Article

Not peer-reviewed version

Characterization of Postbiotics Derived from *Bifidobacterium bifidum* Strains and Influence of Postbiotic-Enriched Alginate Coating on Microbial and Physicochemical Quality of Turkey Breast Meat

Emel Cengiz Kaynakcı *

Posted Date: 13 June 2024

doi: 10.20944/preprints202402.0814.v2

Keywords: turkey meat; edible coating; Bifidobacterium bifidum; postbiotics; alginate

 doi:10.20944/preprints202402.0814.v2

Article

Characterization of Postbiotics Derived from *Bifidobacterium bifidum* Strains and Influence of Postbiotic-Enriched Alginate Coating on Microbial and Physicochemical Quality of Turkey Breast Meat

Emel Cengiz Kaynakcı

Akdeniz University, Institute of Health Sciences, Medical Biotechnology Department, Pinarbasi, Antalya, Turkey; ekaynakci@akdeniz.edu.tr Tel.: (+905335446792)

Abstract: Postbiotics are metabolites, cell components, and other bioactive molecules produced during the fermentation process by beneficial microorganisms such as probiotics. They can exhibit various biological activities, including immunomodulatory, antioxidant, anti-inflammatory, and antimicrobial properties. This study aimed to explore the in vitro antioxidant activity of postbiotics derived from *Bifidobacterium bifidum* DSM 20456 and *B.bifidum* BB12 strains, and to evaluate the effects of postbiotic-enriched alginate coatings on the microbial and physicochemical quality of turkey breast meat stored at refrigerator temperature for 7 days. Hence, the antimicrobial properties, total phenolic content (TPC), total flavonoid content (TFC), and 1,1-difenil-2-picrylhydrazyl radical scavenging activity (DPPH) of the postbiotics were determined. The results revealed that the TPC (87.13 ± 2.24-90.89 ± 1.63 mg GAE/100 mL), TFC (18.83 ± 2.21-24.20 ± 0.25 mg CE/ 100 mL), and DPPH (50.28 ± 0.20-51.56 ± 1.63 mg TEAC/100 mL) values of the tested postbiotics exhibited potential antioxidant capacity. The analysis results indicated that alginate coating with postbiotics caused significant differences in pH, b* (yellowness), and yeast/mold values of turkey meat during storage ($p < 0.05$). The yeast and mold values increased significantly, while the pH and b* values of the samples (except for the control and bb groups) decreased during storage ($p < 0.05$). On day 7, the abundance of yeast and mold in the bb group was 1.2 log CFU/g lower than in the control group ($p < 0.05$). In contrast, L* (brightness), a* (redness), lipid oxidation, total aerobic mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria, and *Listeria monocytogenes* counts were not affected for 7 days ($p > 0.05$). Our results showed that Bifidobacterium postbiotic treatment did not adversely affect the microbiological and physicochemical properties of turkey breast meats. In conclusion, our results demonstrate that postbiotic-enriched alginate coatings may be a practical solution for the meat industry to improve and maintain the quality features of poultry meat products during storage.

Keywords: turkey meat; edible coating; *Bifidobacterium bifidum;* postbiotics; alginate

1. Introduction

Turkey meat is a low-fat and -cholesterol food product rich in protein; minerals such as calcium, phosphorus, and potassium; essential amino acids; and vitamins B1, B2, B6, and B12. Additionally, it contains many unsaturated and essential fatty acids (Mojarradi et al., 2024; Keykhosravy et al., 2022). However, the high nutritional value of meat products facilitates spoilage reactions (Ali Eesa et al., 2023). Owing to the high contents of unsaturated fatty acids and free iron in poultry meat products, a primary spoilage concern is lipid oxidation and cross-contamination that can occur in production facilities during transport and storage (Mojarradi et al., 2024; Sajadizadeh et al., 2024). Under these conditions, the foodborne disease hazards of raw meat products should be considered. These foods are not subjected to post-treatment processes that eliminate or reduce pathogens before consumption

 doi:10.20944/preprints202402.0814.v2

(Sajadizadeh et al., 2024; Babaoğlu et al., 2022). Foodborne pathogenic microorganisms can adhere to surfaces, form colonies on meat, and endanger human health (Babaoğlu et al., 2022).

Listeria monocytogenes can be transmitted to meat and its byproducts through cross-contamination, particularly in poultry processing environments, and can cause listeriosis. The US Centers for Disease Control and Prevention reported that approximately 1,600 people are affected by listeriosis annually in the United States; around 95% are hospitalized, and over 15% die (Keykhosravy et al., 2022).

When combined with consumers' preference for fresh, minimally processed, and preservative-free foods, these restrictions have prompted the development of natural antimicrobial substances as innovative additives to ensure food safety (Mojardi et al., 2024; Mohammed et al., 2023). Edible films and coatings produced from natural components such as proteins (zein and whey) and polysaccharides (including gum, alginate, and chitosan) offer alternatives to commercial packaging (Mojarradi et al.; Ali Easa et al., 2023; Mojaddar Langroodiet al., 2021; Milani et al., 2020; Elhadef et al., 2024; Safari et al., 2023; Yang et al., 2023). Sodium alginate is an anionic polysaccharide that can be gelatinized by adding divalent cations, such as Ca^{2+}, and is used in meat and meat products for coatings and films owing to its good film-forming ability, low price, easy availability, and biodegradability (Chenet al., 2023; Tsitsos et al., 2023; Montone et al., 2023). Components of microbial origin (such as probiotics, postbiotics, and bacteriocin) and their metabolites have recently been included in edible coatings to improve their biological functions (e.g., antimicrobial and radical scavenging) (Abbasi et al., 2023).

