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Thermal and morphological properties and kinetics of diffusion of antimicrobial films on food and a simulant

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ABSTRACT

Seeking to control the release of allyl isothiocyanate (AIT), AIT was incorporated in cellulose acetate films, in encapsulated or non-encapsulated form, with and without carbon nanotubes (CNT). The films were evaluated by thermal analysis (TGA and DSC), microscopy (SEM) and kinetic diffusion into simulant and food. All the films showed similar Tg (187–192 °C) and Tm (222–224 °C) values. Films containg non-encapsulated AIT had smooth surface, while those with encapsulated AIT appeared less homogeneous. CNT associated with encapsulated AIT produced a porous and cracked structure. Encapsulated AIT was more efficient to control the antimicrobial release in hexane and in food. The CNT influenced more the diffusion in hexane than in food, but in both it acted increasing the diffusion coefficient for the film with encapsulated AIT. It was concluded that AIT encapsulated in CD is the most appropriate method to control AIT release in packaging for food.

1. Introduction

Among the bioactive compounds, allyl isothiocyanate (AIT) is a volatile, non-phenolic and natural compound found in the stems, roots, leaves and seeds of plants belonging to the family *Crucifereae* (Lim & Tung, 1997). Several studies have reported its antimicrobial efficiency against various pathogenic microorganisms, including food-borne microorganisms (Dias et al., 2013b; Isshiki, Tokuoka, Mori, & Chiba, 1992; Luciano & Holley, 2009; Nadarajah, Han, & Holley, 2005a; Nielsen & Rios, 2000, Rhee, Dougherty, & Kang, 2002). Olaimat and Holley (2015) demonstrated that κ-Carrageenan/chitosan-based coatings containing AIT have been effective against *C. jejuni*on on chicken breasts.

The limitation of the traditional method of applying additives to food is that the active compounds are degraded during processing and due to reactions with constituents of the food, and for these protection ceases and the quality of food degrades at an increased rate (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010). Another limitation is its inability to selectively target the food surface where most spoilage reactions occur; as a result, an excess of active compound is also unnecessarily added to the food product (Dias et al., 2013a). Controlled release packaging and the encapsulation of active compounds can overcome these two limitations by continuously replenishing active compounds to the food surface, compensating for the consumption or degradation of active compounds, so that a predetermined concentration of active compound is maintained in the food to achieve the desired shelf life (Malheiros, Daroit, Silveira, & Brandelli, 2010; Mastromatteo et al., 2010).

A carbon nanotube (CNT) is characterised by the winding of one or several graphene sheets concentrically with a diameter of nanometre dimensions and an internal hollow cavity (Zarbin, 2007), indicating the possibility of entrapping active substances such as volatile antimicrobials that must be encapsulated. Nano-encapsulation involves the incorporation, absorption or dispersion of bioactive compounds into small vesicles with nano- (or submicron-) sized diameters (Bouwmeester et al., 2009).

The use of nanoparticles for the controlled release of active agents was reported by Sanchez-Garcia, Ocio, Gimenez, and Lagaron (2008), who developed nanocomposites of polycaprolactone and natural mica modified for controlled-release of thymol, a natural biocide. Tunç and Duman (2010) reported that the use of montmorillonite in methyl cellulose film decreased the release of carvacrol. Specifically regarding carbon nanotubes, a previous study showed that the amount of AIT in cellulose-based films with CNTs was proportional to the CNTs amount, confirming their efficacy to retain the volatile AIT in the films (Dias

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et al., 2013b).

Knowledge of the release rate and therefore, of the diffusivity of the active agents from the film matrix into the food, is a determinant factor in the development of an antimicrobial film (Appendini & Hotchkiss, 2002; Bierhalz, Silva, & Kieckbusch, 2012). Mathematical modelling of mass transfer is necessary for in-depth understanding and optimising active systems. Although models to describe controlled release mechanisms exist, most of these studies have been conducted using food simulating systems without taking into account the potential effects of such a type of release system on actual food (Mastromatteo et al., 2010)

The diffusion of active agents from the package is related to the morphology and thermal properties of the material and can be evaluated using scanning electron microscopy and differential scanning calorimetry, among other analytical methods. Through these analyses, it is possible to evaluate if the film features a compact or porous structure, the content of crystallinity and the glass transition temperature, which is the temperature from which diffusion becomes more relevant.

In preliminary studies, cellulose-based antimicrobial films with 20% of AIT exhibited antimicrobial activity against *Salmonella choleraesuis* in vitro and in food during 40 days of conditioning (Dias et al., 2013b). Based on these results, the aims of this study were the following: (i) to determine the thermal and morphological properties of films with encapsulated and non-encapsulated AIT, with and without CNTs, (ii) to analyse the kinetics of diffusion of AIT from the cellulose acetate films into hexane and shredded cooked chicken and (iii) to compare the diffusion of AIT in these two systems.

2. Material and methods

2.1. Materials

The materials used for this research were the following: cellulose acetate (CA) polymer (Rhodia Co., Courbevoie, France); acetone (Merck, Darmstadt, Germany); AIT from oil of mustard (94%, Sigma-Aldrich Chemie, Steinheim, Germany); β-cyclodextrin (CD) (Sigma); nanoparticle CNT (CNT; Ahwahnee Technology, San Jose, CA, USA) and hexane (Sigma).

