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Short Communication

Decontamination of fresh-cut cucumber slices by a combination of a modified chitosan coating containing carvacrol nanoemulsions and pulsed light

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ABSTRACT

In this study, the impact of the combination of pulsed light (PL) treatments with antimicrobial coatings, consisting of modified chitosan suspensions incorporating carvacrol nanoemulsions, was investigated on the decontamination of fresh-cut cucumber slices.

The upper surface of the cucumber slices, with or without the coating deposition, was inoculated with *Escherichia coli* ATCC 26 to reach a final concentration of 10^7 CFU/g of the vegetable. PL treatments were conducted at different fluence (4, 8, and 12 J/cm^2) on the inoculated surface of cucumber slices.

Results showed that the microbial reduction was only marginally affected by the coating formulation. A slight increase was observed when the carvacrol nanoemulsions were embedded in the chitosan matrix, but microbial reduction levels remained always below 1 log cycle. In contrast, the different PL treatments resulted in a statistically significant increase in inactivation with increasing the treatment fluence, reaching 2.6 log cycles at the maximum fluence.

Remarkably, the combination of the antimicrobial coating with the most intense PL treatments resulted in a strong synergistic effect. For example, by combining a PL treatment at 12 J/cm^2 with one of the antimicrobial coatings a microbial reduction > 5 log cycles was reached.

Therefore, it can be concluded that the combination of antimicrobial coatings and PL treatment is a promising method for surface decontamination of fresh-cut vegetables, which could be exploited in view of ensuring their microbiological safety.

1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the most popular and widely grown vegetables all over the world. Its attractive color, distinctive taste and aroma make it an ideal ingredient in raw salads. Cucumbers contain approximately 95% water, which causes their shelf life under refrigerated conditions to be relatively short (< 14 days) and their quality to be significantly reduced immediately after harvesting (Mohammadi et al., 2016).

Considering that fresh-cut produce is frequently eaten raw, its microbial safety has become an important priority for agrifood and public health authorities (World Health Organization (WHO), 2008). Fresh-cut vegetables are extremely sensitive to contamination by cutting or slicing operations. These processes, in fact, increase tissue damage, causing the release of the intracellular compounds and the increase of the activity of microorganisms (Sessa et al., 2015). The increasing trend towards minimally processed vegetables, together with the consideration that heat treatments are not suitable for fresh-cut vegetables, promoted the research of novel non-thermal technologies to ensure, at the same time, both safety and quality of the product (Birmpa et al., 2013).

In particular, the use of antimicrobial coatings has attracted a significant attention, because they can be formulated with naturally derived compounds, which do not alter the appearance of the product, and which are able to form a physical barrier, with the capability of controlled release of natural antimicrobials. Among biopolymeric matrices for edible coatings, chitosan, in addition to being generally recognized as safe (GRAS), biocompatible, and biodegradable, also exhibits excellent antimicrobial properties (Mohammadi et al., 2015). The incorporation of nanoencapsulated essential oils (EOs) in chitosan

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coatings has been reported to further strengthen the observed antimicrobial activity when applied to fruits and vegetables (Chen et al., 2016; Donsì et al., 2015; Severino et al., 2014).

Moreover, the combination of chitosan-based coatings with other non-thermal technologies, such as high hydrostatic pressure, pulsed light (Donsì et al., 2015), γ-radiation, ozone or UV light (Severino et al., 2014) resulted, in general, in an increased overall lethal effects on different vegetables.

Pulsed light (PL) is a promising technology for the inactivation of pathogenic or spoilage microorganisms on the surfaces of vegetables or fruits. It consists of the exposure of a food product to successive repetition of short (100 ns–1 ms), high-intensity pulses (flashes) of polychromatic light (200 nm–1100 nm) produced by a xenon flash lamp with approximately 40% of the emitted light corresponding to the UV region (Oms-Oliu et al., 2010). The microbial inactivation effect of PL is primarily achieved by damaging the microorganism DNA (Li and Farid, 2016). PL has a considerable potential to be implemented in the food industry, as discussed in details in the review of Oms-Oliu et al. (2010). Although the US FDA established 12 J/cm^2 as the maximum allowed fluence for food decontamination (FDA, 2003), in EU there is no standard specific to PL for food treatments.

In a previous study, we have identified *in vitro* different formulations of modified chitosan-based coatings, containing carvacrol nanoemulsions (Tastan et al., 2016), which were most active against *Escherichia coli* and *Listeria innocua*. Here we propose to study in product the combination of such antimicrobial coatings with PL treatments, with the goal of achieving the surface decontamination of fresh-cut cucumber slices inoculated with *E. coli*.