Postbiotics are extensively studied natural antimicrobial agents that benefit health when applied to foods; they are bioactive soluble substances secreted by probiotic microorganisms. Probiotics (e.g., lactic acid bacteria (LAB), *Bifidobacterium spp.*, *Saccharomyces cerevisiae*, and *Bacillus spp.*) produce postbiotics in culture medium (or during fermentation), food, or the gastrointestinal tract. Postbiotics are a mixture of organic acids, exopolysaccharides, bacteriocins, bioactive peptides, enzymes, and other components, and they are applied in household and meat products through methods such as direct application, dipping, and polymer coatings/films (Toushik et al., 2023).

No study has reported the effects of *Bifidobacterium* spp. postbiotics on preserving turkey breast meat. Hence, this study aimed to determine the microbial stability and physicochemical properties of raw turkey breast meat coated through dipping in a postbiotic–alginate edible coating obtained from *B. bifidum* DSM 20456 and *B. bifidum* BB12 strains during storage at 4 °C for 7 days. Similarly, the efficiency of the postbiotic–alginate edible coating for *L. monocytogenes* inhibition in raw turkey meat was investigated.

2. Materials and Methods

2.1. Preparation of Postbiotics

Postbiotics were prepared according to the method of Incili et al. (2021) with some modifications. B. bifidum DSM 20456 and B. bifidum BB12 strains (Akdeniz University, Department of Food Engineering, Turkey) were obtained. The cultures were grown on MRS agar (Lactobacillus Agar Acc. To De Man, Rogosa and Sharpe, BID, Germany) at 37°C for 24-48 hours and the number of colonies was determined by densitometer to approximately 12 McFarland per tube. The 12 McFarland *B.bifidium* probiotic solutions in tubes were centrifuged at 9,400 g for 10 minutes at 4 °C (NF 800 R, Nüve, Turkey). After centrifugation, postbiotics were obtained by filtration using a 0.22 μm syringe filter (Laborgerâte GmbH, Germany). Freshly obtained postbiotics were adjusted to 8% concentration in the immersion solution for use in this study.

 doi:10.20944/preprints202402.0814.v2

2.2. Characterization of Postbiotics

2.2.1. DPPH (1,1-Difenil-2-Picrylhydrazyl Radical Scavenging Activity), Total Phenolic Compounds (TPC), and Total Flavonoid Compounds (TFC)

These analyses were conducted at the Akdeniz University Food Safety and Agricultural Research Centre and Western Mediterranean Agricultural Research Institute Directorate (Antalya/Turkey).

2.2.2. Minimum Inhibitory Concentration (MIC) and Disk Diffusion Of Postbiotics (DDF)

To determine the MIC of *B. bifidum* DSM 20456 and *B. bifidum* BB12 postbiotics against *L. monocytogenes* ATCC 19118, *Salmonella enterica* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Bacillus cereus* ATCC 11778, each well of a 96-well plate (Corning) was filled with 0.1 mL of postbiotic (10–100 mg/mL) and serially diluted using TSB (Milani et al., 2020). The most diluted well with no growth was expressed as the MIC, which inhibited the microorganisms' growth.

The agar disk diffusion method was used for the postbiotics (CFSs) of *Bifidobacterium spp.* to determine their antimicrobial activity. Lawns of *L. monocytogenes* (~6 log10 CFU/mL) were prepared on Oxford Agar. Grade 3 Whatman filter paper was impregnated in the corresponding postbiotic solution (10 mg/mL) and placed in the center of the inoculated plate. Subsequently, the plates were incubated at 37 ± 1 °C for 24 h, and the inhibition zone diameter was measured using a digital caliper in triplicate (Toushik et al., 2023).

2.3. Preparation of L. monocytogenes Pathogenic Bacteria Inoculum

Listeria monocytogenes ATCC 19118 was used in this study. *Listeria monocytogenes* ATCC 19118 maintained at 4–7 °C in blood agar (Merck, 110886, Germany) was taken with a sterile core, switched to a special selective medium for each bacterial culture, and incubated for 24–48 h under appropriate conditions. After incubation, pathogens were taken from the separated colonies with a sterile core and suspended in tubes containing 9 mL of sterile Ringer's solution (Merck, 115525, Germany). The inoculum suspension density was adjusted according to the 0.5 McFarland standard with a densitometer (Biosan, 1 B, Turkey).

2.4. Preparation of the Experiments

Fresh turkey breast fillets (without skin) were purchased from Bahar Turkey Meat Industries A.Ş (Antalya/Turkey). The turkey breast meat was immediately transported to the laboratory under cold chain. Then, turkey breast meats cut into 25 g pieces with a sterile knife and stored at 4°C. The turkey breast pieces were used for microbial and chemical analyses. The turkey breast samples were randomly distributed into five groups, as presented in Table 1. The turkey breast meat samples inoculated with pathogenic bacteria without treatment (postbiotic and alginate solution) served as the controls. For inoculation, 100 µL inoculum cocktail was spread on the surfaces of the 25 g turkey breast pieces with a Drigalski spatula. All samples were contaminated, subjected to bacterial attachment, and kept at room temperature. The other coated samples were immersed for 2 min in 200 mL of the coating solution. The fillets were removed and drained for 1 h at 20 °C in a biosafety cabinet. Subsequently, the groups were contaminated with *L. monocytogenes* as described above. All samples were stored at 4 °C in sterile bags. Each experiment was conducted in triplicate.

