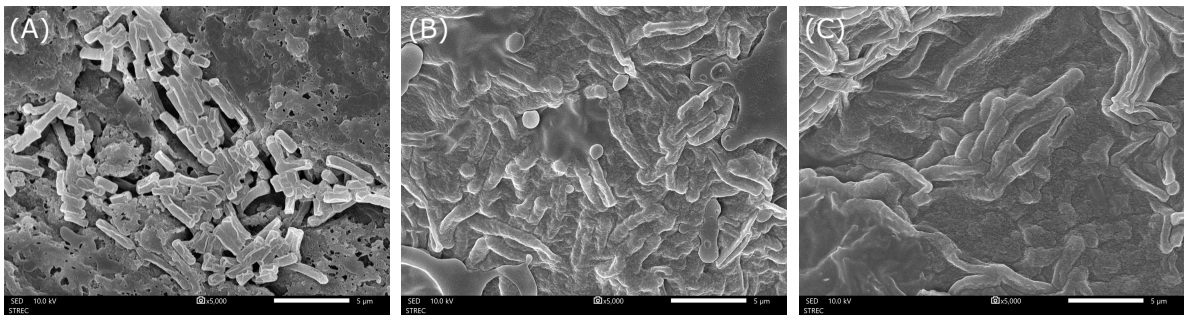

Fig. 4 SEM micrographs showing the location and attachment of *Li. fermentum* Pom5 on dried bacon slices. The micrographs (5000×) depict the following conditions: (A) Coated slices, (B) SA-coated slices, and (C) SA-G-coated slice. These images provide insights into the interaction and distribution of *Li. fermentum* Pom5 on the different coatings applied to the dried bacon slices.

the results demonstrated that the liver and bacon surface tissues had an appropriate structure and enough space for the attachment of bacterial cells. The use of SEM allowed for a detailed analysis of the surface morphology of the liver slices and provided evidence for the successful incorporation of probiotics onto the liver tissue.

CONCLUSION

This study suggests that alginate-coated dried liver and bacon slices enriched with *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 can indeed serve as a stable delivery system for probiotics. The alginate coating, especially when combined with glycerol, proves to be highly effective in protecting the probiotics from the harsh conditions within the intestinal tract of dogs. This protection ensures a higher concentration of viable probiotics reaches the gut. The SEM results provide further evidence of successful probiotic impregnation onto the liver surface, affirming that the liver slices can act as a suitable matrix for probiotic delivery. These compelling findings open up new possibilities for the development of functional pet food products that can contribute to the overall well-being and health of dogs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2024.106.

Acknowledgements: This work was supported by the Srinakharinwirot University Fund (grant number 409/2565) and Faculty of Science, Srinakharinwirot University Fund (grant number 409/2567). This work received support from the Srinakharinwirot University Fund (grant number 376/2567).

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Appendix A. Supplementary data

