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Short communication

Essential oil from pink pepper as an antimicrobial component in cellulose acetate film: Potential for application as active packaging for sliced cheese

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ABSTRACT

Active packagings (antimicrobial) control microbial contamination and reduce the need for preservatives added directly to food. Their active character can be obtained by adding essential oils (EO), which are natural extracts with antimicrobial properties. This work aimed to produce active films of cellulose acetate by incorporation of pink pepper EO, evaluating this action by diffusion in solid medium (agar), dispersion in liquid medium (broth), volatilization (micro-atmosphere), and *in situ* (sliced mozzarella cheese) against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* Typhimurium. The concentrations of 2, 4 and 6% of EO added to the films made them active against *L. monocytogenes* and *S. aureus* in all evaluated media. *Escherichia coli* was sensitive in liquid medium, micro-atmosphere and *in situ*, while *S.* Typhimurium showed sensitivity to the films in liquid medium and *in situ*. The *in situ* tests demonstrated that the affinity between nonpolar molecules of EO and the lipid components of cheese allows the EO of the polymer to migrate to food, indicating favorable characteristics for its use as active packaging, by direct contact.

1. Introduction

Traditionally, packages have passive action in relation to food, merely acting as a barrier between it and the external environment. However, the incorporation of active substances can promote desirable interactions with food, such as antimicrobial activity (Hafsa et al., 2016). Among the active substances, EO gain prominence because their natural origin gives a sense of security to consumers (Calo, Crandall, O'Bryan, & Ricke, 2015). Active packaging, antimicrobial, increases microbiological safety (Rizzolo et al., 2016), and allows producers to reduce the use of synthetic additives added directly to food (Moradi, Tajik, Mehdi, Rohani, & Mahmoudian, 2016).

In previous studies, the pink pepper EO (PPEO), *Schinus terebinthifolius* Raddi, demonstrated antibacterial activity against foodborne pathogens in *in vitro* tests and *in situ* (cheese), but with undesirable sensory interference due to its direct application on food (Dannenberg, Funck, Mattei, Silva, & Fiorentini, 2016). However, this problem can be minimized by the application of EO in the packaging, avoiding direct addition and focusing its action on the food surface, where microbial contamination is more intense (Appendini & Hotchkiss, 2002; Coma, 2008).

The volatile nature of EO makes them sensitive to thermal processes used in the production of packaging (Fabra, López-Rubio, & Lagaron, 2016). However, cellulose acetate (CA) is a polymer capable of forming films at low temperatures (Gouvêa, Mendonça, Soto, & Cruz, 2015).

In view of the potential of PPEO as antibacterial agents in food (Dannenberg et al., 2016), and the lack of studies incorporating this EO in films, this study aimed to apply PPEO as an active component in CA films and to characterize it's *in vitro* and *in situ* action. The study evaluated its action *in vitro* in different application media (solid, liquid and vapor phase), and subsequently *in situ* (sliced cheese), to test its use as active packaging in controlling foodborne pathogens.

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2. Material and methods

2.1. Material

For the preparation of films Cellulose Acetate (CA - Mn ~ 50,000; Aldrich®), Acetone (Synth®) and Tween 80 (Synth®) were used. For the cultivation of bacteria Brain Heart Infusion broth (BHI - Acumedia®) and the following agars were used: Chromogenic (CR - Oxoid®), Baird-Parker (BP - Oxoid®), MLCB (MLCB - Oxoid®), EMB (Eosin Methyene Blue - Oxoid®) and Mueller-Hinton (MH - Oxoid®).

2.2. Bacteria

Four foodborne pathogens were used: *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739) and *Salmonella* Typhimurium (ATCC 14028).

2.3. Essential oil

The PPEO was obtained according to Dannenberg et al. (2016). The chromatographic analysis (GC/MS) detected 18 components, four monoterpenes and 14 sesquiterpenes, including β-myrcene (41%), β-cubebene (12%) and limonene (9%) as the majority compounds (unpublished data).

2.4. Development of active films

The films were produced by casting technique. The filmogenic solution (FS) was formed by CA in acetone (3% w/v). PPEO was added to the FS at concentrations of 0, 2, 4 and 6% (v/v - EO/FS), generating the T0, T1, T2 and T3 treatments. The mixtures were homogenized in Ultra-Turrax (15.000 rpm/5 min). After that, aliquots of 5 mL were spread on glass plates (90 mm diameter), dried at 25 °C for 3 h, sterilized (UV 15 min) and stored (4 °C).

The applied concentrations were based on the results of minimum inhibitory concentration (MIC), previously observed for *L. monocytogenes* and *S. aureus* (MIC = 1.36 mg/mL, unpublished data) to give the MIC per unit area: T1 = 1.36 mg/cm^2, T2 = 2.73 mg/cm^2 and T3 = 5.45 mg/cm^2.