2.5. Microbial Analysis

Microbiological counts were determined by homogenizing a 25 g sample in 225 mL of 0.1% MRD with a stomacher. The total viable bacterial counts were obtained using the spread plate method on plate count agar (PCA, BID, Germany). The plates were incubated at 37 °C for 24–48 h for the total viable count, and at 7 °C for 10 days for the psychotropic count. The LAB were counted on MRS agar (BID, Germany) at 37 °C for 2 days. To enumerate mold and yeast, Dichloran Rose Bengal

 doi:10.20944/preprints202402.0814.v2

Chloramphenicol agar (DRBC, BID, Germany) was incubated at 25 °C for 3 days (İncili et al., 2021). All microorganism counts were reported as log10 CFU/mL. All analyses were performed on storage days 0, 2, 4, and 7.

2.6. Physicochemical Analyses of Coated Turkey Meat During Storage

Physicochemical analyses of the control and postbiotic-enriched alginate-coated turkey meat were performed on days 0, 2, 4, and 7 of refrigerated storage (+4 °C). Each analysis was performed in three replicates and two parallels.

2.6.1. TBARS Analysis

Lipid oxidation in the postbiotic-enriched alginate-coated turkey breast meat samples was monitored through TBARS analysis using the method of Kilic et al. (2003), with some modifications. The results were calculated using a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP) and expressed as μmol of MDA per kg of meat. Furthermore, a 2 g sample was homogenized with 2 mL of trichloroacetic acid (TCA) solution for 15–20 s. The homogenized sample was filtered through Whatman no. 1 filter paper, 1 mL of the filtrate was removed, and 1 mL of thiobarbituric acid (TBA) solution was added. For the blind solution, TCA and TBA solutions (1 mL each) were used. The resulting mixture was kept in a water bath (BM 302, Nüve) at 100 °C for 40 min, cooled to room temperature, and centrifuged at 4100 *g* for 10 min (F 800, Nüve). The supernatant's absorbance after centrifugation was measured at 532 nm.

2.6.2. pH

The pH of the turkey breast meat was measured using a Hanna HI981036 meat pH meter specially designed for meat and meat products (Kaynakcı and Kilic, 2020).

2.6.3. Color Evaluation

The surface of the turkey meat covered with edible films was measured at four locations. L*, a*, and b* values were determined with a spectrocolorimeter (LS172, China) using a 22 mm aperture and a 10° observer and adjusted with a white tile. The L* value indicates the brightness level (from 0 to 100); a* and b* values range from -120 to +120, where a* indicates greenness and redness levels, while b* indicates blueness and yellowness levels (Kaynakcı and Kilic, 2020).

2.7. Weight Loss (WL)

WL was calculated as the weight difference of turkey breast meat samples using the following equation and expressed as a percentage:

$W_1 = (W_2 - W_1) : W_1 \times 100$

where W_1 is the weight of the turkey meat sample, and W_2 is the weight of the sample after 0, 2, 4, and 7 days of chilled storage (Kaynakcı and Kilic, 2020).

2.8. Statistical Analyses

All analyses were performed in triplicate. Experimental data are reported as the mean and standard deviation and were subjected to variance analysis. The significance of differences ($p < 0.05$) among samples was determined using Tukey's test with SPSS software version 23.0 for Windows (SPSS, Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Antimicrobial Activity of Postbiotics

This study investigated the antimicrobial properties of *Bifidobacterium* postbiotics against some pathogenic bacterial species using MIC and DDF antimicrobial tests. *Bifidobacterium* postbiotics were

 doi:10.20944/preprints202402.0814.v2

evaluated against the indicator pathogens *L. monocytogenes* ATCC 19118, *S.enterica* ATCC 14028, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *B. cereus* ATCC 11778. The DDF test showed that *Bifidobacterium spp.* postbiotics were more effective against Gram (+) bacteria than Gram (-) bacteria, and the average inhibition zone was between 11.5 and 15.00 mm. Among the tested pathogens, *Bifidobacterium spp.* strains were the most effective against *L. monocytogenes* (Table 2). In a previous study, films supplemented with *Lactobacillus sakei* postbiotic exhibited inhibition zones of 4.83-± 0.01 and 4.61 ± 0.02 mm for *E. coli* and *Listeria monocytes*, respectively. The *Bifidobacterium bifidum* postbiotics used in our study were more effective than *L. sakei* (Serter et al., 2024). The Gram-negative bacterial species had no inhibition zones. This is believed to be because the cell walls of Gram-negative bacteria contain lipoproteins and lipopolysaccharides, making them more resistant to antimicrobial agents (Abbasi et al., 2023). The MIC values showed that the postbiotics were *effective against L. monocytogenes* at lower concentrations than against other food pathogens.

3.2. Antioxidant Activity, Total Phenolic Content, and Total Flavonoid Content of Postbiotics

There are no studies on using postbiotics of the probiotic bacteria *Bifidobacterium spp*. in coatings for fresh meat and poultry. As postbiotics contain phenolic compounds, short-chain fatty acids, and exopolysaccharides, they have high antioxidant activities and positive health effects (including immune-system-boosting and antitumor effects). Many studies have been conducted on their use as functional foods and nutraceuticals (Gurunathan et al., 2023; Ku et al., 2016). Probiotic yeasts and bacteria and their postbiotic metabolites have significant phenolic and flavonoid compounds that directly influence their antioxidant activities (Abbasi et al., 2023).

The antioxidant activities of *B. bifidum* DSM 20456, *B. bifidum* BB12, and *B. bifidum* DSM 20456 + *B. bifidum* BB12 were 50.28 ± 0.20, 51.05 ± 0.68, and 51.56 ± 1.63 mg TEAC/100 mL, respectively; their TPCs were 87.13 ± 2.24, 88.24 ± 1.65, and 90.89 ± 1.63 mg GAE/100 mL, respectively; and their TFCs were 24.20 ± 0.25, 18.83 ± 2.21, and 21.34 ± 0.96 mg CE/ 100mL, respectively.