2.2. The production of the films

The antimicrobial films (Table 1) composed of cellulose acetate (CA) were obtained using a casting technique. The film solution was prepared by dissolving the cellulose acetate in acetone (Soares, 1998). AIT (20 mL of AIT/100 g of polymer) was dissolved in the film solution in both encapsulated and non-encapsulated forms. Encapsulation of AIT in β-cyclodextrin (CD) was performed according to the method of Zhang, Jiang, and Li (2007). The process for encapsulating the AIT consisted basically of an ethanol solution CD and AIT stirred in ice bath for 6 h and subsequent filtration on filter paper. CNT nanoparticles (0 and 0.1 g of CNT/100 g of polymer) were also added to the film solution according to the method described in patent PI 1004748-4 (Dias, Soares, Borges, & Medeiros, 2011). The film solution was applied to a support/mould using a casting machine (K-Paint applicator, Model 202, Litlington, Royston, UK), and then the solvent was evaporated. The four types of film that were developed based on AIT and CNT amount are described in Table 1.

2.3. Thickness

The film thickness was measured at 10 different points on each film using a micrometre (Mitutoyo 0.01 mm, Mitutoyo Co., Musashi Shinden, Tokyo, Japan). The results shown are the average thickness of three replicates of each film.

2.4. The concentration of AIT in the films

To quantify the AIT present in the films, three samples with an area of 16 cm^2 were taken from each film and placed in screw-cap test tubes. It was added hexane (10 mL, Sigma) to each film sample and mixed at 2500 rpm for 1 min. The samples were left overnight at 4 °C. After this period, the tubes were shaken again for one minute and the hexane was filtered through a 0.22-μm Millipore membrane ® (Millipore Corp. Billerica, Mass., U.S.A.) and stored in vials of approximately 2 mL (Chacon, Buffo, & Holley, 2006). With the aid of a gastight syringe (Hamilton), an aliquot (10 μL) was taken from the vial through the septum and injected into a gas chromatograph coupled to a mass spectrometer (GC/MS; QP 5050, DB5 column, Shimadzu Co., Kyoto, Japan) and an autosampler (Shimadzu Co., Kyoto, Japan). The operating conditions of the GC–MS were the following: column starting temperature, 60 °C; maintained for 2 min; increased to 90 °C at a rate of 12.5 °C/min and maintained for 45 s at 90 °C. The injector temperature was 250 °C and helium was used as the carrier gas at a flow rate of 1 mL/min (Nadarajah et al., 2005a).

To quantify the AIT compound, a standard curve was obtained using a range of known concentrations of AIT. Based on preliminary tests, the method used for the extraction was able to extract 86% of the AIT.

2.5. Thermal properties of the films

2.5.1. Thermogravimetric analysis (TGA)

The samples thermal stability and degradation profile were determined by thermogravimetric analysis (TGA) using a DTG-60H Shimadzu. It was used nitrogen atmosphere with 50 mL/min of flow, a temperature range of 25 °C–600 °C and heating rate of 10 °C/min.

2.5.2. Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (DSC TA 60, TA Instrument, New Castle, DE, USA) was used to determine the glass transition and melting temperatures (Tg and Tm) and percentage of crystallinity (Xc). Film samples were heated at a heating rate of 10 °C/min. Given the samples degradation temperature observed on TGA analysis, the following sequence was adopted for DSC analysis: 1) an initial heating run from 20 to 240 °C was performed, and the material was maintained at this temperature for 5 min to eliminate the thermal history; 2) the material was cooled from 240 to −100 °C, and the material was

Table 1
Matrix of the designed films with their compounds and the experimental results.

Film	Type of AIT	CNTs (%)	Thickness (cm)	AIT in the films (%)	Tg (°C)	Tm (°C)	Xc (%)	D_1 ($cm^2 s^{-1}$)	D_2 ($cm^2 s^{-1}$)	n
1	Non-encapsulated	0	0.0033 ± 0.0006	74.4	187.8	230.8	0.9	$2.40{\cdot}10^{-11} \pm 1.07{\cdot}10^{-12}$	$2.80{\cdot}10^{-11} \pm 7.02{\cdot}10^{-12}$	$0.5732 \pm 2.83{\cdot}10^{-2}$
2	Non-encapsulated	0.1	0.0033 ± 0.0006	81.0	188.1	227.8	2.1	$2.01{\cdot}10^{-11} \pm 9.71{\cdot}10^{-13}$	$2.63{\cdot}10^{-11} \pm 4.65{\cdot}10^{-12}$	$0.5319 \pm 4.17{\cdot}10^{-2}$
3	Encapsulated	0	0.0036 ± 0.0006	97.7	192.9	–	–	$3.58{\cdot}10^{-11} \pm 3.70{\cdot}10^{-12}$	$2.62{\cdot}10^{-11} \pm 8.69{\cdot}10^{-12}$	$0.4898 \pm 5.78{\cdot}10^{-2}$
4	Encapsulated	0.1	0.004 ± 0.0003	87.1	189.9	294.7	16.0	$7.28{\cdot}10^{-10} \pm 6.00{\cdot}10^{-11}$	$5.69{\cdot}10^{-10} \pm 1.52{\cdot}10^{-10}$	$0.9796 \pm 9.58{\cdot}10^{-4}$

[1] The mean values of D ± standard error obtained using Eq. (1) (analytical solution)
[2] The mean values of D ± standard error obtained using Eq. (2) (short time)