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Cellulose acetate active films incorporated with oregano (*Origanum vulgare*) essential oil and organophilic montmorillonite clay control the growth of phytopathogenic fungi

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ABSTRACT

The aim of the present study was to produce active films based on cellulose acetate (CAc) incorporated with different concentrations of oregano essential oil (OEO) and organophilic montmorillonite clay (MMT30B) to control the growth of phytopathogenic fungi. The active films were characterized by ATR-FTIR, XRD, TEM, mechanical resistance, WVTR, OTR, thermal stability, and antifungal activity against the phytopatogens *Alternaria alternata*, *Geotrichum candidum*, and *Rhizopus stolonifer*. The CAc-based active films presented high antifungal activity against the tested phytopathogens, mainly in vapor phase. MMT30B exhibit good dispersion in CAc matrix, resulting in a partially exfoliated conformation. In this way, MMT30B significantly increased films' rigidity and thermal resistance while reduced OTR. OEO acted as plasticizer, facilitating MMT30B dispersion. Also, OEO increased extensibility and decreased WVTR of CAc-based films. Both OEO and MMT30B slightly improved the thermal resistance of films. The active films developed here denote a potential packaging system for postharvest conservation purposes.

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1. Introduction

Active packaging has recently aroused much attention of the scientific community, especially those that feature antimicrobial activities. The ability to interact with food and extend its shelf life provides this type of packaging with a range of potential applications. Packaging materials may play active roles through different mechanisms, including sachets and pads (Otoni, Espitia, Avena-Bustillos, & McHugh, 2016), films (Mellinas et al., 2016), and coatings (Atarés & Chiralt, 2016). Antimicrobial films are a particular class of active packaging that denotes a promising means of increasing food safety by interacting with food and/or its surrounding environment aiming to retard, reduce, or even inhibit the growth of pathogenic and spoilage microorganisms (Espitia et al., 2013). This approach is of special interest to commonly spoiled foods, including meat, dairy and bakery products as well as fruits and vegetables (Corbo, Campaniello, Speranza, Bevilacqua, & Sinigaglia, 2015). The application of antimicrobial active packaging systems has been extensively reviewed elsewhere (Kapetanakou & Skandamis, 2016).

Among active compounds commonly used to produce antimicrobial packaging, oregano (*Origanum vulgare*) essential oil (OEO)

Abbreviations: ATR-FTIR, attenuated total reflectance Fourier transform infrared spectroscopy; CA, ccellulose acetate; DTG, derivative thermogravimetry; EB, elongation at break; EO, essential oil; MMT, montmorillonite clay; GRAS, generally recognized as safe; MMT30B, organophilic montmorillonite clay Cloisite® 30B; M_W, molecular weight; OEO, oregano essential oil; OTR, oxygen transmission rate; PDA, potato dextrose agar; SD, substitution degree; TEC, trietyl citrate; TEM, transmission electron microscopy; TG, thermogravimetry; TS, tensile strength; WVTR, water vapor transmission rate; XRD, X-ray diffraction; YM, Young's modulus.

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has been successfully applied against bacteria, yeasts, and fungi, including *Rhizopus stolonifer* and *Alternaria alternata* (Barbosa et al., 2016; Espitia et al., 2012; Jouki, Yazdi, Mortazavi, & Koocheki, 2014; Pesavento et al., 2015; Souza, Stamford, Lima, & Trajano, 2007). OEO is listed as generally recognized as safe (GRAS) for food for human consumption by the US Food and Drug Administration (FDA) (FDA, 2015). The major active component of OEO is carvacrol, corresponding to approximately 80% of its content, but thymol, γ-terpinene, β-caryophyllene and others compounds are also present and act synergistically to give OEO its active properties (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004). Nonetheless, the direct application of OEO and other essential oils (EOs) on food products can lead to undesirable sensory changes. Thus, the application of EOs into packaging systems provides the latter with an active action of the resulting, but with the advantage of reduced sensory alterations because the application is no longer direct to food (Becerril, Nerin, & Gomez-Lus, 2012). A major packaging system that allows the application of EOs as active compounds comprises polymeric thin films.

The use of biopolymers as matrix for packaging production is increasingly growing. The large amount of plastic waste generated every year in the world is widely concerned, making biopolymers an alternative to overcome this hurdle. This way, biopolymers (*e.g.*, cellulose, and starch, chitosan, to mention a few) stand out as the main materials for the development of sustainable materials. The production of plastic films based on cellulose derivatives has received great attention since they are obtained from the most abundant natural polymer in the environment (Rodriguez, Galotto, Guarda, & Bruna, 2012).