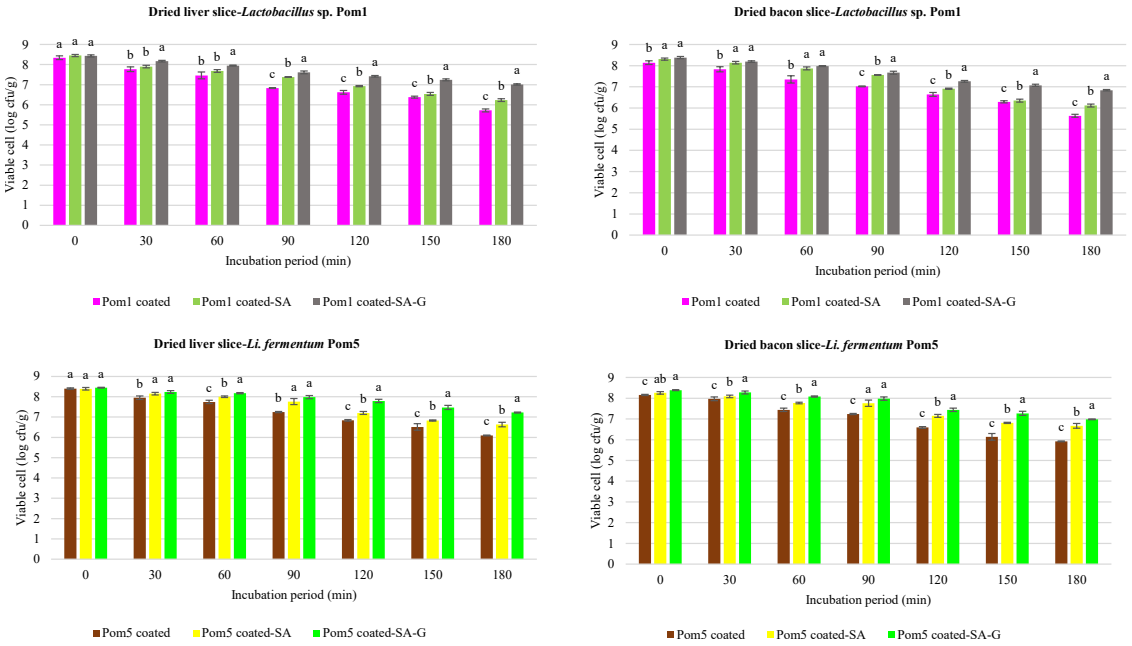

Fig. S1 Viability of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in artificial gastric juice (AGJ) at pH 2 in dried liver and bacon slices with various coatings (coated, alginate-coated (coated-SA), and alginate glycerol-coated (coated SA-G)). Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different letters indicate statistically significant differences at each condition of the experiment with a significance level of $p \leqslant 0.05$.

Table S1 Viability of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in dried liver and bacon slices with various coatings (coated, alginate-coated (coated-SA), and alginate glycerol-coated (coated SA-G)) during the 28-day storage at room temperature.

Product	Condition	Log cfu/g				
		Day 0	Day 7	Day 14	Day 21	Day 28
Dried liver slice						
impregnated with *Lactobacillus* sp. Pom1	coated	8.32 ± 0.07^{Aa}	8.08 ± 0.09^{Ba}	7.70 ± 0.16^{Ca}	7.15 ± 0.10^{Da}	6.42 ± 0.14^{Ec}
	coated-SA	8.34 ± 0.11^{Aa}	8.13 ± 0.09^{Ba}	7.74 ± 0.02^{Ca}	7.35 ± 0.11^{Da}	6.88 ± 0.06^{Eb}
	coated-SA-G	8.36 ± 0.11^{Aa}	8.17 ± 0.09^{Aa}	7.92 ± 0.09^{Ba}	7.38 ± 0.13^{Ca}	7.15 ± 0.11^{Da}
impregnated with *Li. fermentum* Pom5	coated	8.29 ± 0.20^{Aa}	7.97 ± 0.04^{Bb}	7.54 ± 0.13^{Cb}	7.34 ± 0.07^{Db}	6.53 ± 0.09^{Ec}
	coated-SA	8.43 ± 0.09^{Aa}	8.25 ± 0.13^{Ba}	7.79 ± 0.10^{Ca}	7.36 ± 0.05^{Db}	6.93 ± 0.06^{Eb}
	coated-SA-G	8.26 ± 0.08^{Aa}	8.19 ± 0.08^{Aa}	7.88 ± 0.03^{Ba}	7.70 ± 0.07^{Ca}	7.27 ± 0.03^{Da}
Dried bacon slice						
impregnated with *Lactobacillus* sp. Pom1	coated	8.18 ± 0.09^{Aa}	7.96 ± 0.10^{Ba}	7.64 ± 0.11^{Cb}	7.28 ± 0.16^{Db}	6.16 ± 0.05^{Eb}
	coated-SA	8.23 ± 0.01^{Aa}	8.11 ± 0.12^{Aa}	7.54 ± 0.07^{Bb}	7.26 ± 0.05^{Cb}	6.77 ± 0.12^{Db}
	coated-SA-G	8.19 ± 0.03^{Aa}	8.10 ± 0.02^{Aa}	7.99 ± 0.01^{Ba}	7.43 ± 0.14^{Ca}	6.91 ± 0.10^{Da}
impregnated with *Li. fermentum* Pom5	coated	8.15 ± 0.01^{Aa}	8.01 ± 0.02^{Aa}	7.54 ± 0.07^{Bb}	7.22 ± 0.04^{Cc}	6.35 ± 0.06^{Dc}
	coated-SA	8.21 ± 0.08^{Aa}	8.05 ± 0.03^{Ba}	7.94 ± 0.02^{Ca}	7.57 ± 0.03^{Db}	6.80 ± 0.09^{Eb}
	coated-SA-G	8.26 ± 0.04^{Aa}	8.12 ± 0.02^{Aa}	8.02 ± 0.03^{Ba}	7.66 ± 0.07^{Ca}	7.18 ± 0.18^{Da}

Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different capital letters indicate statistically significant differences at each condition of the experiment (column), and different small letters indicate statistically significant differences in each collection sample time of the experiment (row), $p \leqslant 0.05$.