The results of the GC-MS (gas chromatography–mass spectrometry) for the volatile compounds of *Bifidobacterium spp*., the postbiotic strain used in the coating, are given in Table 3. Upon GC-MS analysis, 24 compounds (including hydrocarbons and lipids) were detected from the postbiotics. L-monopalmitin (monoglyceride), n-heptadecane, n-hexadecane, and n-tetradecane were the most frequently identified in the postbiotic content analyses. Similarly, 1-monopalmitin is a major postbiotic bioactive component (26.67–34.11%). Muhammet et al. (2022) reported that the GC-MS analysis of postbiotics revealed the presence of alkanes, aldehydes, hydrocarbons, propionic acid, fatty acids, fatty acid esters, and certain antibacterial and antifungal compounds, such as 2,4-di-tert-butyl phenol and dotriacontane.

In the literature, 1-monopalmitin has recently attracted attention as a potential cancer therapeutic and chemotherapeutic adjuvant (Niu et al., 2023). Therefore, investigating the cytotoxic effects of 1-monopalmitin isolated from *Bifidobacterium spp*. on cancer cells is essential. In addition, some studies have reported antimicrobial activities of this compound, primarily against Gram-negative pathogens and fungi such as *Fusarium spp*., *Aspergillus spp*., and *Penicillium spp.* (Benramdane et al., 2022). In contrast, monopalmitin or monostearin had no antibacterial activity against Gram-positive or Gram-negative bacteria (Wang et al., 2020; Jumina et al., 2018).

İncili et al. (2022 b) showed that the 1-difenil-2-picrylhydrazyl radical scavenging activity (DPPH) and TPC of the postbiotic used in their study were 439.439 ± 1.24 mg TEAC/L and 1708.15 ± 93.28 mg GAE/L, respectively. In another study using the same bacterial strain, 1291 ± 1.5 mg/L TEAC and 2336.11 ± 2.36 mg/L GAE were the values observed for DPPH and TPC, respectively (Huang et al., 2021). The postbiotic DPPH and TPC values used in this study were higher than those of the *B. bifidum* postbiotics used in our study. In a study that investigated the antioxidative properties of *B. bifidum* bacteria, the DPPH radical scavenging activity of *B. bifidum* culture filtrate was increased in a dose-dependent manner. The radical scavenging activity was 56.5 ± 0.64, 71 ± 4.7, and 94 ± 1.3% for the 2.5%, 5.0%, and 7.5% bacterial filtrates, respectively (Ku et al., 2016). In a study where postbiotics (cell-free supernatant) were used against *Clostridium perfringens* infection in poultry meat, the *L.*

 doi:10.20944/preprints202402.0814.v2

rhamnosus postbiotic had a high antioxidant capacity (172.08 mg/mL) owing to its significant phenolic component levels.

The TFCs of *L. rhamnosus* EMCC 1105, *L. fermentum*, *P. acidilactici*, and *P. acidilactici* postbiotics were 17.22, 11.42, 15.79, and 14.6 mg/mL, respectively (Hamal et al., 2020). The TFCs of the postbiotics used in our study (18.18–24.20 mg/mL) were higher.

3.3. pH, TBARS, and Water-Holding Capacity (WHC)

The effects of storage time and alginate base with the edible postbiotic coating treatments on the pH of turkey meat are presented in Table 4. The pH values of the samples decreased until the 7th day of storage ($p < 0.001$). The pH value of the bb group showed a significant decrease from the first day ($p < 0.001$). The pH values were between 5.48 and 5.84 on day 0 ($p < 0.05$), and those of groups a and b1 were the lowest on day 0 of storage. Group b2 had the lowest pH value among the groups on the last day of storage. The pH reduction during the storage period may be attributed to LAB growth ($p < 0.05$). This is similar to the findings of Ansar et al. (2024), who observed decreased pH values in minced meat coated with a novel nanopolymer enriched with *Lactobacillus rhamnosus* postbiotic. However, Mojarradu et al. (2024) reported that the pH values of turkey meat coated with *Lallemantia iberica* seed mucilage enriched with an *M. Sylestris* biological edible film increased during storage.

Fresh meat is a product of concern because it is susceptible to oxidation. Hydroperoxides are produced through lipid peroxidation, and their degradation yields secondary oxidative products, causing unpleasant odors and flavors in meat (Gautam et al., 2023). Malondialdehyde is formed during oxidative degradation of lipids (Elhadef et al., 2023). The TBARS value in high-quality products should be <3 mg MDA/kg, whereas, in acceptable-quality materials, it should be ≤5 mg MDA/kg. TBARS levels ≥ 5 mg MDA/kg in meat include the threshold for distinguishing off-odors and off-taste for humans; the TBARS values of our samples ranged from 0.93 to 1.54 mol/kg (Hamad et al., 2020; Kilinc et al., 2009). The TBARS values of the groups were <5 mg MDA/kg. Fluctuations were observed during storage, as evidenced by the degradation of MDA by spoilage microorganisms and secondary oxidation, causing the formation of metabolites that do not react with TBA (İncili et al., 2023). The TBARS changes in edible coatings with *Bifidobacterium spp.* postbiotics for turkey breast meats are presented in Table 4. No significant differences were observed between the groups and sampling days for TBARS ($p > 0.05$). The TBARS values of the samples were similar, except for those of the b2 and bb groups ($p > 0.05$). The control group had the highest TBARS value (1.54 μmol/kg) at the end of the storage period ($p > 0.05$). İncili et al. (2021) found that the postbiotic *Pediococcus acidilactici* did not affect TBARS values during storage ($p > 0.05$). Furthermore, Kuley et al. (2021) reported that *Lactobacilli reuteri* supernatant alone had a weak antioxidative effect on sardine burgers, as also observed in our study.

