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Active packaging containing encapsulated carvacrol for control of postharvest decay

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ABSTRACT

Roughly, one-third of the food produced globally for human consumption is lost or wasted. These losses occur at all stages of the food value chain and across all types of food. Active packaging already plays a vital role in preventing wastage and further innovation is imperative to streamlining the food supply chain. Herein, we present an antimicrobial packaging based on polyamide (Nylon 6), containing a model essential oil (carvacrol). The volatile carvacrol molecules are encapsulated with Halloysite nanotubes (HNTs), which are naturally occurring aluminosilicate. The resulting polyamide films had an outstanding *in vitro* antifungal properties, with a broad spectrum of inhibitory activity against a wide range of fungal molds: *Alternaria alternata*, *Botrytis cinerea, Penicillium digitatum*, *Penicillium expansum* and *Aspergillus niger*. Furthermore, the active polyamide-based plastic bags were used for fresh produce packaging and their fungicidal and/or fungistatic effects on postharvest pathogens of cherry tomatoes, lychee and grapes were investigated. These *in vivo* experiments have resulted in reduced decay development and significantly extended shelf life. The presented technology holds a great potential for the development of custom-made active packaging for the food and postharvest industries, in a global effort to reduce food loss and waste.

1. Introduction

Food security and food wastage are of major concern worldwide, estimated at 1.3 billion tons of food wastage (Gustavsson et al., 2011). Reduction of fresh produce losses requires accurate horticultural management practices throughout the supply chain, including postharvest sanitation, pre-cooling procedures, maintenance of shipments at optimal holding temperatures, as well as, packaging (Kader, 2002; Soliva-Fortuny and Martı'n-Belloso, 2003).

Food packaging plays a critical role throughout the supply chain in reducing food waste. Packaging acts as a physical barrier, excluding the produce within from bacterial and/or fungal contaminants in storage environments, hence, limiting decay development (D'Aquino et al., 2016; Kader et al., 1989). To extend the shelf life of highly perishable commodities various substances, such as ethylene absorbers, oxygen absorbers or antimicrobials, are incorporated into the packaging material. In food packaging, antimicrobials have an added value in managing the harmful impact of human disease-causing pathogens (Suppakul et al., 2003). Currently, commercially available antimicrobials are primarily oxidizing ions, such as cupper or silver ions (Llorens et al., 2012; Rai et al., 2009; Ruparelia et al., 2008). These compounds are contact biocides and are limited in their ability to control bacteria or molds that promote human diseases and decay in packaged fruits or vegetables. Therefore, antimicrobials such as essential oils (EOs), released as vapor from the film's matrix into the packaging headspace, are capable of sanitizing both the surface of the produce and the headspace environment. Such active packaging systems may extend the shelf life of fresh produce, while controlling the development of decay and disease causing microorganisms, thus maintaining food safety and reducing wastage (Appendini and Hotchkiss, 2002; Labuza and Breene, 1989; Peretto et al., 2014; Vermeiren et al., 1999).

Essential oils are natural aromatic compounds extracted from plants and are approved by the Food and Drug Administration

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(FDA) for food usage as GRAS (generally recognized as safe) (Burt, 2004; Vergis et al., 2015). EOs possess high efficacy in eradicating bacteria and fungi by direct contact, as well as, in vapor phase (Chavan and Tupe, 2014; Farzaneh et al., 2015; Hammer et al., 1999; Kuorwel et al., 2011; Li Destri Nicosia et al., 2016; Lopez et al., 2005). Carvacrol is a terpenoid phenol and is the major component in the EOs of both oregano and thyme (Rao et al., 2010). Carvacrol exhibits potent antifungal activity against a wide range of pathogens *e.g.*, *Candida albicans* (Rao et al., 2010), as well as against food-pathogenic fungi, such as *Aspergillus* spp. and *Botrytis cinerea (B. cinerea)* (Abbaszadeh et al., 2014; Kordali et al., 2008; Rodriguez-Garcia et al., 2016). However, being volatile in nature creates difficulties in incorporating EOs into polymers via high-temperature extrusion technologies. In recent years, Halloysite nanotubes (HNTs), which are naturally occurring aluminosilicate clays and characterized by a hollow tubular nanostructure, arise as promising nanoscale containers for the encapsulation of different active molecules (Abdullayev and Lvov, 2011; Cavallaro et al., 2014; Liu et al., 2014; Lvov and Abdullayev, 2013; Lvov et al., 2015; Lvov and Price, 2008; Lvov et al., 2008; Veerabadran et al., 2007). Importantly, HNTs can be used naturally as nanocapsules without further chemical modification and exhibit a high level of biocompatibility and very low toxicity (Abdullayev et al., 2012; Vergaro et al., 2010; Yah et al., 2012). In our recent study (Shemesh et al., 2015), we demonstrated that HNTs can be used as active carriers for carvacrol, used as a model EO, within low density polyethylene (LDPE) films, processed at 140 °C, while exhibiting potent antimicrobial properties. The resulting nanocomposites displayed much higher carvacrol content in the final film in addition to a sustained release profile of the encapsulated carvacrol in comparison to the control films with no HNTs as carriers (Shemesh et al., 2015).