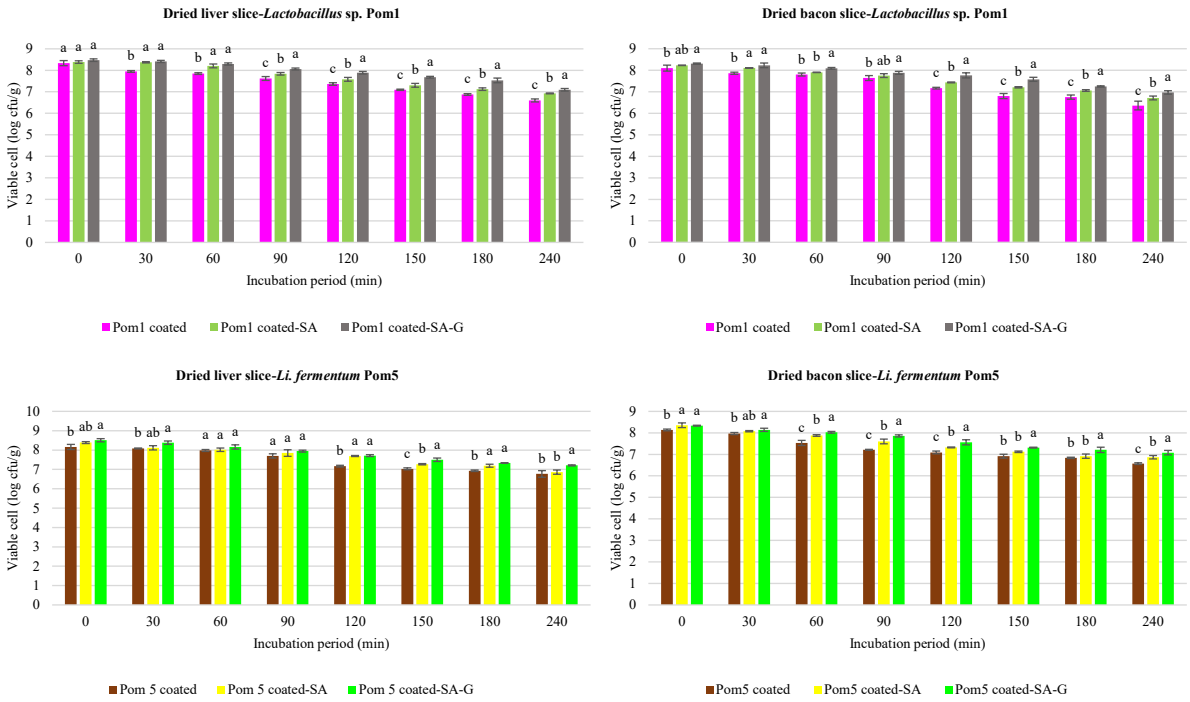

Fig. S2 Viability of *Lactobacillus* sp. Pom1 and *Li. fermentum* Pom5 in artificial intestinal juice (AIJ) at pH 8 in dried liver and bacon slices with various coatings (coated, alginate-coated (coated-SA), and alginate glycerol-coated (coated SA-G)). Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different letters indicate statistically significant differences at each condition of the experiment with a significance level of $p \leqslant 0.05$.

Table S2 Total aerobic counts of dried liver and bacon slices with different coatings (coated, alginate-coated (coated-SA), alginate-glycerol-coated (coated-SA-G), and uncoated) during the 28-day storage at 4 °C.

