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Physical and antifungal properties of β-cyclodextrin microcapsules and nanofibre films containing cinnamon and oregano essential oils

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ABSTRACT

The aim of this study was to investigate the physical and antimicrobial properties of fabricated nanofibre for active packaging systems. Active microcapsules and nanofibres derived from precipitating β-cyclodextrin (β-CD) with cinnamon (CIN) and oregano (OREG) essential oils (EOs) were developed and their *in vitro* antifungal activity against *Botrytis* sp., an important fruit pathogen was determined. Solutions of β-CD and CIN- or OREG-EOs were subjected to co-precipitation to induce micro-encapsulation. Thereafter, the properties of the complexed microcapsules were assessed. Solid-state nuclear magnetic resonance (NMR) showed the presence of new carbon peaks in the β-CD complex spectra, which were absent in the plain β-CD spectrum confirming encapsulation of EOs. Encapsulation of EOs was also confirmed by thermogravimetric analysis (TGA), which indicated that the thermal degradation of β-CD-EO complex occurred over a lower temperature (270 °C) compared to plain β-CD (300 °C). Gas chromatography-mass spectrometry (GC-MS) showed that the amount of volatile EOs released was lower for OREG-EO compared to CIN-EO. Electrospun active nanofibres (~100–300 nm) were successfully fabricated from chitosan/polyvinyl alcohol/β-cyclodextrin (CH/PVA/β-CD) solution with EOs. The nanofibres ability to release EO volatiles and antifungal activity were confirmed.

1. Introduction

The use of antimicrobial packaging materials offers the potential to retard the growth rate of spoilage microorganisms. Several antimicrobial agents have been reported in literature. These include the use of biologically active compounds such as plant extracts. Essentials oils (EOs) often described as concentrated hydrophobic liquids containing volatile aroma compounds and are considered 'green' alternatives due to their numerous bioactive properties (Makwana, Choudhary, Haddock, & Kohli, 2015). Rodriguez, Batlle, and Nerin (2007) tested the antimicrobial, vapour-phase activity of several EOs including cinnamon and oregano coated onto a paraffin paper. Cinnamon EO was the most effective EO and could provide complete protection of strawberries kept for 7 d storage at 4 °C from *Aspergillus niger* without any apparent visible or organoleptic changes (Rodriguez et al., 2007). Cinnamon EO was also used against *Botrytis cinerea* in table grapes stored at 10 °C for 15 d (Melgarejo-Flores et al., 2013). The inhibitory effects of the cinnamon as a vapour were effective against the *Botrytis* sp. starting from 0.196 g/L and was highest at 0.588 g/L of concentration (Melgarejo-Flores et al., 2013).

The volatility of EOs limits their application as food preservatives as they are thermally unstable and require encapsulation to achieve prolonged release. Woranuch and Yoksan (2013) reported that encapsulating eugenol in chitosan nanoparticles using an emulsion-ionic gelation crosslinking method enhanced its

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stability against light oxidation. Microencapsulation of EOs with encapsulating agents such as β-cyclodextrin (β-CD) could protect against oxidation, heat degradation, and evaporation. Cyclodextrins (CD) are cyclic oligosaccharides that exhibit a truncated hollow cone, made up of glucose monomers linked by α-(1.4) bonds (Kayaci, Sen, Durgun, & Uyar, 2014). Cyclodextrins can form non-covalent host-guest inclusion complexes with several molecules including EOs, fragrances/flavours and antioxidants (Kayaci et al., 2014).

Delivery of active antimicrobial agents enhances inhibitory and antioxidant activities compared to traditional materials such as low-density polyethylene terephthalate, polyvinyl chloride and polypropylene (Lou et al., 2017; Rieger, Eagan, & Schiffman, 2015). Previous studies have demonstrated that EOs can be incorporated into nanofibres through electrospinning (Rieger & Schiffman, 2014; Wen et al., 2016). Nanofibre technology can be used to prepare more controlled release structures with greater order and layering in the next step to prepare antimicrobial materials (Rieger & Schiffman, 2014; Wen et al., 2016). While fibres and particles are interesting, the principle methods can also be used to prepare bubbles for application in bio-coatings (Ekemen, Ahmad, Stride, Edirisinghe, & Kaplan, 2013). Materials used for food packaging should not contain any potentially toxic substances. From this perspective, polymeric materials such as chitosan and polyvinyl alcohol are recommended as safe, biodegradable and more biocompatible over conventional materials (Wen et al., 2016).

The market value of pomegranate, a high value crop, is limited due to postharvest decay (30–50%) caused by *Botrytis* grey mould (Munhuweyi, Lennox, Meitz-Hopkins, Caleb, & Opara, 2016). This highlights the need for effective control of decay via natural bioactive agents. The present work evaluated the antifungal activities of encapsulated cinnamon and oregano EOs against a *Botrytis* sp. originally isolated from pomegranate fruit. The use of β-CD and active nanofibres have been shown to ensure prolonged delivery of the EOs (Wen et al., 20160). Thus, the focus of this work was to investigate the physical and antimicrobial properties of encapsulated cinnamon- and oregano-EO using β-CD, and electrospun chitosan/PVA/β-CD/EO nanofibrous films for future use as active packaging materials.

