

Research Article

Potential application of *Leuconostoc mesenteroides* postbiotics and Zinc-carbon dots in fresh pasta filata cheese by aerosolization method

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Abstract

In this research, the white postbiotics (P) powder of *Leuconostoc mesenteroides* and white zinc carbon dots (Z-CDs) from zinc acetate, which were prepared in our previous studies, were used. Hydroxyl, carbonyl, and carboxyl groups in the P were confirmed by FTIR spectra to affect the functions, antioxidant, and antimicrobial capabilities. Antioxidant activity of P was confirmed based on DPPH (IC₅₀ values, 0.28 mg/mL) and FRAP (total antioxidant capacity, 73.5 nM of Fe₂SO₄/L) methods. The antimicrobial properties of P solutions at concentrations of 150, 300, and 450 mg/mL were evaluated through the agar well diffusion assay against *Escherichia coli*, which showed acceptable antimicrobial effects. *E. coli* was sensitive at 4 °C and 25°C in photodynamic treatments. The effects of P (300 mg/mL), Z-CDs (5 mg/mL), and their combination (Z-CDs-P) as additives in fresh pasta filata cheese were studied by aerosolization method. The results showed that the highest inhibitory effects were observed in P, Z-CDs-P, and Z-CDs_{420nm}-Ps treatments; so that *E. coli* reached 1 log₁₀ CFU/g on days 18, 14, and 12, respectively. We concluded that the aerosolization method of P and CDs had a novel antibacterial activity and can be used for the packaging of cheese.

Keywords: Active packaging, Cheese application, Photodynamic, Spray Method.

Introduction

Fresh pasta filata, a soft Italian cheese, is produced from cow or buffalo milk, which is typically kept in water or brine, containing about 50–60% moisture, and has a shelf life of 5 to 7 days when stored at 4 °C (Azhdari & Moradi, 2022). Psychrophilic bacteria and pathogens are the core microorganisms whose enzymatic activities can alter the cheese's texture, color, and aroma (Sharafi et al., 2022). Various approaches, such as film wrapping, dipping, coating, and spraying, have been explored to incorporate antimicrobial agents into active packaging for cheese

(Costa et al., 2018). Aerosolization is a process that transforms liquid into fine droplets, typically with an average diameter exceeding 5 µm, using spraying methods. Additionally, this technique has been employed to apply both natural and synthetic antimicrobial agents to cheese and other products (Sullivan et al., 2020). Natural antimicrobials such as chitosan and natamycin have been administered using nebulizers and sprays on hake fish fillets and cheese (Sullivan et al., 2020) as well as on fresh pasta filata cheese (Divsalar et al., 2025; Torrijos et al., 2022).

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Postbiotics encompass a wide range of metabolic byproducts that are produced and released by probiotics during their growth in culture media, food systems, or within the digestive tract (Sharafi et al., 2024). They include compounds such as organic acids, exopolysaccharides (EPS), bacteriocins, and enzymes that contribute to probiotic health effects. Being stable at different pH levels and not requiring refrigeration, they are easier to store and can be added to various foods (Divsalar et al., 2025). *Leuconostoc* species used in food production generate antimicrobial agents and EPS such as dextran, levan, and glucan, which possess immunomodulatory, antioxidant, and anticancer properties (Bisson et al., 2023). The effects of UF whey containing postbiotics from *Lactobacillus acidophilus* (P-LA5), *Bifidobacterium animalis* (P-BB12), and their combination were evaluated on microbial and sensory properties of high-moisture mozzarella cheese. Postbiotics, produced in a whey-based medium, reduced microbial counts by 1.5–2 log₁₀ CFU/g, with P-LA5 and P-BB12 showing the strongest activity against mesophiles and psychrophiles, respectively. Yeasts and molds were the most sensitive. Overall, postbiotics effectively preserved the microbial quality of mozzarella cheese, indicating their potential as natural preservative fluids (Sharafi et al., 2022).

Carbon dots (CDs) are sub-10 nm carbon particles with unique characteristics that can replace conventional antimicrobials in active packaging (Moradi et al., 2022). Their antibacterial action arises from their structure, surface properties, and ability to produce reactive oxygen species (ROS), which damage bacterial cells (Lysenko et al., 2024). The CDs also act as photosensitizers (PS) in photodynamic inactivation (PDI), generating ROS to eliminate microbes. Doping, such as with zinc, further enhances their optical, antibacterial, and antioxidant properties (Divsalar et al., 2023). A zinc-doped hydroxyapatite was synthesized with different zinc concentrations and found that these biomaterials exhibited antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. The formulation containing 2% zinc showed the strongest antibacterial effect (de Lima et al., 2021). Synthesized CDs from whey via a hydrothermal method and applied them as alginate-based coatings in fiordilatte cheese packaging. The CDs (2500–5000

mg/L) significantly improved the microbiological stability and sensory quality of cheese, doubling its shelf life compared to the uncoated control, which spoiled within 3.5 days. This sustainable approach highlights the strong potential of whey-derived CDs for food industry applications (Lacivita et al., 2023).

The white P powder of *L. mesenteroides* and white Z-CDs, produced in our earlier experiments, were applied in this study. The characterization and functional properties were investigated by antimicrobial and photodynamic methods. Finally, P and Z-CDs were used alone, and in combination (Z-CDs-P), by aerosolization against *E. coli* in fresh pasta filata cheese. The use of an aerosol delivery system by nebulizer due to its availability, relative ease of application, higher surface coverage, production of small droplets in the form of a fine "mist", and greater antimicrobial penetration into cheese compared to other spray methods, was used in this research. The P solution was used as a dilution and carrier solution for aerosolization. In addition, due to the appropriate antimicrobial properties of Z-CDs, and P have the same antibacterial mechanisms, Z-CDs-P application and synergistic effects were carried out on fresh pasta filata cheese.

