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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

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Abstract: Firstly, postbiotics of *Lactobacillus paracasei* ATCC 55544 (PLP) were isolated and characterized. Secondly, PLP has been utilized in *Malva sylvestris* mucilage (MSM) coating, and its antioxidant and antimicrobial properties on lamb meat slices were assessed in 10 days at 4 °C. The main chemical compounds of the PLP were identified and quantified by a gas chromatograph coupled to a mass spectrometer. Various microbiological and sensory assessments were also applied regarding the coated lamb meat slices. PLP contains pyrrolo [1,2-a] pyrazine-1,4-dione, and 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine as well as various organic acids with strong radical scavenging activity and antibacterial effect against investigated pathogenic microorganisms. The PLP-loaded MSM edible coating greatly postponed the growth of microorganisms and extended the product life (>10 days). Moreover, sensory parameters of the samples were preserved more efficiently during cold storage when the PLP-enriched edible coating, particularly MSM+8% PLP, was used. Using edible coating based on MSM and PLP effectively reduces microbial growth in lamb meat during storage.

Keywords: postbiotic; edible coating; cell-free supernatant; *Malva sylvestris* mucilage; microbiological stability; sensory properties.

1. Introduction

Meat is susceptible to various microbial contaminations from production to consumption [1]. Furthermore, the quality and nutritional characteristics of meat products could be greatly decreased due to lipid oxidation. These deteriorations lead to health risks, economic losses, and consumer rejection [2-4]. Applying the antimicrobial and antioxidant coating is a very promising solution to preserve these perishable foods' qualitative properties and nutritional value [5].

Various substances could be used for the production of coating or films. This utility is dependent on their barrier properties and capability to retard spoilage through antimicrobial or

antioxidant activity [3,6,7]. Due to the negative impact of synthetic packaging on the environment, the food industry and producers have developed bio-based packaging, such as edible coatings, with excellent barrier ability to gases, long shelf life, economic feasibility, and potential to carry antibacterial and antioxidant agents [4,8]. Plant-based biopolymers such as mucilage are widely applied for these purposes. Plants are common and old sources of mucilage and polysaccharides with high-molecular-weight. It is extensively employed as a gelling agent, food additive, and an excellent ingredient in producing edible films and coatings [1,9].

Malva sylvestris, also called mallow, is an annual plant with purple flowers and shallowly lobed leaves. It is named Panirak in Iran, and its flowers are commonly used as a remedy for cut wounds, bronchitis, dermal infected wounds, inflammation, and digestive problems [10,11]. Studies have shown the presence of various compounds, mainly polysaccharides in *Malva* spp., that improve the physicochemical properties of various food matrices and influence the function of the host immune system [12,13]. In this regard, Samavati and Manoochehrizade [14] studied the effects of extraction time, extraction temperature, water-to-raw material ratio, and the number of extractions on the extraction yield of mucilage from *M. sylvestris* leaves. The highest polysaccharide extraction yield of 8.37% was obtained under optimal conditions: extraction temperature of 90 °C, extraction time of 4 h, the water-to-raw material ratio of 21, and extraction number of 2 [14]. To the best of our knowledge, there is no report in the literature about the potential use of *M. sylvestris* mucilage (MSM) in edible coating production.

However, it is noteworthy that edible coatings of polysaccharide origin rarely have antioxidative and antimicrobial properties [15,16]. Therefore, food-grade and natural preservatives such as essential oils are often added to the edible coatings to increase their techno-functionality and biological activity (e.g., antioxidant and antimicrobial effects) [17,18].

The concept of 'postbiotic' is associated with deactivated microbial cell structures, cell segments (endo- and exo-polysaccharides, teichoic acids, cell-surface proteins, and peptidoglycan-derived muropeptides), and/or cell metabolites (enzymes, organic acids, bacteriocins, and Short-Chain Fatty Acids (SCFAs)) that are made mainly through the fermentation process by live probiotic cells or made in response to environmental circumstances (e.g., the dominance of dysbiotic microbiota in the Gastrointestinal Tract (GIT)), as well as produced by laboratory methods in pure form, which provide several biological health benefits to the host when consumed in sufficient quantities [19,20]. In addition, postbiotics with specific chemical structures can modulate certain physiological pathways, regulatory responses, metabolism, and/or behavioral feedbacks related to the activity of the host's commensal microbiota [21-23]. According to reports, various postbiotic metabolites such as linoleic acid, lactic acid, SCFA, peptides, proteins, peptidoglycans, exopolysaccharides, and glycoproteins have significant antioxidant and antimicrobial potentials [24-26].