In the present work, we develop active antimicrobial packaging films based on polyamide 6 (PA) for fresh produce. PA is characterized by its high melting temperature (220 °C) (Swain and Isayev, 2009), thus requiring harsh conditions of melt compounding and film production. Based on our recent study results (Shemesh et al., 2015), we employ HNTs as nano-carriers for carvacrol, to allow the incorporation of this highly volatile EOs (carvacrol boiling temperature is 236 °C) into PA matrices at elevated temperatures of 250 °C. First, in a pre-compounding step, HNTs/carvacrol hybrids are produced to promote the loading and encapsulation of the carvacrol into the nanotubes. Subsequently, the resulting hybrids are melt-compounded with PA and films are produced by cast extrusion. We demonstrate that the encapsulated carvacrol can withstand processing at extreme temperatures while preserving its antifungal potency. The resulting PA films exhibit outstanding *in vitro* antifungal properties with a broad spectrum of inhibitory activity against the postharvest model fungi *Alternaria alternata*, *B. cinerea, Penicillium digitatum*, *Penicillium expansum* and *Aspergillus niger*. Additionally, *in vivo* trials indicated reduced decay development on various fresh produce (cherry tomato, lychee and grape) packaged in active PA plastic bags.

2. Materials and methods

2.1. Materials

Halloysite Nanotubes (HNTs), supplied by NaturalNano (USA), are characterized by a tubular form with an external diameter typically smaller than 100 nm, internal diameter of 20 nm and length of 0.2–2 mm. Polyamide 6, Ultramid® B40LN 01, was supplied by BASF (USA). Carvacrol (98%), Nutrient Broth (NB) medium, Tween 80, methyl *tert*-butyl ether (MTBE), iso butyl benzene and Potato Dextrose Agar (PDA) were purchased from Sigma Aldrich Chemicals (Israel). NB bacto-agar was purchased from Becton Dickinson (USA).

2.2. Preparation of HNTs/carvacrol hybrids

Halloysite Nanotubes/carvacrol hybrids were prepared as previously described (Shemesh et al., 2015). The HNTs were shear mixed with carvacrol at a weight ratio of 1:2 (respectively), followed by ultrasonication at room temperature at a constant amplitude of 40% (Vibra cell VCX 750, Sonics & Materials Inc., USA). To prevent agglomeration of the HNTs, the nanotubes were added in two portions to the carvacrol, followed by alternating steps of shear mixing (2 min) and ultrasonication (2 min) for 20 min in total.

2.3. Preparation of PA/(HNTs/carvacrol) films

Polyamide 6 (PA) was melt-compounded with HNTs/carvacrol hybrids using a 16 mm twin-screw extruder (Prism, England) L/D ratio of 25:1 with a screw speed of 150 rpm and feeding rate of 2 kg h^{-1} at 250 °C. Table 1 specifies the composition of the different film blends investigated in the present study. Following the melt-compounding process, ~50 μm thick films were prepared by cast extrusion using 45 mm screw diameter extruder (Dr. Collin, Germany) at 250 °C.

2.4. Characterization

2.4.1. Thermal gravimetric analysis (TGA)

Films samples (weight of ~20 mg) were characterized by thermal gravimetric analysis (TGA) using TGA-Q5000 system (TA instruments, USA) at a heating rate of 20 °C min^{-1} under nitrogen atmosphere, starting at room temperature and increasing up to 600 °C. The thermograms were used for determining carvacrol content in the film following melt compounding and cast extrusion. Carvacrol boiling temperature is 236 °C, thus the weight loss between 110 °C to 236 °C is attributed to carvacrol residue in the film. All tests were carried out three times in triplicates and the carvacrol content in the tested films is reported as the mean ± standard deviation of the three tests.

2.4.2. High-Resolution scanning electron microscopy (HRSEM)

The nanostructure of neat HNTs and films was studied using a Carl Zeiss Ultra Plus high-resolution scanning electron microscope (HRSEM) operated at 1 keV accelerating voltage. Films were cryogenically fractured in liquid nitrogen prior to observation.

2.4.3. In vitro *antifungal activity*

In vitro antifungal activity was characterized using a direct contact assay and an indirect headspace assay, as follows:

(i) Direct contact assay: The IPC-TM-650 test method (IPC, 2007) for testing polymeric circuit board resistance to fungal deterioration was used. For the purpose of this trial, the tested fungal molds were modified from those recommended by the IPC

Table 1
The composition of the different PA film blends investigated in the present work.

Sample	Composition (wt%)		
	PA	HNTs	Carvacrol
Neat PA	100	0	0
PA/carvacrol	98	0	2
PA/(HNTs/2%carvacrol)	97	1	2
PA/(HNTs/4%carvacrol)	94	2	4