Materials and Methods

Materials

L. mesenteroides (ATCC 10830), and *E. coli* (ATCC 25922) were purchased from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. All culture media and dilution buffers were sourced from Quelab (Montreal, Canada). Sterile Polytetrafluoroethylene (PTFE) syringe filters were acquired from Millipore Inc (Massachusetts, USA), ethanol from Pars Alcohol, Shiraz, Iran, and other chemicals and reagents were procured from Sigma-Aldrich (St. Louis, MO, USA). Light-emitting diode 420nm (LED_{420nm}) lamps were supplied by Saba Light (Tehran, Iran).

Preparation of postbiotics

In our previous study, P powder derived from *L. mesenteroides* was produced in UF whey culture. To achieve this aim, *L. mesenteroides* was cultivated in De Man, Rogosa, and Sharpe broth (MRS) containing

2% sucrose at 28 °C for 24 h. The bacterial cells were then collected by centrifugation (4000 g for 15 min), washed twice with normal saline, and resuspended in sterile 0.1% peptone water. Following a series of 10-fold serial dilutions, cultures were prepared, and the suspension corresponding to a concentration of $10 \log_{10}$ CFU/mL was selected for subsequent procedures. The UF cheese whey was adjusted to pH 4.5 using 5N hydrochloric acid, autoclaved at 121 °C for 20 min to remove protein precipitates, centrifuged at 4000 g for 15 min to collect the supernatant, and then supplemented with 1% yeast extract after filtration through a 0.45 μ m syringe filter. Finally, the bacterial suspension ($10 \log_{10}$ CFU/mL) was added to whey medium and incubated (28 °C for 24 h), yielding a white powder characterized by gas chromatography-mass spectrometry (GC-MS) (Divsalar et al., 2025). Aerosolized P (300 mg/mL), and Z-CDs-P [P (300 mg/mL) and Z-CDs (5 mg/mL) at a ratio of 2:1] were tested on fresh pasta filata cheese stored at 4 °C for 18 days.

Z-CDs synthesis

In our previous study, we successfully synthesized white Z-CDs powder (Divsalar et al., 2023). Briefly, Z-CDs were synthesized hydrothermally using a 1% zinc acetate solution as both the carbon source and doping agent. The mixture was heated in a 300 mL reactor at 200 °C for 6 h, followed by centrifugation (15000 rpm, 30 min) and filtration through a 0.45 μ m PTFE syringe filter. The purified Z-CDs solution was then freeze-dried and stored under dark, sterile conditions at 4 °C.

Postbiotics characterization

Fourier transform infrared (FTIR) spectroscopy

To evaluate the functional groups and chemical structure of P powder, a Fourier transform infrared spectrometer (FTIR) with a Thermo Nicolet instrument, Nexus® 670 (USA) was used in the infrared range of 4000-450 cm^{-1} and a resolution of 2 cm^{-1} , and analyzed by the Omnic software (version 6.0; Thermo Electron, Madison, WI, USA).

Antioxidant potential

The antioxidant potential of the P was measured by DPPH (Divsalar et al., 2023) and FRAP (Benzie & Strain, 1999) assays.

Antimicrobial potential of postbiotics and Z-CDs

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The broth dilution method was used to determine the MIC and MBC of P and Z-CDs (CLSI, 2012). For this purpose, 160 μ L of Luria-Bertani broth (LB) was added to each well of the microplate. To achieve these final concentrations, 20 μ L of various P concentrations (12.5, 20, 25, 30, 35, 40, and 50 mg/mL) and Z-CDs (0.38, 0.5, 0.76, 1, and 1.52 mg/mL) were added to each well. Following the addition of *E. coli* suspension (20 μ L; $6 \log_{10}$ CFU/mL) to each well, the microplate was incubated at 37 °C for 24 h. Finally, MIC, defined as a 2 $\log_{10}$ CFU/mL reduction in the initial bacterial inoculum, and MBC, defined as a 3 $\log_{10}$ CFU/mL reduction in the initial bacterial inoculum, were determined by the colony count method. LB medium was used as the blank, LB medium containing bacterial suspension without P, Z-CDs was used as a positive control, and LB medium with P and Z-CDs without bacterial suspension served as the negative control.

Agar well diffusion test

These assays were conducted similarly to our previous work (Divsalar et al., 2023). Based on MIC results, 5MIC, 10MIC, and 15MIC of P (150, 300, and 450 mg/mL), based on 2.5MIC, 5MIC, and 10MIC of Z-CDs (1.25, 2.5, and 5 mg/mL), and Z-CDs-P ([the lowest concentration of Z-CDs that was not toxic and had antimicrobial effects: 5 mg/mL]) to 150, 300, and 450 mg/mL concentrations were added to each well. The diameter of the zone of inhibition (ZOI) was measured using a digital caliper after 24 h of incubation at 37 °C.

Antimicrobial photodynamic inactivation (aPDI) assay

The photodynamic properties of the Z-CDs were evaluated in *E. coli* (Divsalar et al., 2023). Z-CDs (final concentrations $\frac{1}{2}$ MIC, MIC, and 2 MIC) were mixed with *E. coli* suspension ($8 \log_{10}$ CFU/mL) in a 1:1 ratio and left in the dark for 15 min. The

suspension was then exposed to optimized LED lamp irradiation (420 nm wavelength; 15 W power; 120 min irradiation time with a distance of 12 cm) at 25 °C and 4 °C (Divsalar et al., 2023). Finally, 100 µL of each suspension was collected, diluted, and spread onto Tryptic soy agar (TSA) medium at 30-min intervals. After incubation for 24 h at 37 °C, the number of colonies was counted ($\log_{10}$ CFU/mL). The treatments were divided into four groups: a) suspension without Z-CDs and irradiation, b) suspension without Z-CDs and irradiation, c) suspension containing Z-CDs and without irradiation, and d) suspension containing Z-CDs and irradiation.

