EVALUATION OF ANTIBACTERIAL ACTIVITY OF COPPER-BASED NANOMATERIALS FOR FOOD PACKAGING APPLICATIONS

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Abstract – The antibacterial activity of a novel nanocomposite film based on polylactic acid film coated by a sol-gel processing with copper nanoparticles (Cu NPs) at different concentrations (0-0.5-1-10-100-500-1000-2000 ppm) was tested against *Salmonella enterica* serovar Typhimurium, a pathogenic bacterium frequently associated with human salmonellosis outbreaks related to the consumption of contaminated pig meat and products thereof. Antibacterial activity was determined quantitatively using a viable cell count method. The film coated with the sol containing Cu NPs at 500 ppm provided a significant bacterial reduction (1.38±0.55 logCFU/cm^2; P<0.05). Data of copper release from the nanocomposite film were matched with bioactivity test.

Key Words – Copper nanoparticles, nanocomposite film, *Salmonella enterica* serovar Typhimurium.

I. INTRODUCTION

Salmonella is the most frequently reported cause of food-borne outbreaks in European Union; *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently related to human salmonellosis. Human *S.* Typhimurium cases are mostly associated with the consumption of contaminated pig meat and products thereof [1]. Antimicrobial active packaging systems release antimicrobial agents into the food surface and are helpful to minimize the growth of microorganisms, improving food safety and extending shelf life. Some metals have been recognized as antibacterial agents. Copper has been shown to provide good antimicrobial activity against a number of microorganisms. Such a property is improved by the synthesis of copper nanostructures: the biological effectiveness of copper nanoparticles (Cu NPs) is demonstrated to be significantly higher than the bioactivity of the bulk metal itself, although a strain-specific variability was observed [2; 3]. Therefore, the aim of this study was to assess the antibacterial activity against a *Salmonella enterica* serovar Typhimurium strain of a nanocomposite film based on polylactic acid (PLA) film coated with Cu NPs by a sol-gel processing.

II. MATERIALS AND METHODS

Tetraethoxysilane (TEOS), (3-glycidoxypropyl)trimethoxysilane (Glymo-98%), PLA film (Taghleef Industries, Italy) and neutral colloidal suspension of Cu NPs (Clusternanotech, UK) at Cu nominal content of 15.000 ppm were used to prepare the nanocomposite film. The active coatings were obtained by a modification of a sol–gel method previously reported [4]. All the sols were produced starting from an aqueous solution of silicon oxide precursors: TEOS/Glymo molar ratio=1; total silicon concentration: 0.5 M. Different amounts of Cu NPs colloidal suspension were added to obtain final concentrations of 0.5-1–10-100-500–1000-2000 ppm. A sol without Cu NPs was produced and used as negative control. Before the deposition of the sols, the PLA films were treated with cold plasma (absolute pressure range of 0.1–1 mbar, 20s in air at 54 W). The coatings were obtained by using a hand coater with a nominal deposit of 12 ml/m^2; nanocomposite films were dried in oven at 40°C for 10 minutes. Cu release from nanocomposite film (5x5cm piece) immersed in 20ml of distilled water (24h at 20°C; RH 50%) was determined by an inductively coupled plasma atomic emission spectrophotometer. *Salmonella enterica* serovar Typhimurium ATCC-14028 stock culture was incubated in 5 ml of tryptic soy broth (TSB) at 37 °C overnight. Bacteria suspension was serially diluted in TSB to obtain inoculum of approximately 1×10^6 colony-forming units (CFU)/ml. Each 0.5 ml of the inoculum was spot-inoculated on one side of the nanocomposite films

(5×5cm piece) placed into sterile petri dishes and incubated 24h at 20°C, RH 50%. Bacteria were recovered with phosphate-buffered saline (pH 7.3), 10-fold serially diluted and incubated at 30°C for 24h to quantify the number of viable bacteria by conventional plate count. All results were expressed in logCFU/cm^2. The experiment was replicated three times with three samples each time (n=9).

III. RESULTS AND DISCUSSION

The highest antibacterial activity against *Salmonella enterica* serovar Typhimurium reference strain was observed in the film coated with the sol containing Cu NPs at 500 ppm (Figure 1), with a reduction of 1.38±0.55 logCFU/cm^2 compared to the negative control (P<0.05). Change in the aggregation state of metal nanoparticles might explain the reduced antibacterial activity of Cu NPs at higher concentrations. Bioactivity was not clearly in relationship with the Cu released by nanocomposite film as shown in Table 1.

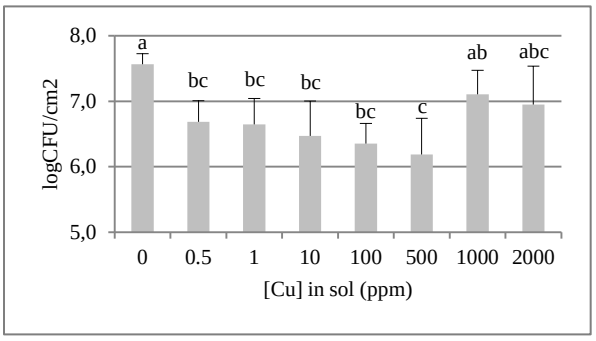

Figure 1. Bacterial cell count measured in the nanocomposite films (Scheffé post hoc test; P<0.05)

Table 1. Copper release from the nanocomposite films (n.d.=not detected; results are expressed as mean±SEM)

[Cu] in sol (ppm)	[Cu] in 5x5cm film piece (μg)	
	Nominal	*Released*
0	0.000	n.d.
0.5	0.015	n.d.
1	0.030	n.d.
10	0.300	0.10 ± 0.06
100	3.000	0.68 ± 0.20
500	15.00	7.32 ± 0.60
1000	30.00	7.25 ± 0.98
2000	60.00	17.7 ± 4.24

IV. CONCLUSION

The novel nanocomposite film based on PLA film coated by a sol-gel processing with Cu NPs has an interesting potential for an antimicrobial active food packaging. This approach might be compatible with a large-scale manufacturing of nanocomposite PLA films, as required by packaging technology.

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