2. Materials and methods

2.1. Fungal culture

Botrytis sp. (STE-U 7866), was obtained from pomegranates (cv. Herskawitz) located in the Wellington area, Western Cape, South Africa (GPS S33° 39.276 E18° 59.399). The pathogen isolate was grown on Potato Dextrose Agar (PDA) (Biolab, Modderfontein, South Africa) for 7–14 days at 25 °C before each trial. Spore concentrations were freshly prepared by filtering each culture grown in PDA through two layers of cheesecloth with distilled water amended with 0.01 mL/L of Tween 20 (Sigma-Aldrich, St. Louis, MO, USA). Spores were counted with a haemocytometer (Neubauer, Marienfeld-Superior, Lauda-Konigshofen, Germany) and an optical microscope (Leica Wild M8 Transmitted Light Stereo Microscope, Wild Heerbrugg, Switzerland), and final concentration was adjusted to 1×10^6 spores/mL.

2.2. Essential oils

Cinnamon leaf (*Cinnamon verum*) oil was obtained commercially from Soil (Durban, South Africa) and oregano (*Origanum vulgare* leaf extract) oil was obtained commercially from Clive Teubes Africa (Randburg, South Africa). The oils were previously extracted from fresh or partly dried leaves by means of steam distillation based on the supply data sheet provided. Samples were kept refrigerated at 4 °C until the start of experiment.

2.3. Microencapsulation process

Microcapsules of cinnamon:β-CD (CIN/β-CD) and oregano:β-CD (OREG/β-CD) were prepared separately as described by Ayala-Zavala et al. (2008). Approximately 50 ± 0.01 g of β -CD (MW: 1134.98 g/mol, Sigma-Aldrich, Steinheim, Germany) was dissolved in ethanol (10 g/L) and then EO was slowly added to the warm β-CD solution to obtain a weight ratio of 16:84 (EO:β-CD) in a 1 L Erlenmeyer flask placed in oil bath set at 55 ± 2 °C on a temperature controlled hot plate (Heidolph MR3001 K, Sigma Aldrich, Germany). Afterwards, the heater was turned off and resultant mixture was covered and stirred for 4 h. Final solution was maintained overnight at 4 °C. Precipitated microcapsules were recovered by vacuum filtration (Whatman No. 1) and freeze-dried (VirTis, BenchTop "K", USA) for 48 h and allowed to air-dry at 25 °C in a desiccator for 24 h (to assure complete dryness). The obtained EO:β-CD microcapsules were weighed at equilibrium and the amount of recovered active-microcapsules (on dry weight basis) was calculated in percentage. Initial weight prior to the addition of active agents was compared to the recovered active-microcapsules. Final active-microcapsules were stored at 25 °C in an airtight bottle. Microcapsules of cinnamon:β-CD (CIN/β-CD), oregano:β-CD (OREG/β-CD), and their control (without EO) were prepared separately in triplicate per sample.

2.4. Nano film preparation

Nano films were prepared as described by Wen et al. (2016) with a few modifications. Chitosan (3% w/w) was prepared by dissolving 3 g of low molecular weight chitosan (Sigma-Aldrich, Steinheim, Germany) in 100 g of 70% acetic acid. Mixture was stirred constantly using a magnetic stirrer (RY5, IKA, Germany) at room temperature for 4 h. Polyvinyl alcohol (PVA) (7% w/w) obtained from Sigma-Aldrich, Steinheim, Germany was dissolved in 100 g distilled water at 80 °C for 3 h under constant stirring. The two solutions were subsequently combined based on weight ratio 30:70 of chitosan (CH) to PVA. Thereafter, β-CD (2 g) was dissolved into the chitosan/PVA (98 mL) solution by constant stirring at 55 °C for 1 h. Then, cinnamon- and oregano-EO (≈3 g) were respectively added to the CH/PVA/β-CD solution and mixed for 3 h. Separate solutions were also prepared by adding the previously fabricated microcapsules to the CH/PVA mix. Final mixture obtained was a turbid white solution. Solutions of CH/PVA and CH/PVA/β-CD were also prepared separately for comparison purposes. Viscosity of the solution (20 g) was measured at 22 ± 1 °C using a DV II + Pro viscometer (Brookfield, Middleboro, USA) with S63 and S64 spindles at 20 rpm. Conductivity of each solution was measured using a conductivity meter (CRISON Instruments, Barcelona, Spain) and presented later in the results and discussion.

2.5. Electrospinning process

Solutions were loaded into a 1 mL syringe fitted with a 21-gauge steel needle (0.8 mm nozzle diameter). This was driven by a syringe pump (Genie Plus, Kent Scientific, Torrington, USA) to give the solution flow rate of 0.2–0.6 mL/h. Electrospinning was conducted by applying a voltage varying from 13 to 15 kV with a power supply. The electrospinning was allowed to run for 3–5 h per sample solution onto a collector. A grounded collecting plate covered by a piece of aluminium foil was used as the collector for the fibre deposition. The distance between needle tip and collector was approximately 20 cm. Room temperature was maintained at