Cheese safety evaluation

Based on our previous work and the antimicrobial/cytotoxicity results, we selected concentrations of 300 mg/mL for P and 5 mg/mL for Z-CDs (Divsalar et al., 2023; Divsalar et al., 2025). Treatments: (a) cheese sample (control), (b) cheese sample with Z-CDs (5 mg/mL), (c) cheese sample with Z-CDs (5 mg/mL) under LED_{420nm} radiation, (d) cheese sample with P (300 mg/mL), (e) cheese sample with a combination of P (300 mg/mL) and Z-CDs (5 mg/mL) at a ratio of 2:1, and (f) cheese sample with a combination of P (300 mg/mL) and Z-CDs (5 mg/mL) under LED_{420nm} radiation at a ratio of 2:1. All samples were inoculated with *E. coli*.

To artificially inoculate samples with *E. coli*, cheeses were submerged in *E. coli* suspension (final 4 $\log_{10}$ CFU/mL) for 5 min, allowed to air dry for 15 min, and then transferred to the aerosolization chamber (3A, Lonato del Garda, Italy). Next, 10 mL of each treatment was poured into the nebulizer tank and sprayed by an aerosol method for 30 min at a speed of 0.35 mL/min; so that all parts of the cheese were sprayed and treated in the chamber (chamber volume: 7.5 liters, measuring 26.5 cm in length, 21 cm in width, and 17.5 cm in height). Cheese samples were placed in sterile plastic containers. Then, they were stored in a refrigerator at 4 °C for 18 days, and microbial evaluation was conducted on days 0, 3, 6, 9, 12, 15, and 18. To this end, 5 g of each sample was accurately weighed and homogenized in 45 mL of peptone water (0.1%) using a stomacher for 2 min at 260 rpm. Subsequently, 10-fold serial dilutions of the homogenized samples were prepared. Aliquots

of each dilution were plated onto MacConkey agar plates using the surface plating method and incubated at 37 °C for 48 h (Al-Nabulsi et al., 2020; Dannenberg et al., 2017).

Statistical analysis

All experiments were conducted in triplicates. Statistical analyses were performed using GraphPad Prism version 9.01 (GraphPad Software, USA). Analysis of variance (ANOVA) was performed on all collected data, followed by Duncan's multiple range test ($P < 0.05$).

Results and Discussion

Fourier transform infrared (FT-IR) spectroscopy

Evaluation of chemical bonds, achieving a more comprehensive description and identification of functional groups are studied by FTIR (**Fig. 1**). Each peak represents the absorption in the corresponding wavenumber and is created by a specific chemical bond (Hou et al., 2018). The main P spectral bands at 3384 cm^{-1} (stretching of O-H groups), 2917 cm^{-1} (C-H bond stretching vibrations), 1613 cm^{-1} (stretching of C=O groups), 1391 cm^{-1} (CH_2 vibration and COOH bending), 1241 cm^{-1} (out-of-plane bending CH_2), which are related to the organic acid groups and polysaccharides. Additionally, the weak absorption peaks located at 883 and 776 cm^{-1} can be related to α -D-glucan structure, which agrees with previous studies (Rasouli et al., 2021; Siedler et al., 2019; Şirin, 2023). In fact, the characterized functional groups such as hydroxyl, carbonyl, and carboxyl on the glucan backbone are key contributors to its antioxidant and antimicrobial activity (Şirin, 2023).

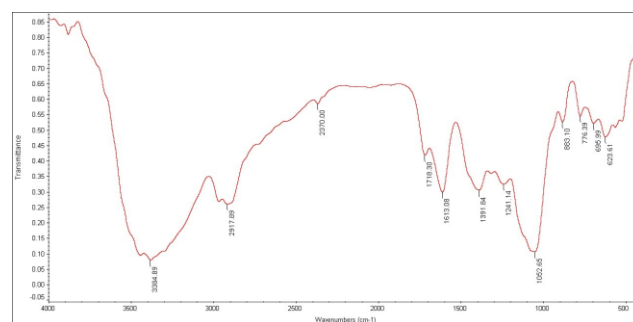


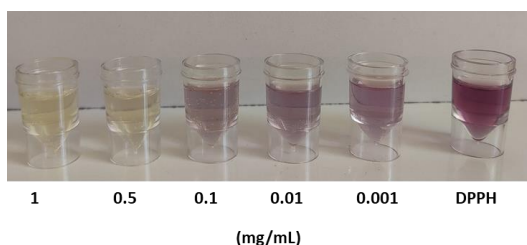
Figure 1. FTIR spectrum of synthesized postbiotics.

Antioxidant potential of postbiotics

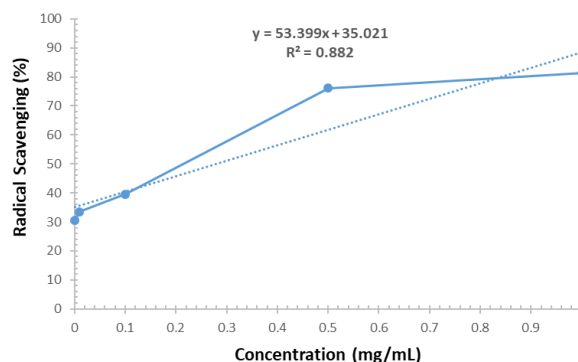
The postbiotics radical scavenging potential were determined (Divsalar et al., 2023). The purple color of the methanolic DPPH solution changed after adding different P concentrations (0.001, 0.01, 0.1, 0.5, and 1 mg/mL) during reaction (**Fig. 2A**). Obviously, the color changes may be due to the presence of a large number of carboxyl groups (Htwe et al., 2019). As reported in previous studies, the antioxidant potential of postbiotics derived from Lactic acid bacteria (LAB) also depends on the bacterial strains (Xing et al., 2015). On the other