Based on the discussions outlined above, this study aimed to produce a novel bioactive edible coating based on PLP and MSM with antioxidant and antimicrobial characteristics. The effects of the PLP-incorporated MSM coating on the microbial growth and consumer acceptance of lamb meat slices were also investigated during refrigerated storage for 10 days.

2. Materials and Methods

2.1. Materials.

Quercetin, gallic acid, and ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) were procured from Sigma-Aldrich Co. (St Louis, MO, USA). The culture media Eosin Methylene Blue (EMB), Mannitol Salt Agar (MSA), Sabouraud Dextrose Agar (SDA), Plate Count Agar (PCA), Mueller Hinton Broth (MHB), and Mueller Hinton Agar (MHA) were purchased from Merck Co. (Darmstadt, Germany).

2.2. Preparation of bacterial strains and cell-free supernatant.

Lactobacillus paracasei ATCC 55544 strain was prepared from Cinnagen Co. (Iran). By sub-culturing of *L. paracasei* ATCC 55544 in MRS broth (containing 2 g/L dipotassium phosphate, 20 g/L dextroses, 8 g/L meet extract, 0.05 g/L manganese sulfate, 10 g/L peptones, 0.1 g/L magnesium sulfate, 4 g/L yeast extract, 5 g/L sodium acetate trihydrate, 1 g/L polysorbate 80, and 2 g/L triammonium citrate), the fresh microbial suspension was prepared and standardized by visible-ultraviolet spectrophotometer (Beckman, MO, USA; model DU-530) was performed in the range of 600 nm.

The first step in the synthesis of postbiotics (Cell-Free Supernatant (CFS)) is the culture of *L. paracasei* ATCC 55544 in MRS broth, which should be done at a temperature of 37 ± 1 °C for 48 h in a CO_2 incubator (BINDER INC., Germany). Due to the significant effect of different microbial strain culture conditions and extraction methods on the postbiotic performance, in order to obtain sufficient and appropriate amounts of CFSs, this method was performed again in 2000 ml Erlenmeyer flasks. Briefly, after incubation for 24-48 h at 37 °C, centrifugation at 6000 × g at 4 °C for 10 minutes was performed to eliminate bacterial cells and obtain CFS, and finally, the culture supernatant was harvested and lyophilized (Biobase, China) (pump pressure: 100 mTorr, freezing temperature: −40 °C, and shelf temperature: −60 °C) and used as CFS in subsequent experiments [27].

2.3. Gas chromatography-mass spectroscopy (GC/MS).

The main chemical compounds of PLP were identified and quantified by a gas chromatograph (Agilent Technologies 7890A, USA) coupled with a mass spectrometer (Agilent Technologies 5975C, USA), according to the method of Moradi *et al*. [28] with some changes. The PLP (0.1 µL) was injected into a capillary column (with dimensions of 30 m × 0.25 mm × 0.25 µm), and the temperature of the column rose from 45 to 210 °C at 3 °C/min. Helium gas with a flow rate of 1 mL/min was used, and the ionization energy was set at 70 eV.

