Influence of supercritical carbon dioxide impregnation conditions on cinnamaldehyde effectiveness as antimicrobial agent in biodegradable films

Souza, A. C.[1]; Dias, A. M. A.[2], Sousa, H. J. C.[2]; Tadini, C. C.[1]
[1]Chemical Eng. Dept., Escola Politécnica, University of São Paulo, SP, Brazil
[2]Chemical Eng. Dept., University of Coimbra, Coimbra, Portugal
catadini@usp.br

Abstract

Bio-polymeric materials, designed to be completely biodegradable, are currently attracting a great deal of attention from researchers and industry. In this way, the development of a biodegradable packaging based on cassava starch, glycerol and clay nanoparticles, and incorporated with cinnamaldehyde as antimicrobial agent showed to be a promising field of study. Biodegradable films previously produced by casting process were loaded with cinnamaldehyde by using a supercritical carbon dioxide (sc-CO_2) impregnation method, performed in high pressure equipment operated at 35 ºC and 250 bar. Cinnamaldehyde amount impregnated was quantified by spectroscopy analysis and the water vapor permeability was calculated by gravimetric method. Films submitted to 15 h of impregnation and at 10 bar·min^{-1} of depressurization rate presented the best result of cinnamaldehyde loaded: (2.49 ± 0.30) mg/g of the film. Regarding the water vapor permeability, the values decreased from (10.09 ± 0.35) g·mm·m^{-2}·day^{-1}·kPa^{-1} for films without impregnation to (4.07 ± 0.82) g·mm·m^{-2}·day^{-1}·kPa^{-1}, when the bio-polymer was impregnated with cinnamaldehyde at the best condition. Furthermore, FTIR (Fourier transform infrared) spectra showed interactions between cinnamaldehyde and polymer molecules, that suggests a good affinity.

Introduction

Consumer demands for safer, environmentally friendly and higher quality products have increased and thus technical solutions for the garbage disposal problem are being pursued. Historically most of this waste material was destined to landfills, but increasing cost of land and diminishing availability of land for this use has limited further employment of this method for dealing with garbage. Incineration also has been frequently used despite issues related to air pollution control. More recently recycling was introduced, but full implementation of these policies worldwide are still deficient and other issues like population growth and increasing industrialization have been the driving force for research for other technical solutions. In this context, development of biodegradable films for packaging materials that can be used as a substitute for petrochemical polymers is an interesting perspective, since it provides an alternative to non-degradable products, and also increases income in the agricultural sector (Souza, Ditchifield, Tadini, 2010).

Although many types of biodegradable polymers are being industrially produced (PLA, PHA, PCL, PEA and others), polymers from agricultural sources are the most studied by researchers, especially polysaccharides. This family is represented by different products such as starch or cellulose based on glucose units linked in macromolecular chains. Among the films made from polysaccharides, those obtained from starch are the most important because starch is one of the most commonly used agricultural raw materials, since it is a renewable source, inexpensive (even cheaper than polyethylene), widely available and relatively easy to handle. Besides, it is found in several forms according to the origin of its raw material (Souza, Ditchifield, Tadini, 2010).

Cassava starch has been extensively used to produce biodegradable films (Müller, Yamashita, Laurindo, 2008; Paes, Yakimets, Mitchell, 2008; Veiga-Santos et al., 2008; Kechichian et al., 2010) and the results indicated that these carbohydrates are promising materials in this regard. Films developed from starch are described as isotropic, odourless, tasteless, colourless, non-toxic and biologically degradable (Flores et al., 2007). Furthermore, Brazil is the second largest cassava producing country, behind only Nigeria, with a production of 26.03 million metric tons (Mt) in 2009, representing approximately 13% of the world production (FAO 2011).

Usually, the second major component of a starch based film is the plasticizer that is used to overcome film brittleness caused by high intermolecular forces. Some authors consider that the glycerol, a polyalcohol found naturally in a combined form as glycerides in animal and vegetable fats and oils, is the best plasticizer for water soluble polymers (Bertuzzi et al., 2007; Müller, Yamashita, Laurindo, 2008).

Another technological driver is the progress of nanotechnology, which also offers new possibilities for bio-based polymers (Souza, Ditchifield, Tadini, 2010). The production of bionanocomposites has proven to be a promising option, since polymer composites are increasingly gaining importance as substitute materials due to their superior tensile properties, making them especially suited for transportation and packaging applications.