hand, some bioactive substances, including EPS, enzymes, Mn^{+2} ions, and bioactive peptides, can be considered responsible for postbiotics' antioxidant activity (Tang et al., 2017). Enzymes present in postbiotics play an essential role in oxidative stress response (Daniela et al., 2010). Radical scavenging was dose-dependent; so, it was increased in concentrations from 0.001 to 1 mg/mL. P display strong antioxidant activity, with an IC_{50} of 0.28 mg/mL (**Fig. 2B**), due to their interaction with free radicals through hydroxyl, carbonyl, and carboxyl functional groups (Şirin, 2023).

(A)



(B)



(C)

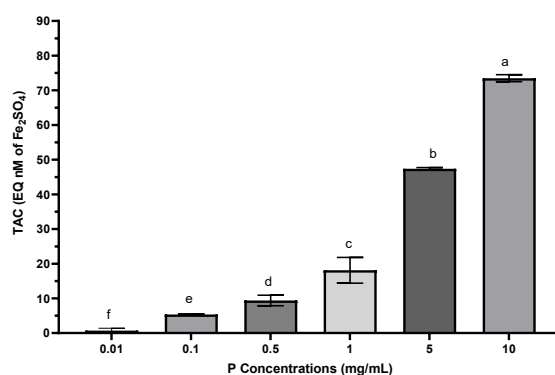


Figure 2. Antioxidant activity of P performed by DPPH (A, B), and FRAP (C) tests. Values with different letters are significantly different ($p \leq 0.05$) from control sample.

The obtained IC_{50} was significantly higher than the IC_{50} of BHT (3.8 μ g/mL) and ascorbic acid (4.3 μ g/mL) (Ghorbani et al., 2022). Postbiotics prevent

excess ROS generation and increase the resistance of cells against oxidative stress by modulating the intracellular antioxidant system (Chávarri Hueda &

Diez-Gutiérrez, 2024). The scavenging potential of P from *L. mesenteroides* and *Saccharomyces cerevisiae* was obtained from 20 to 28% by the DPPH method (de Oliveira Coelho et al., 2019). Metabolites of *L. mesenteroides*, like levan and glucan, showed antioxidant and anticancer activity (Li et al., 2020).

The FRAP assay was used to evaluate the total antioxidant capacity (TAC) of various concentrations (0.01, 0.1, 0.5, 1, 5, and 10 mg/mL) of P through the reduction of the Fe^{3+} -TPTZ complex to Fe^{2+} by blue color. The FRAP and DPPH tests provide important additional information about the postbiotics antioxidant mechanism (Cömert & Gökmen, 2018). The study found that increasing P concentrations from 0.01 to 10 mg/mL boosts antioxidant potential, with a TAC of 73.5 nM $\text{Fe}_2\text{SO}_4/\text{L}$ (Fig. 2C). According to our previous research, GC-MS analysis of *L. mesenteroides* P identified several compounds that may explain their antioxidant activity (Divsalar,

Moradi, et al., 2025). Fatty acid methyl esters like 6-octadecanoic acid methyl ester can inhibit free radicals and lipid peroxidation by stabilizing lipid membranes (Pinto et al., 2017). Hydroxy fatty acid esters, such as 2,3-hydroxypropyl dodecanoic acid ester, contribute by donating hydrogen atoms to neutralize reactive oxygen species. Aromatic amines like 4-ethoxy-N-ethyl-N-methyl-benzenamine may donate electrons to stabilize radicals, though this needs further confirmation. Benzoic acid, a phenolic compound, also enhances antioxidant defense via radical scavenging and metal chelation. Altogether, these compounds likely act synergistically to produce the strong antioxidant effect observed in the P (Isamura et al., 2022). The antioxidant activity of postbiotics is confirmed through the electron transfer reaction mechanism and acts as a reducing agent by transferring a hydrogen to the Fe^{3+} complex through functional groups such as hydroxyl and carboxylic (Bisson et al., 2023).

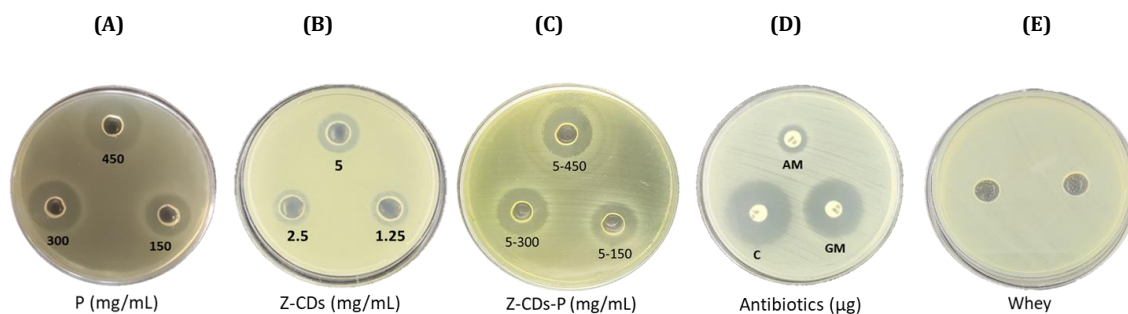


Figure 3. Antimicrobial activity of P (A), Z-CDs (B), Z-CDs-P (mg/mL) (C), antibiotics (μg) (D) and whey (E) against *E. coli* according to agar well diffusion method expressed as a diameter of zone of inhibition (mm) around each well.