2.4. Antioxidant activity.

The antioxidant effect of PLP was measured against ABTS free radicals, utilizing the method of Nantitanon *et al.* [29] with modifications. The ABTS radical monocation was generated by oxidation of 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$. The mixture was stored at ambient temperature for 12 h in a dark place and then diluted to 0.7±0.2 absorbance at 750 nm by ethanol. The PLP or control (0.2 mL) was then added to the solution of ABTS (1.8 mL), and the mixture was subjected to a spectrophotometer to measure its absorbance at 750 nm. The antioxidant activity of CZEO was calculated as below:

$$\text{Antioxidant activity } (\%) = \left(1 - \frac{\text{Absorbance of sample}}{\text{absorbance of control}}\right) \times 100$$

2.5. Antimicrobial activity.

The well diffusion agar (WDA), disc diffusion agar (DDA), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) were used to determine the antibacterial effect of the postbiotics against *Listeria innocua* ATCC 33090, *Bacillus cereus* ATTC 14579, *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 6539, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, through the methods described by Behbahani, *et al.* [30]. Antibiotics chloramphenicol, gentamicin, and tetracycline were applied for antibacterial comparison in the DDA test.

2.6. Mucilage extraction.

The mucilage of *M. sylvestris* (MSM) was extracted according to the optimized extraction conditions reported by Samavati and Manoochehrizade [14]. The leaves of *M. sylvestris* were powdered and then defatted three times (80% ethanol, 60 C, 2 h) to discard colored materials and oligosaccharides. The pre-treated sample (20 g) was extracted by water at an extraction temperature of 90 °C, extraction time of 4 h, the water-to-raw material ratio of 21, and extraction number of 2. The insoluble residues were removed by a nylon cloth, and the filtrate was concentrated and then precipitated by the addition of ethanol. The precipitate (mucilage) was dried (50 °C), ground, packed, and stored at 4 °C.

2.7. Edible coating preparation.

The MSM (5 g) was mixed with Tween 80 (1.75 g; 35% of the mucilage dry weight) and then dissolved in distilled water (100 mL) under heating and stirring periods. The hydrocolloid solution was then incorporated with PLP (0, 0.5, 1, 1.5, and 2% v/v) for coating purposes [1].

2.8. Lamb meat slice coating.

The lamb meat slices with appropriate dimensions were dipped for 1 min in MSM-PLP solution and then withdrawn and air-dried (10 min). The coated samples were then stored at 4 °C for 10 days. The non-coated sample was used as a control [31].

2.8.1. Microbiological analyses.

Peptone water (45 g; 0.1% v/v) was added to the meat sample (5 g), and the mixture was homogenized at 200 rpm for 1 min in a Stomacher. The serial dilutions (10^{-1}-10^{-6}) were made with 0.1% peptone water and then added to the culture medium in plates, and the following microbial tests were performed:

- Total viable count (TVC) was measured in the PCA medium by incubating at 37 °C for 48 h.
- Psychrotrophic count (PTC) was evaluated in the PCA medium through incubation at the medium at 7 °C for 10 days.
- *E. coli* count was determined in EMB medium through incubation at 37 °C for 24 h.

- *S. aureus* count was measured in an MSA medium by incubating the medium at 37 °C for 24 h).
- Fungi count was measured in the SDA medium by incubating at 27 °C for 72 h [32].

2.9. Sensory evaluation.

The lamb meat samples were evaluated by 25-well trained panelists (12 females and 13 males) in terms of odor, color, appearance, and overall acceptance, through a nine-point hedonic scale test (9 = like extremely to 1 = dislike extremely). The sensory parameters with scores higher than 4 were considered acceptable [33].

2.10. Statistical analysis.

Data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) *via* SPPS software was applied to process the data, and the difference between the means was determined by the Duncan test ($p<0.05$). The experiments were performed in three replications.