3.4. Color Changes of Turkey Breast Fillets

Food appearance is important to customers when purchasing food. Postbiotic and sodium alginate treatments did not change the L* values between the groups during the storage period ($p > 0.05$; Table 5), except for those of the b1 and b2 groups, which increased during storage ($p < 0.05$). The a* values showed no statistical significance ($p > 0.05$) between the groups on all storage days, except for the a and b2 groups ($p < 0.05$). The a* values showed a significant difference between days 0 and 2, and the bb group had the highest a* value on day 0. However, no differences were observed between the a* values of all groups on days 4 and 7 of storage ($p > 0.05$). The b* values showed a significant difference between the groups on all storage days except for days 4 and 7 ($p < 0.05$). The b* values during storage showed significant changes only in the a, b1, and b2 groups on days 2 and 4 ($p < 0.05$). The b* color value of the a group had the highest values on days 0 and 2 than the other groups ($p<0.05$) (Table 5).

İncili et al. (2020) used *Lb. Plantarum* postbiotic in meat marination, reporting that the a* and b* values changed and the L* value decreased. In another study conducted by the same authors, they found that postbiotics and chitosan did not affect the color properties (L*, a*, and b*) of chicken breast fillets ($p > 0.05$) (İncili et al., 2021).

 doi:10.20944/preprints202402.0814.v2

3.5. Microbiological Analysis of Turkey Breast Meat

The postbiotic and alginate edible coatings did not affect the total aerobic mesophilic bacteria, psychotropic bacteria, or LAB ($p > 0.05$). LAB and psychotropic bacteria are the leading causes of spoilage in vacuum-packed or modified-atmosphere meat and meat products, as they can grow at refrigeration temperatures (Luong vd., 2020). The microbial effects of alginate-based edible films containing postbiotics on *L. monocytogenes* inoculated on turkey breast meat are shown in Figure 1. There were no significant differences between the mean total aerobic bacterial and psychotropic bacterial counts of the samples on any of the storage days ($p > 0.05$) (Figure 1a,b). The control, a, b1, b2, and bb groups showed increases of 2.65, 2.95, 2.98, 3.05, and 2.99 log CFU/g from days 0 to 7 of storage, respectively ($p < 0.05$). Except for the control group, all groups' mean total aerobic bacteria counts showed a statistically significant increase according to the analyzed storage days ($p < 0.05$). Although the turkey meat was brought from the factory to the laboratory without breaking the cold chain, it reached 7 log CFU/g on the 2nd day of storage. The control group and other alginate-based postbiotic-coated turkey breast meat samples spoiled after day 4. This is believed to have been due to the factory conditions under which the turkey meat samples were taken, as well as the meat's contamination with psychotropic bacteria (such as *Pseudomonas spp.*).It is thought that postbiotics would have been more effective if turkey breast meat with low microbial initial load and high quality could have been obtained elsewhere. LAB, which are significant in the deterioration of raw chicken meat, can multiply under refrigeration conditions (Jay et al., 2008). Except for the b1 and b2 groups, there were no differences in the mean LAB counts during storage ($p > 0.05$) (Figure 1d). The highest LAB count was found in the b2 group (5.78 ± 0.42) and was 1.2 log higher than that in the control on day 0. At the end of the storage period, the LAB counts in the control, a, b1, b2, and bb groups were 5.65, 6.81, 7.03, 7.16, and 6.80 log CFU/g, respectively. After the storage, the mean LAB count of the b2 group was significantly higher than that of the control group ($p < 0.05$). From these results, it can be concluded that postbiotics have no potential to inhibit the main flora that are resistant to chilling in turkey breast fillets.

Yeast and mold counts differed between the groups on days 0 and 2, but not on days 4 and 7 ($p < 0.05$) (Figure 1c). The bb group was more effective in terms of yeast and mold growth on days 4 and 7 than the other groups ($p > 0.05$). After the storage, the control, a, b1, b2, and bb groups showed +5.37, +5.28, +4.74, +4.68, and +4.11 log increases, respectively ($p < 0.05$). It was observed that the bb group affected yeast and mold growth the most ($p < 0.05$).

The number of *L. monocytogenes* in the treatment groups was higher than that in the control on day 0 ($p < 0.05$) (Figure 1e). The control, a, b1, b2, and bb groups showed increases of 2.06, 1.54, 1.49, 1.40, and 0.70 log CFU/g, respectively, from days 0 to 7 of storage ($p > 0.05$). Although the bb group did not show significant differences, it was effective against *L. monocytogenes* compared with the other groups. As a result of the MIC analysis performed in this study, the two strains of *Bifidobacterium bifidum* appeared to be effective against *L. monocytogenes*; however, the same effect was not observed in the food matrix. İncili et al. (2021) reported that the levels of the same compounds detected in both studies, including ferulic acid and gallic acid, significantly differed in their two studies with *Pediococcus acidilactici* postbiotic.

Serter et al. (2023) observed that the effects of *L. sakei* and *L. plantarum* postbiotics on *L. monocytogenes* were observed after the 4th day ($p < 0.05$). They noted that this may have been because the postbiotics showed a bacteriostatic rather than a bactericidal effect. In addition, the fact that *L. monocytogenes* is a pH-resistant bacterium that can grow slowly at low pH complicates the elimination of this pathogen.