The antioxidant effects of EPS from *L. mesenteroides* were investigated, where 20 mg/mL of EPS led to scavenging of 20% of free radicals, which may be attributed to the presence of dextran. These results were consistent with the FRAP test (Bisson et al., 2023; Yilmaz et al., 2022). Many research studies have shown that postbiotics can reduce oxidative stress both in vitro and in vivo (Villarini et al., 2008). The postbiotics derived from *Lactobacillus plantarum* can prevent DNA damage and aflatoxin B1 absorption through their antioxidant activity (Khani et al., 2023).

Antimicrobial potential of postbiotics

The study evaluated the P's antimicrobial activity of *L. mesenteroides* by microbroth and agar well diffusion assay against *E. coli*. The MIC/MBC was acquired at 30 mg/mL. The findings showed that the average ZOI of P at 150, 300, and 450 mg/mL concentrations against *E. coli* were dose-dependent (Fig. 3A). Antibiotics and whey were considered as positive and negative controls, respectively (Fig. 3D and 3E). Whey, as the culture medium used to prepare P, did not show antimicrobial activity against *E. coli* (Table 1). Based on our previous study, P were non-toxic up to 500 mg/mL on the L929 cell line.

Generally, postbiotics have many advantages over probiotics, including longer shelf life, lower temperatures, easy transportation, storage, and safety; they are recognized as safe antimicrobial compounds (Rao et al., 2023). *L. mesenteroides* is one of the common probiotics, and its metabolites have broad antibacterial activity against many food pathogens such as *E. coli*, *L. monocytogenes*, *S. aureus*, and *Pseudomonas aeruginosa* (Rao et al., 2023; Touthik et al., 2022). The antimicrobial potential of *L. mesenteroides* is attributed to substances such as short-chain fatty acids, enzymes, EPS, peptides, organic acids, vitamins, plasmalogens, teichoic acids, hydrogen peroxide, and bacteriocins. Based on our previous GC-MS analysis profile of P, the antibacterial activity of 6-octadecanoic acid methyl ester disrupts bacterial membranes, increasing permeability and causing cell lysis. 2,3-hydroxypropyl dodecanoic acid ester, due to its amphiphilic nature, interferes with membrane enzymes and nutrient transport, inhibiting bacterial growth. The aromatic amine 4-ethoxy-N-ethyl-N-methyl-benzenamine may act by inducing oxidative stress or disturbing bacterial metabolism (Casillas-vargas et al., 2021). Benzoic acid inhibits microbial growth by lowering intracellular pH and disrupting enzymatic activity. Together, these compounds act synergistically to enhance the antibacterial effects of the P through membrane disruption, metabolic interference, and acidification (Divsalar et al., 2025). However, researchers attribute the inhibitory activity of postbiotics to the action of organic acids (such as acetic acid, benzoic acid, and lactic acid), hydrogen peroxide, and bacteriocin (Touthik et al., 2022). Postbiotics of *L. mesenteroides* had an acidic pH (pH: 3.5), which led to cell membrane damage by organic acids. It is considered one of the most important antibacterial mechanisms of postbiotics, effectively destroying Gram-negative and Gram-positive pathogens (Atassi et al., 2019). Protonated organic acid molecules diffuse directly through the cell membrane and inhibit cell growth. The presence of fatty acids can help organic acids to pass through membranes. Organic acids can also disrupt cell wall synthesis in prokaryotes (Zhang et al., 2021).

On the other hand, bacteriocins produced by *L. mesenteroides*, such as mesentericin, carnosine, and leucosin, are active against food pathogens; mesentericin is relatively resistant to heat (up to

100°C) and pH (4.5). The commercial application of mesentericin in the stability development of dairy products has been reported (Zhang et al., 2021). Bacteriocins employ common mechanisms: increasing membrane permeability by forming a complex of bacteriocin monomers, destabilizing the cytoplasmic membrane, and leaking vital components (Hussein et al., 2022). Dextran and xanthan from *L. mesenteroides* have important technological and antimicrobial properties (Bisson et al., 2023; Hussein et al., 2022). The mechanism of EPS antimicrobial activity involves interfering with cellular homeostasis processes, leading to cell destruction, inhibition of cell division, and DNA damage (He et al., 2010). However, some substances, such as hydrogen peroxide in lyophilized form, are lost, so it seems that antimicrobial activities are largely related to organic acids and bacteriocins (Moradi et al., 2021). The peptides and organic acids present in postbiotics of *L. mesenteroides* were confirmed to have antibacterial activity against *E. coli* and *Salmonella paratyphi A* compared to *L. monocytogenes* and *S. aureus* (Özogul et al., 2017). Another study verified the antibacterial activity of *L. mesenteroides* postbiotics against various pathogens such as *E. coli*, *L. monocytogenes*, *S. aureus*, and *S. Typhimurium*, which were related to bacteriocins, organic acids, and other antimicrobial substances (Koo et al., 2015).

Antimicrobial potential of Z-CDs

Antimicrobial activity

According to the obtained results, the Z-CDs were found to be spherical, crystal-shaped, and ranged in size from 2 to 20 nm. The presence of C, O, and Zn in the CDs structure were confirmed using XPS, and the presence of hydroxyl and carboxyl groups was confirmed by FTIR analysis. Antimicrobial and photodynamic mechanisms of action were comprehensively examined in both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Salmonella Typhimurium*) bacteria. The antioxidant properties of the CDs were evaluated using DPPH (IC₅₀ 1.14 mg/mL) and FRAP (total antioxidant capacity 46 nM of Fe₂SO₄/L) assays. In addition, the prepared CDs showed no cytotoxicity in the L929 cell line at concentrations below 5 mg/mL (Divsalar et al., 2023).