3. Results and Discussion

3.1. Chemical composition of postbiotics.

The chemical profiles, retention time, and the main constitute percentages (> 1%) of postbiotics from *L. paracasei* ATCC 55544 are presented in Table 1. In different types and percentages, postbiotics were characterized by their abundance of organic acid, peptides, fatty acid-containing compounds, alcohols, esters, and aldehyde. Pyrrolo[1,2-a] pyrazine-1,4-dione, benzoic acid and 5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine are found in investigated postbiotics. The mentioned agent has been previously recognized as a well-known antimicrobial and antioxidant agent [34]. Among identified compounds, a waxy, 18-carbon chain saturated fatty acid known as laurostearic acid was found in postbiotic metabolites. Laurostearic acid could act as a biosurfactant and helps to remove the biofilm of pathogens from the surface [35]. Besides, the presence of 1, 4-diaza-2, 5-dioxo-3-isobutyl bicyclo[4.3.0]nonane among the identified compounds could indicate the potential anti-biofilm and antimicrobial function of the prepared postbiotic solution [36]. Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'alpha)- is other identified compound with the promising antimicrobial, and anti-inflammatory properties [37]. The production of organic acids, including lactic acid and acetic acid, is one of the characteristics of lactic acid bacteria. Despite the presence of some organic acids, lactic acid was not found in examined postbiotic metabolites. The results of studies showed that the method of analysis plays an important role in the kind and the contents of individual compounds [38,39].

Table 1. Chemical composition of postbiotics of *Lactobacillus paracasei* ATCC 55544 (PLP) identified and quantified by GC/MS.

NO.	Compounds	Retention time (min)	%
1	Ethanol	6.058	1.15
2	Butric acid	7.559	9.33
3	2,3-Butanediol	8.15	2.11
4	2-Methylpiperidine	11.656	0.45
5	Laurostearic acid	18.135	8.6

NO.	Compounds	Retention time (min)	%
6	Pyrrolo[1,2-a]pyrazine-1,4-dione	24.197	23.2
7	Benzoic acid	24.896	5.54
8	3-Methyldecane	25.942	2.3
9	1, 4-diaza-2, 5-dioxo-3-isobutyl bicyclo[4.3.0]nonane	27.375	3.6
10	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1',2'-d]pyrazine	27.592	4.2
11	Ergotaman-3',6',18-trione, 12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'alpha)-	33.150	1.6

3.2. Antioxidant activity.

Polyphenols can neutralize free radicals *via* their inherent redox properties; thus, these bioactive compounds have preventive effects on various degenerative diseases such as cardiovascular diseases and diabetes, along with their potential to improve the immune system [40]. The ABTS assay is based on the direct oxidation of ABTS with $K_2S_2O_8$ to yield a stable $ABTS^{\bullet+}$, with a blue-green color. In the presence of antioxidants, the decolorization (reduction) extent of the radical solution is then evaluated at 750 nm [41]. The PLP had a markedly high antioxidant activity (78.60 ± 0.50%). This remarkably high antioxidant effect is mainly due to the presence of phenols, flavonoids, and the main chemical constituents of the PLP [42]. The antioxidant property of the PLP has been reported in some studies [43]. Therefore, the PLP could be used to delay or inhibit the free radical reactions and associated oxidative damages.

3.3. Antibacterial activity.

In this study, the antimicrobial tests, including DDA, WDA, MIC, and MBC were used to investigate the antibacterial activity of the PLP against some pathogenic and spoilage bacterial species. The antibacterial effect of the PLP was dependent on the type of bacterial species, and the Gram-positive bacteria were generally more sensitive to the microbial metabolites than the Gram-negative species (Tables 2 and 3). The mean inhibition zone for Gram-positive bacteria was 26.53 mm in the DDA and 29.93 mm in the WDA test; however, the Gram-negative bacterial species had lower mean inhibition zones of 19.59 mm (in DDA) and 22.13 mm (in WDA). The MIC and MBC tests showed similar results, and lower PLP concentrations were needed to inhibit the growth of or kill the Gram-positive bacteria compared to the Gram-negative ones (Table 3).

Table 2. The mean inhibition zone diameter (mm) of postbiotics of *Lactobacillus paracasei* ATCC 55544 (PLP), on some pathogenic microorganisms by Disc Diffusion Agar (DDA) method.

