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Title: Evaluation of aerosolized form of *Leuconostoc mesenteroides* postbiotics and Zinc-doped carbon quantum dots in preservation of Mozzarella cheese

Abstract

High-Moisture Mozzarella Cheese (HMMC) is one of the most popular cheeses in the world. It is made in different sizes and packaged in different preservative solutions. HMMC has a short shelf life. In this study, white postbiotics from *Leuconostoc mesenteroides* were prepared in ultrafiltration cheese whey. According to the DPPH (IC₅₀ 0.28 mg/mL) and FRAP methods (Total antioxidant capacity 73.5 nM) the antioxidant activity were confirmed. The dose-dependent properties of postbiotics against *E. coli* were evaluated at concentrations of 150, 300, and 450 mg/mL using the agar well diffusion method; so that the ZOI of Z-CQDs concentrations (150, 300 and 450 mg/mL) was 18.34±0.31, 22.32±0.48 and 23.35±0.52 mm, respectively. In addition, the MTT assay results showed that the different postbiotics concentrations (0.5, 50, 100, 250 and 500 mg/mL) were non-toxic in L929 cell line. In this study, carbon quantum dots (CQDs) with a white appearance were synthesized from zinc acetate by hydrothermal method. The Z-CQDs were characterized by particle size analysis, electronic microscopy, UV-visible spectroscopy, FTIR and XPS spectroscopy. The antioxidant properties of the CQDs were evaluated using both DPPH (IC₅₀ 1.14 mg/mL) and FRAP (Total antioxidant capacity 46 nM) assays. According to the MTT assay, the Z-CQDs were non-toxic at a concentration of 5 mg/mL in the mouse fibroblast cell line (L929). Z-CQDs (~9.8 nm) showed dose-dependent antimicrobial activity against *Escherichia coli*; so that the ZOI of Z-CQDs concentrations (1.25, 2.5 and 5 mg/mL) was 13.65±0.40, 14.46±0.36 and 17.52±0.46 mm, respectively. In addition, observations. Furthermore, SEM observations confirmed this activity. The photodynamic properties of Z-CQDs as a photosensitizer were examined with a focus on Z-CQDs concentration, irradiation and temperature [i.e., time (0–120 min), temperature (4 and 25 °C), and LED lamps (420 nm wavelength)]. It was evident that *E. coli* was susceptible to photodynamic treatment at 420 nm when both temperatures were utilized. Remarkably, the bacterial cell membranes were significantly destroyed by Z-CQDs and photodynamic treatments. According to the results, postbiotics (300 mg/mL), Z-CQDs (5 mg/mL), and postbiotics-Z-CQDs (300/-5 mg/mL) solutions were sprayed as additives on HMMC. The results showed that postbiotics, Z-CQDs, and their combination, improved the shelf life of HMMC; the combination (postbiotics-Z-CQDs 420nm) was more effective than separation. During storage, lactic acid bacteria (1.38 log₁₀ CFU/g), aerobic-anaerobic mesophilic bacteria (1.69 log₁₀ CFU/g), psychrophilic bacteria (2.16 log₁₀ CFU/g), coliforms (2.14 log₁₀ CFU/g), yeast-mold (1.90 log₁₀ CFU/g) and *Pseudomonas* spp. (2.97 log₁₀ CFU/g) over 18 days at 4 °C compared with the control. The pH index also significantly were lower than the control (p ≤0.05), which could be due to the acidic nature of the postbiotics and Z-CQDs. The Weight loss (%) values in all treatments were lower than that of the control, confirming the positive effect of the spraying

method. In terms of sensorial indicators, postbiotics-Z-CQDs420nm increased the overall acceptance of HMMC samples. In particular, the aromatic and smoky compounds of Z-CQDs created a smoky odor in HMMC, and its tissue improved owing to the presence of exopolysaccharide compounds of postbiotics. Based on the antimicrobial, chemical, and sensorial assessments, postbiotics from *L. mesenteroides* and Z-CQDs could be a desirable additive in HMCC to prevent adverse sensorial effects.

Keywords: Cell-free supernatant, Carbon dots, Active packaging, Photodynamics, Nanomaterials.