The study measured the antimicrobial activity of Z-CDs by microbroth and agar well diffusion assay against *E. coli*. The MIC/MBC was acquired at 0.5 mg/mL. The sensitivity of microorganisms to CDs was evaluated based on the ZOI. The average ZOI of

Z-CDs against *E. coli* is shown in **Figure 3B** and **Table 2**. Based on the sensitivity classification of nanomaterials (Zanetti et al., 2019), Z-CDs at 1.25, 2.5, and 5 mg/mL concentrations were in extremely sensitive, very sensitive, and sensitive categories.

Table 1. Antimicrobial activity (zone of inhibition; mm) of postbiotics against *E. coli*.

P (mg/mL)			Control (Antibiotics)		
150	300	450	Ampicillin (10 µg)	Gentamicin (10 µg)	Chloramphenicol (30 µg)
18.34 ± 0.31 ^b	22.32 ± 0.48 ^a	23.35 ± 0.52 ^a	12.13 ± 0.17	23.70 ± 0.26	25.34 ± 0.32

*Within each row, values with different letters are significantly different ($p \leq 0.05$).

Table 2. Antimicrobial activity (zone of inhibition; mm) of Z-CDs against *E. coli*.

Z-CDs (mg/mL)			Control (Antibiotics)		
1.25	2.5	5	Ampicillin (10 µg)	Gentamicin (10 µg)	Chloramphenicol (30 µg)
13.65 ± 0.40 ^b	14.46 ± 0.36 ^b	17.52 ± 0.46 ^a	12.13 ± 0.17	23.70 ± 0.26	25.34 ± 0.32

*Within each row, values with different letters are significantly different ($p \leq 0.05$).

Table 3. Antimicrobial activity (zone of inhibition; mm) of Z-CDs-P against *E. coli*.

Bacterial strain	Z-CDs-P (mg/mL)		
	5-150	5-300	5-450
<i>E. coli</i>	19.36 ± 0.32 ^b	24.17 ± 0.29 ^a	25.32 ± 0.23 ^a

*Within each row, values with different letters are significantly different ($p \leq 0.05$).

Several factors are responsible for CDs' antibacterial activity, such as microbial strains, CDs concentrations, and their intrinsic properties (particle size, surface functional groups, and charge) (Li et al., 2022). Due to the presence of anionic sites, the bacterial peptidoglycan layer is porous, thus facilitating the electrostatic interactions of the CDs. For example, the zeta potential of *E. coli* is -4.95 mV (Kousheh et al., 2020). CDs with negative and neutral surface charge have weaker antimicrobial activity than positive surface charge (Moradi et al., 2022). The differences in CDs' antimicrobial activities are due to the variation of CDs' charge and different sources. Also, the bacterial cell charge may play an important role in their antimicrobial effects. For

example, negatively charged CDs (-24.5 mV) have antibacterial activity against *E. coli* (-4.95 mV), which may be due to electrostatic repulsion (Kousheh et al., 2020). CDs with zeta potentials of -19.5, +27.6, and +0.94 mV showed different antibacterial activity against *E. coli* (Bing et al., 2016). The zeta potential of the synthetic Z-CDs was -11.4 mV, confirming the presence of negatively charged groups on the surface of CDs (Divsalar et al., 2023). Gram-positive bacteria are sensitive to ROS; however, Gram-negative bacteria have a more complex cell wall, which leads to their resistance (Ghorbani et al., 2022). CDs will interact with specific sites in the cell membrane of bacteria (Zhao et al., 2019). Metal doping changes the surface charge and overall properties of CDs and

thus affects their antibacterial activity (Long et al., 2021). As a result, zinc doping can positively affect the performance of CDs by increasing the active sites and critical properties such as photocatalytic activity, doping efficiency, and bacteriostatic effect. Due to the favorable overlap between the photoluminescence ranges of zinc and CDs, there is a possibility of energy and charge transfer between them. Therefore, Zn-doped CDs show high doping efficiency, which leads to beneficial effects on their bacteriostatic activity (Das et al., 2018). ROS generation and the release of Zn^{2+} ions damage the cell wall and lead to cell death. These metal oxides act as effective antibacterial agents due to ROS generation, which includes O_2^- , OH^- , and H_2O_2 (Ijaz et al., 2020).

Antimicrobial potential of Z-CDs-P

The agar well diffusion method was used to investigate the antimicrobial Z-CDs-P effects: Z-CDs (the lowest concentration that was not toxic and had antimicrobial effects: 5 mg/mL) and P (150, 300, and 450 mg/mL) (**Fig. 3C**). According to **Table 3**, Z-CDs-P treatments showed sensitivity to *E. coli*. Since the mechanism of P and Z-CDs is primarily the cell wall permeability, the effectiveness and synergism of them were to be expected (Zhang et al., 2021). Subsequently, the ZOI of P at 300 and 450 mg/mL concentrations were not significantly different; the combination of P at 300 mg/mL and Z-CDs at 5 mg/mL was chosen for cheese application.

Antimicrobial photodynamic inactivation (aPDI) assay

The aPDI performance of Z-CDs as PS was examined for factors related to Z-CDs concentrations: at 0.25, 0.5, and 1 mg/mL, irradiation (time (0–120 min), temperature (25 °C and 4 °C), and light source ($\text{LED}_{420\text{nm}}$ 15w) with an initial *E. coli* concentration of $7 \log_{10}$ CFU/mL.