Cellulose acetate (CAc) is one of the most important cellulose esters (Rudaz & Budtova, 2013). CAc is produced by the replacement of hydroxyl groups from cellulose backbone chain by acetate groups by means of a reaction of native cellulose with acetic anhydride using sulfuric acid as catalyst (Wan Daud & Djuned, 2015). However, as most of the biopolymers, CAc presents limited mechanical, thermal, and barrier properties when compared with traditional, petroleum-derived polymers (Rodriguez, Galotto, et al., 2012). An alternative to handle these limitations is the application of nanoparticles as fillers for reinforcement purposes.

In case of applying these nanomaterials into food contact packaging, many people fear the risk of indirect exposure due to a potential migration from the film to the packaged food (Silvestre, Duraccio & Cimmino, 2011). The health risk of consuming food containing nanoscale compounds transferred from the packaging is not yet fully understood, since it will depend on the particle toxicity, size, morphology, and the rates of migration and ingestion (Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2012). The nanoparticles (NPs) have potential to migrate to the packaged foodstuff, but migration assays and risk assessment are still not conclusive (Souza & Fernando, 2016). This scarce availability of migration studies is explained by the difficulties in characterizing the NPs in such complex matrices as food (Huang, Li, & Zhou, 2015). The pioneers on the study of NPs migration into foodstuff concluded that nanoclay content in vegetables packaged with biodegradable starch/clay nanocomposite films was in conformity with current regulations and European directives (European Commission, 2007), demonstrating that these materials can be utilized in the food packaging sector (Avella et al., 2005). Thus, when more toxicity data become available from studies with nanocomposites, nanotoxicology and exposure stimates should be re-evaluated (Souza & Fernando, 2016).Organophilic montmorillonite (MMT) clay is one of the most common nanostructures applied to biopolymers. MMT is a layered clay formed by an octahedral sheet of aluminum hydroxide located between tetrahedral sheets of silica (Silvestre et al., 2011). MMT is frequently submitted to organophilization, which increases its lamellar distance and its organic character in a way that its compatibility with natural polymers (*e.g.*, CAc) is enhanced. Also, increased lamellar distances favor the interaction and the penetration of polymer into clay layers (Paiva, Morales, & Díaz, 2008). Although there are some reports of nanocomposites based on CAc and MMT (Park, Misra, Drzal, & Mohanty, 2004; Rodriguez, Sepulveda, Bruna, Guarda, & Galotto, 2013; Rodriguez, Coloma, Galotto, Guarda, & Bruna, 2012; Rodriguez, Galotto, et al., 2012), the influence of MMT on the production of antimicrobial packaging as well as the investigation of physical-mechanical and active properties of CAc-based films is still limited, especially when its application is against phytopathogenic microorganisms for the postharvest preservation of fruits and vegetables.

Therefore, this work set out to develop CAc-based active films incorporated with OEO and MMT organoclay to come up with a novel material that meets the mechanical and barrier requirements for food packaging applications, with the advantage of playing an antimicrobial action in an effort to increase food safety. Also, this work aimed to evaluate the physical-mechanical properties of the produced films, including spectroscopy, microscopy, tensile, permeability, and thermal analyses, as well as *in vitro* antifungal evaluation against the phytopathogens *A. alternata*, *R. stolonifer*, and *Geotrichum candidum*.

2. Materials and methods

2.1. Materials

CAc (SD = 2.5; M_W = 2,024,000 g mol^{-1}) was kindly provided by Rhodia Solvay Group (Santo André, SP, Brazil). OEO was purchased from Ferquima (Vargem Grande Paulista, SP, Brazil). MMT clay Cloisite® 30B (MMT30B) was kindly provided by Southern Clay Products (Gonzales, TX, USA). Acetone was purchased from Vetec Química (Duque de Caxias, RJ, Brazil).

2.2. Film production

Eight grams of CAc were dissolved in 80 mL of acetone during 24 h at room temperature. OEO was directly added into the film-forming solution. MMT30B (0.2 g) was dispersed in 25 mL of acetone and stirred for 24 h in a closed bottle. Using a lid with a hole in the middle to cover the MMT30B suspension bottle and an ice bath, the suspension was sonicated in a probe ultrasonicator, model DES500 (Unique Group, Indaiatuba, SP, Brazil) during 30 min using a 1.3-cm-diameter probe and 400 W of power to increase the MMT30B dispersion. The components were added in different ratios to form different films (Table 1).

After sonicating the film-forming solutions for 30 min at 400 W, also using an ice bath, they were poured (30 mL) into glass plates

Table 1
Oregano essential oil (OEO) and montmorillonite clay Cloisite® 30B (MMT30B) contents in the aqueous, cellulose acetate-based film-forming solutions.

Film	OEO (%, wt.)[a]	MMT30B (%, wt.)[a]
A (control)	0.0	0.0
B	20.0	0.0
C	40.0	0.0
D	60.0	0.0
E	0.0	2.5
F	20.0	2.5
G	40.0	2.5
H	60.0	2.5

[a] Additive (*i.e.*, OEO or MMT30B):cellulose acetate mass ratios, multiplied by 100 to give percentage values.

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