	Antimicrobial substance			
Microorganism	**PLP**	**In (Chl + PLP)**	**In (Gen + PLP)**	**In (Tet + PLP)**
Pseudomonas aeruginosa	15.11 ±0.26 d	22.00 ±0.30 b	27.81±0.21 a	19.10 ±0.54 c
Escherichia coli	7.10 ±0.17 d	25.20 ±0.11 b	28.10 ±0.12 a	17.10 ±0.16 c
Salmonella typhi	10.21 ±0.27 d	21.12 ± 0.13 b	24.25 ± 0.24 a	18.11 ± 0.41 c
Staphylococcus aureus	19.31 ±0.34 c	17.3±0.22 d	28.10 ±0.21 a	27.10 ±0.12 b
Bacillus cereus	19.21 ±0.27 d	31.26±0.17 b	35.18 ±0.11 a	29.05 ±0.24 c
Listeria innocua	25.30 ±0.38 b	24.12 ±0.41 c	26.14 ±0.15 b	36.32 ±0.22 a

Means within the same row with different small letters differ significantly ($p<0.05$).

Table 3. The well diffusion agar (WDA), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the postbiotics of *Lactobacillus paracasei* ATCC 55544 (PLP) on some pathogenic microorganisms.

Microorganism	WDA (mm)	MIC (mg/mL)	MBC (mg/mL)
Pseudomonas aeruginosa	22.25 ±0.33 d	5.65	18.75
Escherichia coli	21.10 ±0.12 f	10.37	15.2

Salmonella typhi	23.06 ± 0.34 e	10.38	37.6
Staphylococcus aureus	25.12 ±0.13 c	3.35	9.68
Bacillus cereus	30.33 ±0.25 b	3.36	10.37
Listeria innocua	34.35 ±0.30 a	3.42	10.68

Means within the same column with different small letters differ significantly ($p<0.05$).

According to the outcomes of the DDA and WDA tests, *L. innocua* and *E. coli* received the maximum and minimum inhibition zones, respectively. Furthermore, the inhibition zone in the WDA method was remarkably higher than in the DDA assay. This is mainly due to the direct contact of the PLP with the bacterial species in the former method compared to the latter one [44-46]. It could also be noteworthy that there was a synergistic effect between the PLP and antibiotics; the interaction between PLP and antibiotics resulted in a generally higher antibacterial effect than the PLP alone (Table 1). Based on the MIC and MBC tests, these results have been attributed to the differences in the bacterial cell wall composition. A thicker mucopeptide layer is observed in the former group than in the latter. Additionally, lipoproteins and lipopolysaccharides are the main compositions of the wall structure of the Gram-negative bacterial species, which lead to their higher resistance to antimicrobial compounds [8,47]. The antibacterial activity of the PLP has been attributed to the ability of its compounds to inhibit the synthesis of bacteria's essential enzymes and/or cause damage to the bacteria's cell wall [48,49].

3.4. Microbiological properties of the lamb meat slice.

The TVC results of the non-coated and coated lamb meat slices during 10 days' storage at 4 °C are indicated in Table 4. The TVC of the samples increased significantly ($p<0.05$) as the storage time increased, except for the MSM +8% PLP sample, which showed a non-significant increase in TVC during the storage period ($p>0.05$). A TVC change of 10.14, 6.51, 5.03, 3.24, 3.18, and 0.5 Log CFU/g were observed for the control, MSM +0% PLP, MSM +2% PLP, MSM +4% PLP, MSM +6% PLP, and MSM +8% PLP, respectively, as the storage time increased from first to tenth day. The International Commission of Food Microbial Standards reported that fresh meat's maximum permitted microbial load is 7 Log CFU/g or 10^7 CFU/g [50,51]. Accordingly, the shelf lives for the control and MSM +0% PLP samples were found to be 4 and 7 days, respectively; surprisingly, the lamb meat slices wrapped with PLP-rich MSM edible coating never exceeded the permitted limit throughout the storage period. The PTC values of the samples were also changed by the edible coating and storage time ($p<0.05$) (Table 4).

Table 4. Effect of different concentrations of postbiotics of *Lactobacillus paracasei* ATCC 55544 (PLP) added to *M. sylvestris* mucilage (MSM) based edible coating on microbial load changes of lamb meat slices during 10 days of storage at 4 ºC.