2. Materials and methods

2.1. Materials

Cucumbers (*Cucumis sativus*) were purchased from a local market in Fisciano (Salerno, Italy), and selected for uniform shape, size and color, and without mechanical damage. Carvacrol EO with a purity > 98% (Sigma-Aldrich s.r.l., Italy) was used as antimicrobial agent. Food-grade modified chitosan (3% *N*-palmitoyl chitosan), prepared according to a previously developed method (Le Tien et al., 2003) by *N*-acylation of native chitosan (Kitomer™, MW 1600 kDa, 83% deacetylation, Marinard Biotech, Canada) using palmitoyl chloride, was used as a matrix for the antimicrobial coatings. A combination of glycerol monooleate and Tween 20 (both from Sigma-Aldrich S.R.L., Italy) at 1:1 weight ratio, as defined in previous studies (Donsì et al., 2012, 2014; Sessa et al., 2013; Spigno et al., 2013; Tastan et al., 2016), or whey protein isolates (Volactive UltraWhey 90, a kind gift of Volac International Limited, UK) were used as emulsifying agents.

2.2. Test microorganism

The Gram-negative bacterium *E. coli* ATCC 26, often used as a nonpathogenic surrogate for *E. coli* O157:H7, was used in this study. In fact, *E. coli* is one of the most important pathogens implicated in foodborne outbreaks of fresh produce (European Food Safety Authority (EFSA), 2013). The bacterial suspension was prepared by suspending 100 μL of a pre-culture of *E. coli* (ATCC 26) in 100 mL of sterile tryptic soy broth (TSB, Oxoid, UK). After 18 h of incubation at 37 °C without shaking in a Function Line 7000 incubator (Heraeus Instruments, Germany), the broth reached the early stationary phase with a microbial concentration of approximately 10^8 CFU/mL. Then, it was diluted with buffered peptone water (BPW Oxoid, UK) to reach a final concentration of the working suspensions of 10^7 CFU/mL.

2.3. Carvacrol nanoemulsions

Nanoemulsions were formulated and prepared by high pressure homogenization (HPH), according to previous studies (Donsì et al., 2012, 2014; Severino et al., 2014; Tastan et al., 2016). The compositions of the two carvacrol nanoemulsions tested (EM1 and EM2), previously optimized and characterized (Tastan et al., 2016), are shown in Table 1.

Table 1
Formulations of nanoemulsions (EM1 and EM2) incorporating carvacrol essential oil and corresponding mean droplet size, polydispersity index and zeta potential (Tastan et al., 2016).

	EM1	EM2
Carvacrol	1%	3%
Sunflower oil	5%	3%
Glycerol monooleate	3%	–
Tween 20	3%	–
Whey protein isolate	–	6%
Water	88%	88%
Mean droplet size (nm)	113 ± 5	115 ± 10
Polydispersity index (PDI)	0.30 ± 0.02	0.24 ± 0.04
Zeta potential (mV)	− 27.1 ± 5.3	− 33.0 ± 6.0

2.4. Preparation of antimicrobial coating-forming solutions

The modified chitosan (MC) coating suspensions were prepared by dissolving modified chitosan in 1% (*v*/v) acetic acid solution and stirring for 24 h to ensure total solubility. Subsequently, the nanoemulsions (EM1 and EM2) were added and mixed vigorously using an Ultra Turrax T25 (IKA Labortechnik, Germany) high shear mixer at 19,000 rpm for 5 min. Coating suspensions were vacuum degasified at room temperature.

The first coating suspension to be tested (MC + EM1) consisted of 3% (w/w) nanoemulsion and 2% (w/w) modified chitosan. The second coating suspension instead consisted of 2.5% (w/w) nanoemulsion and 2% (w/w) modified chitosan. The two formulations were previously optimized (Tastan et al., 2016). In MC + EM1 suspension, carvacrol concentration was 0.03% (w/w), whereas in MC + EM2 suspension, it was 0.08% (w/w). In addition, also a coating suspension without any nanoemulsion addition (MC) was prepared at 2% (w/w) modified chitosan.

2.5. Coating of fresh-cut cucumbers and sample inoculation

Whole cucumbers were prewashed with a chlorinated water solution (250 mg/kg) for 5 min in order to reduce the natural microbiota and then washed for 5 min in sterile water to remove any residual chlorine, according to a previously validated procedure (Donsì et al., 2015). Samples were dried on sterile aluminum foils in a biological safety cabinet for 1 h. Cucumbers sections with a diameter of 3.5 ± 0.1 cm were cut into slices (5 mm thickness) with a sterile knife. The antimicrobial coatings (MC, MC + EM1, and MC + EM2) were applied on cucumber slices by immersion method for 3 min. The coated samples were allowed to dry for 1 h on sterile aluminum foils placed in a biological safety cabinet (25 °C, 50% RH). Afterward, the upper side of the slices was inoculated with 500 μL of the working suspension of *E. coli* in sterile Petri dishes. Samples were then treated by PL on the inoculated side. Control samples, consisting of uncoated cucumber slices, were also inoculated.

This sequence (coating followed by inoculation) was selected, in agreement with a previous study (Donsì et al., 2015), considering that that, in industrial applications, the coating layer would reasonably be applied to the cucumber slices immediately after the washing and slicing phases. Therefore, any further contamination would be likely to occur after the coating application, during the phases of product manipulation, eventual packaging, or transport. Moreover, the application of the coating layer after *E. coli* inoculation, would have been washed away part of the microbial population, with the risk of making difficult