4. Conclusions

There are no studies in the literature on the characterization of *Bifidobacterium spp.* postbiotics and their effects on meat and meat products' shelf life and physicochemical properties. In this study, *B. bifidum* postbiotics enriched with alginate did not negatively affect turkey breast meat's microbial stability and physicochemical properties. Thus, a novel, natural, and edible coating material for meat and meat products has been developed. Among this study's limitations may be the irregularity in the

 doi:10.20944/preprints202402.0814.v2

formation of metabolites during the microbial growth of *B. bifidum postbiotics spp*. strains. Therefore, the cultivation medium, incubation times, and colony numbers of the strains from which the postbiotics were derived seem to be issues that need to be planned out in more detail. Standardization of the derived postbiotics seems to be very critical. To increase the efficacy of postbiotics derived from *Bifidobacterium spp.* against spoilage pathogens in poultry meat, future studies should test the addition of natural antimicrobial and antioxidant substances to alginate-based edible coatings.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: I would like to thank Prof. Dr. Ahmet Yılmaz Çoban, who permitted me to work in the laboratory; the lecturer Dr. Kübra Yıldırım, who shared her knowledge with me; and my master's degree students İrem Serra Pekşen, Aleyna Kavalcı, and Meryem Ece Mutlu.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Šojić, B., Ikonić, P., Kocić-Tanackov, S., Peulić, T., Teslić, N., Županjac, M., ... & Pavlić, B. (2023). Antibacterial activity of selected essential oils against foodborne pathogens and their application in fresh Turkey sausages. *Antibiotics*, *12*(1), 182. https://doi.org/10.3390/antibiotics12010182.
2. Mojarradi, F., Bimakr, M., & Ganjloo, A. (2024). Effect of bio-edible coating based on Lallemantia iberica seed mucilage incorporated with Malva sylvestris leaf bioactive compounds on oxidative stability of turkey meat. *Journal of Food Measurement and Characterization*, *18*(1), 402-412. https://doi.org/10.1007/s11694-023-02160-8.
3. Keykhosravy, K., Khanzadi, S., Hashemi, M., & Azizzadeh, M. (2022). Protective effect of chitosan-loaded nanoemulsion containing Zataria multiflora Boiss and Bunium persicum Boiss essential oils as coating on lipid and protein oxidation in chill stored turkey breast fillets. *Journal of Food Science*, *87*(1), 251-265. https://doi.org/10.1111/1750-3841.16011.
4. Mohammed Ali Eesa, Ammar, Behnaz Bazargani-Gilani, and Shaimaa Obaid Hasson. "Comparison of green and synthetic silver nanoparticles in zein-based edible films: Shelf-life study of cold-stored turkey breasts." *Food Science & Nutrition* 11.11 (2023): 7352-7363. https://doi.org/10.1002/fsn3.3661.
5. Sajadizadeh, Hosna; et al. "Antimicrobial activity of Oliveria decumbens vent. Essential oil and Ferula asa-foetida extract on Turkey breast meat: Antimicrobial activity of Oliveria decumbens vent. Essential oil and Ferula asa-foetida extract." *Journal of Food Measurement and Characterization* (2023): 1-11. (2024) https://doi.org/10.1007/s11694-023-02155-5.
6. Babaoğlu, A.S., Ainiwaer, T., Özkan, H. *et al.* Grapefruit and pomelo peel extracts as natural antioxidants for improved storage stability of Turkey patties during refrigerated storage. J Food Sci Technol **59**, 4067–4074 (2022). https://doi.org/10.1007/s13197-022-05458-5.
7. Mojaddar Langroodi, A., Nematollahi, A. & Sayadi, M. Chitosan coating incorporated with grape seed extract and *Origanum vulgare* essential oil: An active packaging for turkey meat preservation. *Food Measure* **15**, 2790–2804 (2021). https://doi.org/10.1007/s11694-021-00867-0.
8. Milani, Morteza Adeli; et al. "Effect of novel bioactive coating enriched with nanoemulsion of mustard essential oil on the quality of turkey meat." *Journal of Food & Nutrition Research* 59.1 (2020).
9. Elhadef, Khaoula; et al. "Gelatin-sodium alginate packaging film with date pits extract: An eco-friendly packaging for extending raw minced beef shelf life." *Meat Science* 207 (2024): 109371. https://doi.org/10.1016/j.meatsci.2023.109371.
10. Safari, R., Yaghoubi, M., Marcinkowska-Lesiak, M., Paya, H., Sun, X., Rastgoo, A., ... & Alirezalu, K. (2023). The effects of double gelatin containing chitosan nanoparticles–calcium alginate coatings on the stability of chicken breast meat. *Food Science & Nutrition*, *11*(12), 7673-7685. https://doi.org/10.1002/fsn3.3686.
11. Yang, X., Zhao, D., Ge, S., Bian, P., Xue, H., & Lang, Y. (2023). Alginate-based edible coating with oregano essential oil/β-cyclodextrin inclusion complex for chicken breast preservation. *International Journal of Biological Macromolecules*, 2023 251, 126. https://doi.org/10.1016/j.ijbiomac.2023.126126.
12. Chen, X; Li, L; Chen; L; Shao, W; Chen, Y; Fan, X., ... & Feng, X. (Tea polyphenols coated sodium alginate-gelatin 3D edible scaffold for cultured meat. *Food Research International* 2023, *173*, 113267.https://doi.org/10.1016/j.foodres.2023.113267.