Parameters	Days of storage	Edible coating					
		Control	MSM +0% PLP	MSM +2% PLP	MSM +4% PLP	MSM +6% PLP	MSM +8% PLP
TVC (log CFU/g)	1	2.59±0.12 Da	2.62±0.04 Da	2.59±0.14 Ca	2.51±0.22 Ca	2.53±0.15 Ca	2.35±0.34 Aa
	4	7.48±0.18 Ca	4.5±0.45 Cb	4.01±0.25 Cb	3.91±0.16 Cb	2.87±0.3 Cb	2.63±0.29 Ab
	7	8.81±0.14 Ba	7.42±0.44 Bb	6.59±0.4 Bbc	5.9±0.19 Bcd	4.32±0.15 Bd	3.91±0.2 Ae
	10	12.73±0.35 Aa	9.13±0.18 Ab	7.62±0.35 Ac	5.75±0.33 Ac	5.71±0.23 Ac	3.03±0.31 Ad
PTC (log CFU/g)	1	1.2±0.19 Db	2.06±0.16 Da	2.06±0.15 Ca	2.05±0.02 Ba	2.05±0.05 Ca	2.05±0.16 Ba
	4	5.02±0.4 Ca	4.38±0.28 Ca	3.29±0.16 Cb	3.24±0.22 Bb	2.11±0.11 Cb	2.08±0.05 Bb
	7	7.33±0.07 Ba	5.64±0.17 Bb	4.6±0.16 Bbc	3.07±0.3 Bd	2.78±0.07 Bcd	2.63±0.75 Bcd
	10	9.89±0.2 Aa	6.55±0.18 Ab	5.72±0.33 Abc	4.88±0.19 Acd	4.10±0.07 Ad	4.01±0.54 Acd
***E. coli* (log CFU/g)**	1	0.83±0.03 Da	0.8±0.04 Ca	0.79±0.44 Ca	0.75±0.08 Ba	0.80±0.23 Aa	0.78±0.23 Aa
	4	2.92±0.04 Ca	1.8±0.38 Cab	1.28±0.04 Cab	0.92±0.44 Bb	0.87±0.02 Ab	0.79±0.04 Ab

Parameters	Days of storage	Edible coating					
		Control	MSM +0% PLP	MSM +2% PLP	MSM +4% PLP	MSM +6% PLP	MSM +8% PLP
	7	4.08±0.19 Ba	3.97±0.18 Ba	3.50±0.09 Ba	2.22±0.07 Bb	2.12±0.68 Ab	0.94±0.14 Ab
	10	4.86±0.16 Aa	4.79±0.26 Aa	4.66±0.09 Aa	3.32±0.19 Ab	2.82±0.05 Ac	1.15±0.05 Ad
***S. aureus* (log CFU/g)**	1	0.95±0.02 Cd	8.04±0.05 Ab	9.13±0.04 Aa	9.15±0.02 Aa	9.12±0.03 Aa	8.1±0.04 Ac
	4	3.49±0.03 Ca	2.86±0.05 Db	2.25±0.05 Dc	2.01±0.02 Dd	1.92±0.04 Dd	1.81±0.02 Bd
	7	4.02±0.06 Ba	3.85±0.06 Ca	3.72±0.06 Ca	3.34±0.01 Ca	2.15±0.04 Bb	2.21±0.47 Bb
	10	4.87±0.08 Aa	4.5±0.06 Ba	3.89±0.07 Ba	2.83±0.09 Bb	1.52±0.01 Cc	1.3±0.49 Bc
Fungi (log CFU/g)	1	2.23±0.03 Da	1.03±0.02 Db	1.02±0.08 Cb	0.96±0.06 Cb	0.95±0.09 Bb	0.35±0.01 Cc
	4	2.89±0.01 Ca	2.79±0.05 Ca	2.31±0.09 Bb	2.26±0.04 Bbc	1.89±0.06 Bc	1.75±0.01 Ab
	7	3.98±0.03 Ba	3.25±0.05 Ab	3.1±0.08 BCd	2.98±0.03 Ac	2.34±0.03 Ac	1.55±0.09 Be
	10	5.05±0.1 Aa	4.04±0.02 Bb	3.94±0.03 Ab	3.56±0.08 Ac	2.54±0.08 Acd	1.41±0.09 Ad

Means within the same row with different small letters differ significantly ($p<0.05$). Means within the same column with different capital letters differ significantly ($p<0.05$).