Product	Condition	Log cfu/g				
		Day 0	Day 7	Day 14	Day 21	Day 28
Dried liver slice						
impregnated with *Lactobacillus* sp. Pom1	uncoated	2.31 ± 0.03^{Aa}	2.55 ± 0.06^{Ba}	2.90 ± 0.14^{Ca}	3.17 ± 0.05^{Da}	3.58 ± 0.14^{Ea}
	coated	2.18 ± 0.16^{Aa}	2.47 ± 0.06^{Ba}	2.77 ± 0.08^{Ca}	2.99 ± 0.05^{Dc}	3.21 ± 0.10^{Eb}
	coated-SA	2.26 ± 0.11^{Aa}	2.54 ± 0.07^{Ba}	2.88 ± 0.10^{Ca}	$3.10 \pm 0.04D^{ab}$	3.20 ± 0.10^{Eb}
	coated-SA-G	2.31 ± 0.17^{Aa}	2.47 ± 0.16^{Ba}	2.87 ± 0.09^{Ca}	$3.09 \pm 0.09D^{ab}$	3.46 ± 0.17^{Ea}
impregnated with *Li. fermentum* Pom5	uncoated	2.17 ± 0.17^{Aab}	2.34 ± 0.07^{Bb}	2.90 ± 0.18^{Cab}	3.21 ± 0.07^{Da}	3.38 ± 0.16^{Ea}
	coated	2.28 ± 0.07^{Aa}	2.52 ± 0.03^{Ba}	3.02 ± 0.05^{Ca}	3.25 ± 0.07^{Da}	3.50 ± 0.13^{Ea}
	coated-SA	2.37 ± 0.05^{Aab}	2.44 ± 0.09^{Bab}	2.92 ± 0.04^{Cab}	3.11 ± 0.06^{Dab}	3.29 ± 0.12^{Ea}
	coated-SA-G	2.12 ± 0.06^{Ab}	2.48 ± 0.10^{Bab}	2.80 ± 0.09^{Cb}	2.98 ± 0.11^{Db}	3.22 ± 0.03^{Ea}
Dried bacon slice						
impregnated with *Lactobacillus* sp. Pom1	uncoated	2.14 ± 0.16^{Aa}	2.43 ± 0.06^{Ba}	2.65 ± 0.18^{Ca}	3.05 ± 0.03^{Da}	3.10 ± 0.11^{Ea}
	coated	2.16 ± 0.11^{Aa}	2.38 ± 0.07^{Ba}	2.71 ± 0.14^{Ca}	2.93 ± 0.04^{Db}	3.12 ± 0.06^{Ea}
	coated-SA	2.22 ± 0.19^{Aa}	2.53 ± 0.14^{Ba}	2.73 ± 0.09^{Ca}	3.04 ± 0.12^{Dab}	3.10 ± 0.04^{Ea}
	coated-SA-G	2.18 ± 0.03^{Aa}	2.46 ± 0.08^{Ba}	2.66 ± 0.12^{Ca}	2.97 ± 0.06^{Dab}	3.08 ± 0.04^{Ea}
impregnated with *Li. fermentum* Pom5	uncoated	2.14 ± 0.14^{Aa}	2.40 ± 0.04^{Bb}	2.55 ± 0.18^{Ca}	2.98 ± 0.06^{Da}	3.16 ± 0.04^{Ea}
	coated	2.10 ± 0.05^{Aa}	2.44 ± 0.115^{Bb}	2.73 ± 0.125^{Ca}	3.04 ± 0.04^{Da}	3.22 ± 0.08^{Ea}
	coated-SA	2.27 ± 0.10^{Aa}	2.39 ± 0.08^{Bb}	2.59 ± 0.02^{Ca}	2.97 ± 0.05^{Da}	3.13 ± 0.14^{Ea}
	coated-SA-G	2.15 ± 0.05^{Aa}	2.62 ± 0.05^{Ba}	2.65 ± 0.07^{Ca}	3.02 ± 0.11^{Da}	3.15 ± 0.03^{Ea}

Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different capital letters indicate statistically significant differences at each condition of the experiment (column), and different small letters indicate statistically significant differences in each collection sample time of the experiment (row), $p \leqslant 0.05$.

Table S3 Physical parameters (Aw, pH, and moisture content) of dried liver and bacon slices with different coatings (coated, alginate-coated (coated-SA), alginate-glycerol-coated (coated-SA-G), and uncoated) during the 28-day storage at 4 °C.