13. Tsitsos, A.; Economou, V.; Chouliara, E.; Koutouzidou, G.; Arsenos, G.; Ambrosiadis, I. Effect of Chitosan and Alginate-Based Edible Membranes with Oregano Essential Oil and Olive Oil in the Microbiological, Physicochemical and Organoleptic Characteristics of Mutton. *Microorganisms* **2023**, *11*, 507.
14. Montone, A.M.I.; Malvano, F.; Taiano, R.; Capparelli, R.; Capuano, F.; Albanese, D. Alginate Coating Charged by Hydroxyapatite Complexes with Lactoferrin and Quercetin Enhances the Pork Meat Shelf Life. *Foods* **2023**, *12*, 553. https://doi.org/10.3390/foods12030553.
15. Abbasi, A., Sabahi, S., Bazzaz, S., Tajani, A. G., Lahouty, M., Aslani, R., & Hosseini, H. An edible coating utilizing Malva sylvestris seed polysaccharide mucilage and postbiotic from *Saccharomyces cerevisiae var. boulardii* for the preservation of lamb meat. *International Journal of Biological Macromolecules* 2023, *246*, 125660. https://doi.org/10.1016/j.ijbiomac.2023.125660.
16. Toushik, S. H., Kim, K., Park, S. H., Park, J. H., Ashrafudoulla, M., Ulrich, M. S. I., ... & Ha, S. D. Prophylactic efficacy of *Lactobacillus curvatus* B67-derived postbiotic and quercetin, separately and combined, against *Listeria monocytogenes and Salmonella enterica ser. Typhimurium* on processed meat sausage. *Meat Science*, *197*, (2023). 109065. https://doi.org/10.1016/j.foodcont.2023.110186.
17. Moradi, M., Kousheh, S. A., Almasi, H., Alizadeh, A., Guimarães, J. T., Yılmaz, N., & Lotfi, A. Postbiotics produced by lactic acid bacteria: The next frontier in food safety. *Comprehensive reviews in food science and food safety*, 2020, 19.6: 3390-3415. https://doi.org/10.1111/1541-4337.12613.
18. İncili, G. K., Karatepe, P., Akgöl, M., Kaya, B., Kanmaz, H., & Hayaloğlu, A. A. Characterization of Pediococcus acidilactici postbiotic and impact of postbiotic-fortified chitosan coating on the microbial and chemical quality of chicken breast fillets. *International Journal of Biological Macromolecules*, 2021a, 184: 429-437. https://doi.org/10.1016/j.ijbiomac.2021.06.106.
19. Kilic, B. & Richards, M.P. (2003). Lipid Oxidation in Poultry Döner Kebab: Pro-oxidative and Anti-oxidative Factors. Journal of Food Science. 68. 686 - 689. 10.1111/j.1365-2621. 2003.tb05732.x.
20. Kaynakci, E., & Kilic, B. (2020). Effect of replacing beef fat with safflower oil on physicochemical, nutritional and oxidative stability characteristics of wieners. *Food Science and Technology*, *41*, 52-59. https://doi.org/10.1590/fst.07720.
21. Akman, P. K., Kutlu, G., & Tornuk, F. (Development and characterization of a novel sodium alginate based active film supplemented with *Lactiplantibacillus plantarum* postbiotic. *International Journal of Biological Macromolecules*, 2023, 125240. https://doi.org/10.1016/j.ijbiomac.2023.125240.
22. Ozma, M. A., Abbasi, A., & Sabahi, S Characterization of postbiotics derived from *Lactobacillus paracasei* ATCC 55544 and its application in Malva sylvestris seed mucilage edible coating to the improvement of the microbiological, and sensory properties of lamb meat during storage. *Biointerface Res Appl Chem*, 2022, 13. https://doi.org/10.33263/BRIAC133.267.
23. Niu, L., Li, W., Chen, X., Su, X., Dong, J., Liao, Q., ... & Sun, R. 1-Monopalmitin promotes lung cancer cells apoptosis through PI3K/A kt pathway in vitro. *Environmental Toxicology*, 2023, 38.11: 2621-2631. https://doi.org/10.1002/tox.23897.
24. Benramdane E, Chougui N, Ramos PAB, Makhloufi N, Tamendjari A, Silvestre AJD, Santos SAO. Lipophilic Compounds and Antibacterial Activity of *Opuntia ficus-indica* Root Extracts from Algeria. *International Journal of Molecular Sciences*. 2022; 23(19):11161. https://doi.org/10.3390/ijms231911161.
25. Gautam, Shweta; et al. "Emulsion-Based Coatings for Preservation of Meat and Related Products." *Foods* 12.4 (2023): 832. https://doi.org/10.3390/foods12040832.
26. Elhadef, Khaoula; et al. "Gelatin-sodium alginate packaging film with date pits extract: An eco-friendly packaging for extending raw minced beef shelf life." *Meat Science* 207 (2024): 109371. https://doi.org/10.1016/j.meatsci.2023.109371.
27. Kuley, E., Yazgan, H., Özogul, Y., Ucar, Y., Durmus, M., Özyurt, G., & Ayas, D. Effectiveness of Lactobacilli cell-free supernatant and propolis extract microcapsules on oxidation and microbiological growth in sardine burger. *Food Bioscience*, 2021, 44: 101417. https://doi.org/10.1016/j.fbio.2021.101417.
28. Xiao, Q., Gu, X., & Tan, S. Drying process of sodium alginate films studied by two-dimensional correlation ATR-FTIR spectroscopy. *Food chemistry*, 2014, 164: 179-184. https://doi.org/10.1016/j.foodchem.2014.05.044.
29. Derkach, S. R., Voron'ko, N. G., Sokolan, N. I., Kolotova, D. S., & Kuchina, Y. A Interactions between gelatin and sodium alginate: UV and FTIR studies. *Journal of Dispersion Science and Technology*, 2019. https://doi.org/10.1080/01932691.2019.1611437.
30. Badita, C. R., Aranghel, D., Burducea, C., & Mereuta, P. J. R. J. P. Characterization of sodium alginate based films. *Rom. J. Phys*, 2020, 65: 1-8.
31. Xiao, Q., Gu, X., & Tan, S. Drying process of sodium alginate films studied by two-dimensional correlation ATR-FTIR spectroscopy. *Food Chemistry*, 2014, 164: 179-184. https://doi.org/10.1016/j.foodchem.2014.05.044.
32. ICMSF. (1986). International Commission on microbiological specifications for foods. Microorganisms in foods. 2. Sampling for microbiological analysis. *Principles and Specific Applications*. Author 1, A.B.; Author 2, C.D. Title of the article.