Based on **Figure 4A**, *E. coli* was inhibited at a 1 mg/mL concentration, after 90 min of exposure to $\text{LED}_{420\text{nm}}$ at 25 °C. Also, this trend was observed at 0.25 and 0.5 mg/mL concentrations after 120 min. A decrease of around 2.09 and 2.19 $\log_{10}$ CFU/mL was observed in *E. coli* suspension irradiated with $\text{LED}_{420\text{nm}}$ and containing 1 mg/mL concentration after 120 min, respectively. *E. coli* had an increasing trend in control and reached $9.39 \log_{10}$ CFU/mL. Inhibition of *E. coli* occurred at 1 mg/mL concentration after 60 min of exposure to $\text{LED}_{420\text{nm}}$ at 4 °C, while *E. coli* was inhibited at 0.5 and 0.25 mg/mL concentration, after 90 and 120 min, respectively. A decrease of 2.58 and 2.57 $\log_{10}$ CFU/mL was shown in bacterial suspension with $\text{LED}_{420\text{nm}}$ and bacterial suspension at 1 mg/mL concentration after 120 min, respectively. *E. coli* was increased and reached $9 \log_{10}$ CFU/mL in the control (**Fig. 4B**).

The antibacterial performance of Z-CDs illustrated ROS generation under $\text{LED}_{420\text{nm}}$ and the effect of temperature at 4 °C, which can be considered due to the sensitivity of *E. coli* to cold temperature shock. Antimicrobial mechanisms of PDI against bacteria include cell structure disruption, macromolecule

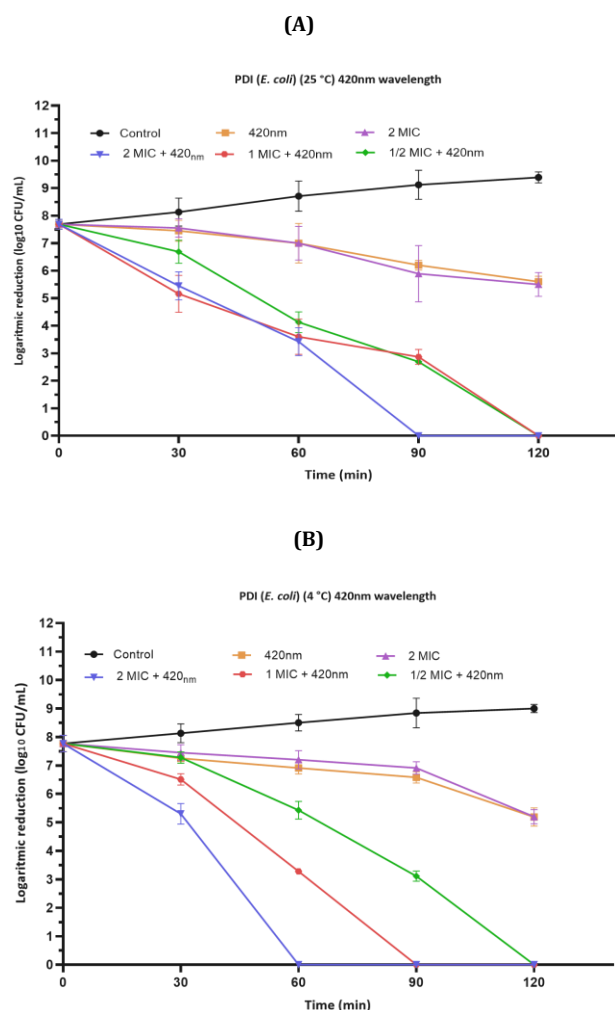


Figure 4. Antimicrobial photodynamic inactivation of Z-CDs graphs against *E. coli* at $\text{LED}_{420\text{nm}}$ for time (0–120 min) at 25 °C (A) and 4 °C (B).

oxidation, such as proteins, extracellular DNA, and polysaccharides, which are influenced by several factors such as light intensity, PS concentrations, physicochemical properties, cell wall structure, and temperature. The lipopolysaccharide layers of Gram-negative bacteria play an important role in PDI resistance (Sheng et al., 2022). LED radiation at 400–460nm effectively inactivates microorganisms due to the stimulation of endogenous porphyrin molecules (Jiang et al., 2019). In the presence of 0.5 µg/mL methylene blue, the protein and nucleic acids leakage in *L. monocytogenes* increased proportionally to the irradiation time (Lin et al., 2012). Exposure of porphyrin-based PS to the blue light decreased the respiratory efficiency of *E. coli* rapidly (Pudziuvyte et al., 2011). Co-culture of *E. coli* and *S. aureus* containing polyacrylamide-polydopamine/AgCl₂ hydrogels and xenon lamp irradiation significantly increased total protein release and beta-galactosidase cytoplasmic enzyme (Mao et al., 2019). The effects of temperature on PDI can be related to the bacterial structure and its resistance: *E. coli* is not resistant to cold temperature stress, but *L. monocytogenes* produces cold shock proteins (Gandhi & Chikindas, 2007).

Cheese safety evaluation

Postbiotics were non-toxic up to 500 mg/mL, and the Z-CDs-P treatment extended the shelf life from about 5.5 to over 11 days, highlighting its potential for dairy preservation (Divsalar et al., 2025). **Figure 5** reports the inhibitory effect of Z-CDs, P, and Z-CDs-P against *E. coli* during 18 days of storage at 4°C in fresh pasta filata cheese. During 18 days of cold storage, the bacterial population in the control sample decreased from 4.20 to 2.67 log₁₀ CFU/g. A similar trend was observed when Z-CDs alone and Z-CDs 420nm were used, reaching 2.02 and 2 log₁₀ CFU/g at the end of storage, respectively. As expected, the highest inhibitory effects were observed in the P, Z-CDs-P and Z-CDs_{420nm}-P treatments; so that *E. coli* reached 1 log₁₀ CFU/g on days 18, 14, and 12, respectively. The decrease in *E. coli* in the control group can be attributed to fatigue caused by the microorganism's attempt to multiply at low temperatures (Divsalar et al., 2018). Postbiotics metabolites and Z-CDs synergistically enhance both the microbial safety and structural stability of fresh pasta filata cheese, particularly