Product	Condition	Aw				pH				Moisture content (%)			
		Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28
Dried liver slice													
impregnated with	uncoated	0.83 ± 0.01^{Aa}	0.82 ± 0.02^{Aa}	0.81 ± 0.01^{Ba}	0.82 ± 0.01^{ABa}	5.98 ± 0.11^{Bb}	6.39 ± 0.04^{Aa}	6.37 ± 0.06^{Aa}	6.39 ± 0.22^{Ab}	21.10 ± 0.08^{Bb}	21.00 ± 0.09^{Cb}	20.79 ± 0.02^{Dc}	21.04 ± 0.03^{Bc}
Lactobacillus sp.	coated	0.81 ± 0.01^{Ab}	0.81 ± 0.02^{Aa}	0.81 ± 0.01^{Aa}	0.81 ± 0.01^{Ab}	6.12 ± 0.21^{ABab}	6.16 ± 0.17^{ABb}	6.09 ± 0.02^{Bb}	6.37 ± 0.16^{Ab}	22.03 ± 0.10^{Aa}	20.28 ± 0.05^{Cd}	21.77 ± 0.12^{Aa}	20.65 ± 0.26^{Dd}
Pom1	coated-SA	0.80 ± 0.02^{Ab}	0.80 ± 0.01^{Aa}	0.81 ± 0.01^{Aa}	0.81 ± 0.01^{Ab}	6.44 ± 0.25^{Aa}	6.38 ± 0.27^{Aa}	6.34 ± 0.13^{Aa}	6.44 ± 0.07^{ABa}	20.26 ± 0.16^{Cc}	20.62 ± 0.03^{Bc}	21.26 ± 0.03^{Bb}	21.75 ± 0.10^{Aa}
	coated-SA-G	0.80 ± 0.01^{Ab}	0.81 ± 0.02^{Aa}	0.81 ± 0.02^{Aa}	0.81 ± 0.01^{Ab}	6.30 ± 0.16^{CBab}	6.31 ± 0.29^{Ba}	6.00 ± 0.14^{Cb}	6.24 ± 0.19^{ABc}	20.02 ± 0.14^{Dd}	21.45 ± 0.20^{Ba}	20.35 ± 0.03^{Cc}	21.54 ± 0.05^{Bb}
impregnated with	uncoated	0.81 ± 0.01^{Ba}	0.82 ± 0.02^{Aa}	0.81 ± 0.03^{Ba}	0.80 ± 0.02^{Ba}	6.26 ± 0.15^{Cc}	6.34 ± 0.09^{Cc}	6.88 ± 0.04^{Ba}	6.26 ± 0.09^{Bc}	21.24 ± 0.04^{Ab}	20.56 ± 0.02^{Cc}	20.06 ± 0.01^{Dc}	20.80 ± 0.10^{Bd}
Li. fermentum	coated	0.81 ± 0.01^{Aa}	0.81 ± 0.01^{Aa}	0.80 ± 0.01^{Aa}	0.80 ± 0.01^{Aa}	6.35 ± 0.08^{ABb}	6.76 ± 0.22^{Aa}	5.86 ± 0.07^{Ca}	6.41 ± 0.08^{Bb}	23.67 ± 0.17^{Aa}	23.62 ± 0.10^{Aa}	23.65 ± 0.03^{Aa}	22.82 ± 0.14^{Ba}
Pom5	coated-SA	0.80 ± 0.01^{Aa}	0.80 ± 0.02^{Aa}	0.81 ± 0.01^{Aa}	0.81 ± 0.01^{Aa}	6.78 ± 0.04^{Aa}	6.41 ± 0.09^{Bb}	6.81 ± 0.03^{Aa}	6.17 ± 0.07^{Cd}	20.80 ± 0.12^{Cc}	19.28 ± 0.03^{Dd}	19.30 ± 0.06^{Dd}	21.81 ± 0.05^{Ac}
	coated-SA-G	0.80 ± 0.02^{Aa}	0.80 ± 0.01^{Ab}	0.81 ± 0.01^{Aa}	0.81 ± 0.01^{Aa}	6.24 ± 0.06^{Bc}	6.15 ± 0.12^{Cd}	6.69 ± 0.06^{Ab}	6.84 ± 0.09^{Aa}	20.65 ± 0.09^{Dc}	21.62 ± 0.04^{Cb}	22.53 ± 0.18^{Bb}	22.25 ± 0.06^{Bb}
Dried bacon slice													