Lamb meat slices experienced a marked rise in PTC over time; however, the rate of PTC increase in the samples coated with PLP-MSM was remarkably lower compared to the non-coated and PLP-free MSM-coated lamb meat slices. PTC is the most common microbial flora of meat and meat products stored under cold conditions. *Pseudomonas* is considered an extremely aerobic bacterial species, and they are not able to survive in the absence of oxygen [52]. On this point, it seems that the PLP-MSM edible coating had an oxygen barrier ability and inhibited oxygen penetration into lamb meat slices and, subsequently, the growth of *Pseudomonas* species. These bacterial strains have the potential to synthesize extracellular proteases and lipases, thereby leading to meat quality deterioration under inappropriate conditions [53,54]. Additionally, the investigated postbiotic had a remarkable antibacterial effect on the growth of *P. aeruginosa* (Tables 2 and 3).

The PLP-MSM coating was also able to manifestly inhibit the growth of fungi, *S. aureus*, and *E. coli* in lamb meat slices during the refrigerated period (Table 4). As can be seen, the maximum and minimum microbial counts were found in control, and MSM+8%PLP coated lamb meat slices, respectively, on day 10. In line with our results, Behbahani and Imani Fooladi [1] reported that the edible coating made by *Lallemantia royleana* seed mucilage and *Allium hirtifolium* essential oil significantly inhibited the growth of microbial pathogens (TVC, PTC, fungi, *S. aureus*, and *E. coli*) in meat slices. This effect was ascribed to the oxygen-barrier ability of the coating and antimicrobial properties of the essential oil rich in phenolic compounds [1]. Similarly, Zanganeh, Mortazavi, Shahidi, and Alizadeh Behbahani [8] successfully developed a novel edible coating (*Citrus paradise* essential oil-loaded *Lallemantia iberica* seed mucilage) to extend the microbiological stability of lamb slices during refrigerated storage. Noshad, Alizadeh Behbahani, Jooyandeh, Rahmati-Joneidabad, Hemmati Kaykha, and Ghodsi Sheikhjan [31] utilized the *Plantago major* seed mucilage comprising *Citrus limon* essential oil as an eatable coating to expand the shelf-life of buffalo meat under refrigeration circumstances, and the bioactive coating was able to suppress the growth of pathogenic and spoilage microorganisms meaningfully. According to the results of this study, the PLP-rich MSM edible coating could be therefore used to increase the microbiological stability, safety, and shelf-life of meat and related products under refrigerated temperatures.

3.5. Sensory properties of the lamb meat slice.

Consumer rejection of foods is also associated with microbial growth and lipid oxidation extent. The sensory parameters of the lamb meat slices are reported in Table 5. Although there were no significant differences between the coated and non-coated samples in

terms of all sensory parameters on day 1 ($p>0.05$), increasing the PLP concentration in the MSM coating led to a significantly lower change in sensory features over time. In this regard, there were no significant changes in color, appearance, and overall acceptance of the MSM+8%PLP coated lamb meat slices throughout the storage period. It has been claimed that meat samples are only accepted for human consumption when the sensory features are perceived to have high scores (> 4) [55,56]. Accordingly, the control and PLP-free MSM-coated lamb meat slices were found to be unacceptable regarding overall acceptance after 4 days of storage at 4 °C; nonetheless, the samples coated with PLP-loaded MSM were acceptable throughout the display.

Table 5. Effect of different concentrations of postbiotics of *Lactobacillus paracasei* ATCC 55544 (PLP) added to *M. sylvestris* mucilage (MSM) based edible coating on sensory properties of lamb meat slices during 10 days of storage at 4 ºC.