33. Gurunathan, S., Thangaraj, P., & Kim, J. H. Postbiotics: Functional Food Materials and Therapeutic Agents for Cancer, Diabetes, and Inflammatory Diseases. *Foods*, 2023, 13.1: 89. https://doi.org/10.3390/foods13010089.
34. Ku, Seockmo, Myeong Soo Park, Geun Eog Ji, and Hyun Ju You Review on *Bifidobacterium bifidum* BGN4: Functionality and nutraceutical applications as a probiotic microorganism. *International journal of molecular sciences*, 2016, 17.9: 1544. https://doi.org/10.3390/ijms17091544.
35. İncili, G. K., Karatepe, P., Akgöl, M., Tekin, A., Kanmaz, H., Kaya, B., ... & Hayaloğlu, A. A. Impact of chitosan embedded with postbiotics from *Pediococcus acidilactici* against emerging foodborne pathogens in vacuum-packaged frankfurters during refrigerated storage. *Meat Science*, 2022, 188: 108786. https://doi.org/10.1016/j.meatsci.2022.108786.
36. Huang, H. C., Huang, W. Y., Chiu, S. H., Ke, H. J., Chiu, S. W., Wu, S. Y., ... & Chang, T. M. Antimelanogenic and antioxidative properties of *Bifidobacterium bifidum*. *Archives of dermatological research*, 2011, 303: 527-531. https://doi.org/10.1007/s00403-011-1135-y.
37. Hamad, G. M., Abdelmotilib, N. M., Darwish, A. M., & Zeitoun, A. M. Commercial probiotic cell-free supernatants for inhibition of *Clostridium perfringens* poultry meat infection in Egypt. *Anaerobe*, 2020, 62: 102181. https://doi.org/10.1016/j.anaerobe.2020.102181.
38. Kilinc, B., Cakli, S., Dincer, T., & Tolasa, S. Microbiological, chemical, sensory, color, and textural changes of rainbow trout fillets treated with sodium acetate, sodium lactate, sodium citrate, and stored at 4 C. *Journal of Aquatic Food Product Technology*, 2009, 18.1-2: 3-17. https://doi.org/10.1080/10498850802580924.
39. Keykhosravy, K., Khanzadi, S., Hashemi, M., & Azizzadeh, M. Chitosan-loaded nanoemulsion containing Zataria Multiflora Boiss and Bunium persicum Boiss essential oils as edible coatings: Its impact on microbial quality of turkey meat and fate of inoculated pathogens. *International Journal of Biological Macromolecules*, 2020, 150: 904-913. https://doi.org/10.1016/j.ijbiomac.2020.02.092.
40. İncili, G. K., Akgöl, M., Karatepe, P., Tekin, A., Kanmaz, H., Kaya, B., & Hayaloğlu, A. A. Whole-Cell Postbiotics: An Innovative Approach for Extending the Shelf Life and Controlling Major Foodborne Pathogens in Chicken Breast Fillets. *Food and Bioprocess Technology*, 2023, 1-23. https://doi.org/10.1007/s11947-023-03009-0.
41. Arrioja-Bretón, D., Mani-López, E., Palou, E., & López-Malo, A. Antimicrobial activity and storage stability of cell-free supernatants from lactic acid bacteria and their applications with fresh beef. *Food Control*, 2020, 115: 107286. https://doi.org/10.1016/j.foodcont.2020.107286 https://doi.org/10.2478/aoas-2023-0081.
42. Serter, B., Önen, A., & Ilhak, O. I. Antimicrobial efficacy of postbiotics of lactic acid bacteria and their effects on food safety and shelf life of chicken meat. *Annals of Animal Science*, 2023, 24.1: 277-287.
43. İncili G.K., Akgöl M., Aydemir M.E., Alan S., Mutlu M., İlhak O.İ., Öksüztepe G. Fate of Listeria monocytogenes and Salmonella Typhimurium in homemade marinade and on marinated chicken drumsticks, wings and breast meat. *LWT*, 2020, 134: 110231. https://doi.org/10.1016/j.lwt.2020.110231.
44. Dong, S., Li, L., Hao, F., Fang, Z., Zhong, R., Wu, J., & Fang, X. (2024). Improving quality of poultry and its meat products with probiotics, prebiotics, and phytoextracts. *Poultry Science*, *103*(2), 103287.
45. Wang, W., Wang, R., Zhang, G., Chen, F., & Xu, B. (2020). In vitro antibacterial activities and mechanisms of action of fatty acid monoglycerides against four foodborne bacteria. *Journal of Food Protection*, *83*(2), 331-337.
46. Jumina, Nurmala, A., Fitria, A., Pranowo, D., Sholikhah, E. N., Kurniawan, Y. S., & Kuswandi, B. (2018). Monomyristin and mono palmitin derivatives: Synthesis and evaluation as potential antibacterial and antifungal agents. *Molecules*, *23*(12), 31-41.
47. Mohammadi, R., Moradi, M., Tajik, H. and Molaei, R. (2022). Potential application of postbiotics metabolites from bioprotective culture to fabricate bacterial nanocellulose based antimicrobial packaging material, International Journal of Biological Macromolecules, Vol. 220, pp. 528-536.
48. Ansari, N., Mahmoudi, R., Ghajarbeygi, P., Mehrabi, A., Alizadeh, A., & Kazeminia, M. (2024). Evaluation effect of antimicrobial nanocellulose film combined with *Lactobacillus rhamnosus* postbiotics in active packaging of minced meat. *Journal of Microbiology, Biotechnology and Food Sciences*, *13*(4), e6209. https://doi.org/10.55251/jmbfs.6209.