impregnated with	uncoated	0.73 ± 0.02^{Aa}	0.74 ± 0.02^{Aa}	0.74 ± 0.01^{Aa}	0.74 ± 0.02^{Aa}	6.76 ± 0.11^{Ba}	7.27 ± 0.17^{Aa}	6.88 ± 0.16^{Bb}	6.26 ± 0.09^{Cb}	13.75 ± 0.06^{Bc}	13.42 ± 0.01^{Cd}	13.28 ± 0.02^{Cb}	14.25 ± 0.04^{Ac}
Lactobacillus sp.	coated	0.73 ± 0.01^{Aa}	0.74 ± 0.05^{Aa}	0.72 ± 0.01^{Bb}	0.72 ± 0.01^{Bb}	6.08 ± 0.10^{Cb}	6.81 ± 0.17^{Bb}	7.19 ± 0.08^{Aa}	6.66 ± 0.17^{Aa}	14.69 ± 0.01^{Cb}	14.67 ± 0.02^{Cb}	15.26 ± 0.06^{Ba}	15.65 ± 0.11^{Ab}
Pom1	coated-SA	0.74 ± 0.01^{Aa}	0.72 ± 0.02^{Ba}	0.72 ± 0.02^{Bb}	0.72 ± 0.01^{Bb}	6.73 ± 0.16^{ACa}	6.56 ± 0.17^{ABc}	6.59 ± 0.23^{Ac}	6.08 ± 0.06^{BCc}	11.50 ± 0.16^{Dd}	14.36 ± 0.09^{Ac}	12.68 ± 0.17^{Cc}	13.27 ± 0.04^{Bd}
	coated-SA-G	0.73 ± 0.03^{Aa}	0.74 ± 0.04^{Aa}	0.74 ± 0.01^{Aa}	0.74 ± 0.01^{Aa}	5.98 ± 0.13^{Cb}	6.33 ± 0.06^{Bc}	6.97 ± 0.17^{Aa}	6.52 ± 0.17^{Ca}	17.74 ± 0.02^{Ba}	18.26 ± 0.04^{Aa}	15.72 ± 0.02^{Da}	16.08 ± 0.03^{Ca}
impregnated with	uncoated	0.73 ± 0.01^{Ba}	0.72 ± 0.02^{Ba}	0.77 ± 0.06^{Aa}	0.73 ± 0.00^{Ba}	6.74 ± 0.14^{Ba}	6.24 ± 0.08^{Cb}	6.81 ± 0.06^{Ba}	6.63 ± 0.20^{Bb}	11.54 ± 0.03^{Bc}	10.59 ± 0.01^{Cd}	10.79 ± 0.01^{Cb}	10.71 ± 0.08^{Cd}
Li. fermentum	coated	0.73 ± 0.03^{Aa}	0.73 ± 0.02^{Aa}	0.72 ± 0.01^{Ab}	0.73 ± 0.02^{Aa}	6.71 ± 0.04^{Aa}	5.84 ± 0.11^{Ab}	6.49 ± 0.15^{Cb}	6.25 ± 0.06^{Dc}	13.65 ± 0.07^{Aa}	13.75 ± 0.08^{Aa}	10.98 ± 0.01^{Db}	13.44 ± 0.02^{Bb}
Pom5	coated-SA	0.74 ± 0.05^{Aa}	0.73 ± 0.02^{Aa}	0.73 ± 0.03^{Ab}	0.73 ± 0.02^{Aa}	6.41 ± 0.08^{Bb}	6.27 ± 0.09^{Db}	7.15 ± 0.11^{Aa}	6.56 ± 0.05^{Ab}	13.69 ± 0.08^{Aa}	11.21 ± 0.11^{Cc}	12.85 ± 0.01^{Ba}	12.85 ± 0.07^{Bc}
	coated-SA-G	0.72 ± 0.01^{Ab}	0.72 ± 0.01^{Aa}	0.73 ± 0.04^{Ab}	0.75 ± 0.02^{Aa}	6.53 ± 0.08^{Ab}	6.63 ± 0.03^{Aa}	6.08 ± 0.08^{Cc}	7.22 ± 0.09^{Aa}	12.85 ± 0.03^{Db}	13.11 ± 0.01^{Cb}	13.20 ± 0.04^{Ca}	14.37 ± 0.02^{Aa}

Data are expressed as average values ± standard deviation (SD) with 3 replicates. Different capital letters indicate statistically significant differences at each condition of the experiment (column), and different small letters indicate statistically significant differences in each collection sample time of the experiment (row), $p \leqslant 0.05$.