Parameters	Days of storage	Edible coating					
		Control	MSM +0% PLP	MSM +2% PLP	MSM +4% PLP	MSM +6% PLP	MSM +8% PLP
Odor	1	8.86±0.53 Aa	8.8±0.39 Aa	8.9±0.27 Aa	7.89±0.45 Aa	7.98±0.41 Aa	9.42±0.33 Aa
	4	7.85±0.42 Aa	6.31±0.53 Bb	8.2±0.24 Aa	7.2±0.44 ABab	6.9±0.31 Bab	9.13±0.43 Aa
	7	6.06±0.2 Bab	5.54±0.39 Bb	6.2±0.5 Bab	6.85±0.39 BCa	6.7±0.29 BCab	8.06±0.33 Ba
	10	2.60±0.42 Cd	1.4±0.35 Ccd	2.4±0.6 Cbc	4.52±0.48 Cab	4.45±0.45 Cb	7.94±0.59 Ba
Color	1	8.73±0.45 Aa	8.22±0.5 Aa	8.2±0.52 Aa	8.26±0.51 Aa	8.05±0.32 Aa	8.6±0.48 Aa
	4	7.25±0.52 Aa	7.75±0.32 Aa	7.51±0.5 Aa	7.52±0.58 Aa	7.36±0.55 ABa	8.1±0.50 Aa
	7	5.14±0.42 Bc	6.4±0.4 Bbc	7.3±0.21 Aa	7.45±0.32 Aba	6.41±0.33 Bab	7.23±0.39 Aa
	10	2.26±0.24 Cc	3.2±0.3 Cbc	4.1±0.31 Bb	5.63±0.15 Ba	5.32±0.25 Ba	6.68±0.33 Aa
Appearance	1	8.21±0.33 Aa	8.05±0.9 Aa	8.37±0.5 Aa	8.32±0.3 Aa	8.24±0.31 Aa	8.38±0.46 Aa
	4	7.46±0.33 Aa	7.45±0.29 Aa	7.56±0.5 Aa	7.69±0.44 Aba	8.01±0.44 Aa	7.99±0.54 Aa
	7	6.06±0.22 Ba	6.12±0.18 Ba	7.3±0.40 Aa	6.7±0.45 Aba	7.78±0.58 Aa	7.46±0.49 Aa
	10	3.95±0.39 Cd	4.1±0.37 Bcd	5.2±0.3 Bbc	6.21±0.38 Bab	7.52±0.53 Aa	6.76±0.38 Aa
Overall acceptance	1	8.79±0.34 Aa	8.32±0.36 Aa	9.31±0.4 Aa	8.36±0.55 Aa	9.15±0.47 Aa	9.31±0.86 Aa
	4	7.59±0.4 Bab	6.81±0.43 Bb	7.5±0.5 Abb	8.1±0.51 Aab	8.13±0.44 Aab	9.18±0.53 Aa
	7	4.53±0.38 Cc	4.23±0.44 Cc	7.2±0.5 BCb	7.56±0.54 Ab	7.69±0.23 Aab	8.69±0.46 Aa
	10	2.47±0.39 Dd	3.15±0.56 Cd	5.8±0.47 Cc	6.42±0.42 Abc	7.43±0.55 Aab	8.57±0.45 Aa

Means within the same row with different small letters differ significantly ($p<0.05$). Means within the same column with different capital letters differ significantly ($p<0.05$).

According to the sensorial essay, it could also be noteworthy that there is a good correlation between sensory results and microbial properties of the lamb meat slices; the samples with high microbial stability perceived the maximum sensory scores. In line with the results of the present study, it has been demonstrated that PLP-loaded polysaccharide-based edible coatings can increase the microbiological stability and shelf-life of meat and related products [6,32,57-61].

4. Conclusions

Consequently, the edible coating comprised of *M. sylvestris* mucilage (MSM) and postbiotics of *L. paracasei* ATCC 55544 (PLP) can improve the shelf-life of lamb meat slices by postponing microbial alterations. Increasing the concentration of PLP in the MSM-based coating preserved the meat quality more efficiently. We found that MSM enriched with 8%PLP provided the inhibition of microbial growth and preservation of sensory properties during cold storage. Due to the consumer demand for the use of natural preservatives, it is recommended to use MSM+8%PLP edible coating to prolong the shelf-life of meat and meat products.

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Conflicts of Interest

The authors declare no conflict of interest.

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