against *E. coli*. The postbiotics, as confirmed by GC-MS analysis, contains functional compounds such as 6-octadecanoic acid methyl ester, 2,3-hydroxypropyl dodecanoic acid ester, 4-ethoxy-N-ethyl-N-methyl-benzenamine, and benzoic acid, in addition to organic acids, hydrogen peroxide, and bacteriocin-like peptides. These metabolites collectively inhibit *E. coli* through multiple mechanisms, including lowering the environmental pH, disrupting bacterial membranes, inducing oxidative stress, and impairing energy metabolism. Fatty acid esters increase the hydrophobicity of the cheese matrix, weakening bacterial membranes and restricting nutrient uptake, while benzoic and lactic acids acidify the cytoplasm and disrupt proton gradients. Hydrogen peroxide and bacteriocin-like peptides further intensify oxidative and membrane damage, leading to cell lysis (Zapaśnik, 2022). Moreover, Z-CDs, owing to their photocatalytic and redox-active properties, produce ROS under irradiation and interact electrostatically with bacterial membranes, causing additional structural and genetic damage (Divsalar et al., 2023).

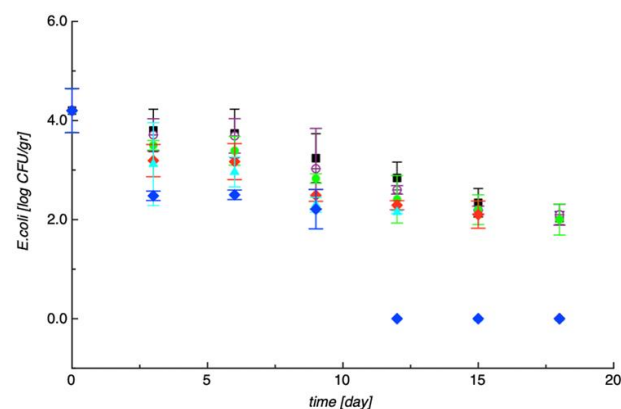


Figure 5. Time course during refrigerated storage of *E. coli*. Control cheese (black); cheese treated by Z-CDs (violet); cheese treated by Z-CDs_{420nm} (green); P (red); Z-CDs-P (sky-blue); Z-CDs_{420nm}-P (blue).

Research investigated the application of aerosolized P derived from *L. mesenteroides*, Z-CDs, and Z-CDs-P to enhance the shelf life of fresh pasta filata cheese. Treatments included P (300 mg/mL), Z-CDs (5 mg/mL), and their mixture, which were applied by aerosolization and stored at 4 °C for 18 days. The shelf life, based on microbiological and sensory

analyses, increased from 5.5 days in the control to over 9 days with Z-CDs-P and 11 days with Z-CDs_{420nm}-P. These findings demonstrate that the aerosolized Z-CDs-P system provides a multifunctional and efficient strategy for preserving freshness and safety of fresh cheese in the dairy industry (Divsalar et al., 2025). In a recent study, developed CQDs from whey through a hydrothermal synthesis and integrated them into alginate-based coatings for fiordilatte cheese. Incorporation of CQDs at concentrations of 2500–5000 mg/L markedly enhanced both microbial stability and sensory attributes of the cheese, effectively doubling its shelf life compared to the uncoated control, which deteriorated within 3.5 days. This innovative and eco-friendly approach underscores the promising role of whey-derived CQDs as functional nanomaterials for extending the freshness and quality of dairy products (Lacivita et al., 2023). In an effort to enhance the preservation of high-moisture mozzarella cheese, the study explored the use of carboxymethyl cellulose (CMC) coatings, both alone and in combination with natamycin, during 8 days of storage at 7 °C. The CMC coating independently led to a marked reduction in all microbial populations, while the addition of natamycin was particularly effective against molds and yeasts, decreasing their counts by 0.6–0.9 log₁₀ CFU/g at concentrations of 0.05–0.5%. Unlike the control samples, which reached spoilage by day 4, the coated cheeses maintained acceptable microbiological and sensory quality up to day 8, thereby doubling the shelf life of mozzarella (Azhdari & Moradi, 2022).

Conclusion

In this study, the effect of P, Z-CDs, and Z-CDs-P as additives were investigated by aerosolization in fresh pasta filata cheese. Z-CDs showed appropriate antimicrobial and photodynamic properties. The results of this study showed that P and Z-CDs solutions individually improved the safety of fresh pasta filata cheese, but the Z-CDs-P was more effective. It has led to the reduction of *E. coli* during 18 days of storage at 4°C, compared to the control. According to antimicrobial and chemical tests, these two compounds can be a suitable additive for cheese. Therefore, their use as antimicrobial agents provide a favorable performance in food products. However, the study faced some limitations, including its lab-

scale design, short storage period, and the need for further safety and regulatory evaluation of carbon dots. Postbiotics variability and the challenges of working with complex dairy matrices also require attention. The aerosolization process needs optimization for consistent industrial use. Future research should focus on scaling up the system, optimizing aerosolized formulations for industrial applications, evaluating their long-term stability and safety, and elucidating the synergistic mechanisms between P and nanostructured materials such as Z-CDs, and testing performance in real dairy processing to develop safe and sustainable clean-label preservation technologies. These efforts could lead to the development of next-generation intelligent sprays and coatings that support sustainable, clean-label preservation in the dairy industry. Expanding this approach to other food systems may enable the creation of natural and eco-friendly preservation strategies that reduce reliance on synthetic preservatives while enhancing food safety and shelf life. Moreover, investigating the molecular interactions between Z-CDs and postbiotics compounds could provide valuable insights into their combined antimicrobial and antioxidant actions. Overall, this work aligns with the global movement toward sustainable, safe, and consumer-friendly food preservation technologies.

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Conflicts of interest

None.

Disclaimer

None.

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