2.5. Antimicrobial activity of the films

2.5.1. Antimicrobial activity on solid medium

The antimicrobial activity by diffusion in solid medium was carried out similarly to the disk-diffusion technique (CLSI, 2015) using *S. aureus*, *L. monocytogenes*, *E. coli* and *S.* Typhimurium. The films were aseptically cut into disks of about 10 mm diameter and added to plates containing solid medium. The results were analyzed for clear zones around the films, showing antimicrobial activity, expressed in mm. The area covered by the films themselves was discounted (Hafsa et al., 2016).

2.5.2. Antimicrobial activity in broth

Cutouts of films (4 cm^2) were placed in tubes with 4 mL BHI broth inoculated with *L. monocytogenes*, *S. aureus, E. coli* and *S.* Typhimurium (10^4 CFU/mL). The system was incubated at 37 °C, removing aliquots at 0, 4, 8, 12 and 24 h for quantification of cell growth (CFU/mL) in MH agar.

2.5.3. Antimicrobial activity in micro-atmosphere

The antimicrobial activity of films by micro-atmosphere was based on the methodology proposed by Ghabraie, Vu, Tata, Salmieri, and Lacroix (2016) for EO evaluation. Cultures of L. *monocytogenes*, *S. aureus, E. coli* and *S.* Typhimurium (10^3 CFU/mL) were inoculated on MH agar plates (15 mL). On the inside face of the plates intact pieces of the films were arranged (90 mm diameter). The plates were inverted, sealed with parafilm, and incubated at 37 °C for 24 h. The results were expressed as the inhibition percentage calculated by the differences between the viable cell counts of the treatments and control.

2.5.4. Antimicrobial activity in situ

The food matrix used for analysis was mozzarella cheese slices (thickness ≈ 1.5 mm) obtained commercially. The slices were initially exposed to Ultraviolet light for 15 min on both sides (Lee, Lee, & Song, 2015). After that, they were experimentally contaminated on one side, by the addition of a cell suspension (10^6 CFU/mL) of *L. monocytogenes*, *S. aureus*, *E. coli* or *S.* Typhimurium, obtaining a final concentration of 10^4 CFU/g in the cheese. After drying the inoculum, the slices were placed on the films with the contaminated side in contact with it. The system was stored for 12 days at 4 °C, being analyzed at 0, 3, 6, 9 and 12 days.

Samples were diluted in peptone water (0.1%) and inoculated into CR agar plates for the quantification of *L. monocytogenes*, BP agar plates for counting *S. aureus*, MLCB agar plates for counting *S.* Typhimurium and EMB agar plates for counting *E. coli.*

2.6. Statistical analysis

The results were compared by analysis of variance (ANOVA) using STATISTICA software (StatSoft, France - version 6.1). The Duncan test was used to detect significant differences ($P \leq 0.05$) between the means.

3. Results and discussion

3.1. Antimicrobial activity on solid medium

The results of the antimicrobial activity analysis of the film, with and without PPEO, by direct contact in solid medium, are shown in Fig. 1.

The film without PPEO (T0) did not present any activity for the evaluated foodborne pathogens. The incorporation of 1.36 mg/cm^2 (T1), 2.73 mg/cm^2 (T2) and 5.45 mg/cm^2 (T3) of PPEO in the films became active against *S. aureus* and *L. monocytogenes*, increasing its activity as the EO concentration increased. *Staphylococcus aureus* presented the highest sensitivity among Gram-positive pathogens, with zones of inhibition of 34.54 ± 1.67 (T1), 37.65 ± 2.79 (T2) and 61.27 ± 2.23 mm^2 (T3). The zones of inhibition observed for *L. monocytogenes* were lower: 12.39 ± 0.7, 06.15 ± 1.60 and 32.91 ± 0.71 mm^2.

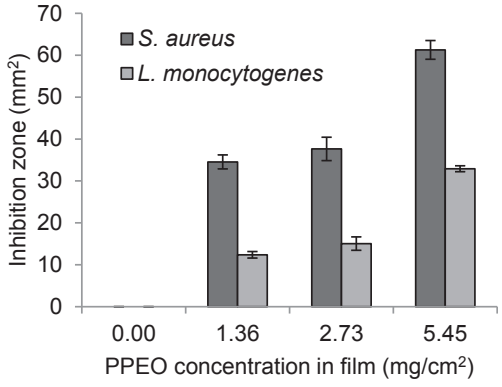

Fig. 1. Antimicrobial activity of films with different concentrations of PPEO against *L. monocytogenes* and *S. aureus*. Results are expressed as mean of zones of inhibition (n = 3) ± standard deviation.