Mineral clays are technologically important and are mainly composed of hydrated aluminosilicate with neutral or negative charged layers (Wilhelm et al., 2003). Clay is a potential filler; itself a naturally abundant mineral that is toxin-free and can be used as one of the components for food, medical, cosmetic and healthcare products (Chen, Evans, 2005). Moreover, clay is environmentally friendly and inexpensive.

Cassava based materials are already known to be applied as interesting packaging material for several food products, mainly because of their good film-forming. In this context, biodegradable films carrying natural additives, such as antimicrobial agents, could be considered the developing tendency of functional food packaging in the near future. Active packaging provides microbial safety for consumers, reducing, inhibiting or retarding the growth of microorganisms, and then, could extend the shelf life of the packaged food.

Cinnamon has been used as a spice for thousands of years. The main constituent of his bark oil is the cinnamaldehyde, a well known agent due to its antimicrobial activity. In this work, cinnamaldehyde were impregnated into cassava starch membranes using a Supercritical Solvent Impregnation (*SSI*) method and employing supercritical carbon dioxide (sc-CO_2) as the carrier solvent and the polymer swelling/plasticizing agent. Besides its greener characteristics, the *SSI* method also permits to tune agent loading and agent penetration by controlling the depressurization step and impregnation period.

The main goal of this work was to develop active and biodegradable films, and also to quantify, by spectroscopy analysis, cinnamaldehyde contents impregnated/released on each condition of impregnation period and depressurization rate studied. Besides, interactions between polymer and antimicrobial agent were studied with FTIR (Fourier transform infrared) spectroscopy and water vapor permeability of produced membranes was compared with results of membranes without impregnation.

Materials and Methods

Materials

Native cassava starch, kindly supplied by Cargill Agrícola, Brazil (amylose: 19.7 g/100 g; amylopectin: 80.3 g/100 g; moisture: 12.5 g/100 g) was used as the film forming component to provide a continuous biodegradable film matrix. Glycerol (Synth, Brazil) was added as plasticizer to improve their flexibility. Natural -Na montmorillonite clay (commercial product Argel T, used as received, without purification, Bentonit União, Brazil) was used as filler. Distilled water and ethanol (Synth, Brazil) were used as solvents for the filmogenic solutions. Cinnamaldehyde was used as antimicrobial agent. Carbon dioxide (CO_2) was used in the impregnation experiments.

Film preparation

The filmogenic solution was prepared in three steps: firstly, 0.10 g of clay nanoparticles were suspended in distilled water for 1 h, and, after a rest period of 24 h, they were blended with a suspension of 5.0 g of starch, 1.5 g of glycerol, and 95 g of distilled water. After homogenization, this solution was heated in a domestic microwave oven until starch gelatinization, which occurs at (69 ± 2) ºC. After cooling, this solution was diluted with 14.25 g of ethanol, and, poured onto

cylindrical plates and dried at (35 ± 2) ºC for (18-24) h, in an oven with forced air circulation. After drying, all films were stored at a controlled relative humidity of 75 % prior to testing.

Supercritical solvent impregnation (*SSI*) experiments

Impregnation process in sc-CO_2 was performed in high pressure equipment running in the batch mode (Cipriano de Sousa et al., 2006). In general terms, an experimental *SSI* assay consists in introducing compressed or supercritical CO_2, until a desired operational pressure is achieved, into the immersed impregnation cell (at operation temperatures leading to supercritical conditions). The cell contains biodegradable films to be impregnated (6 samples of 1.2 cm × 1.2 cm) in a stainless steel support and also the antimicrobial agent. This agent is solubilized in scCO_2 and homogenization is achieved by magnetic stirring. After a pre-established impregnation duration time (the period in which the cinnamaldehyde and the membranes are in direct contact), the system is then depressurized.

Two impregnation duration times (3 h and 15 h) and two depressurization rates (1 bar·min^{-1} and 10 bar·min^{-1}) were chosen for this work. The employed amount of cinnamaldehyde (0.20 g) was calculated according to the operational pressure (250 bar) and the temperature condition (35 °C), and taking in consideration the agent solubility in scCO_2 at the employed operational conditions, the cell volume (10 cm^3) and the membranes weight (~ 50 mg for each sample).

Water vapor permeability (*WVP*)

A gravimetric method based on ASTM E96/E96M-05 (2005), using the Desiccant Method, was applied.

Cinnamaldehyde release experiments

Antimicrobial release studies were performed using a spectrophotometer (JASCO, model 530, Japan) at 289 nm. Antimicrobial agent concentration was calculated using previously determined calibration curve prepared in milliQ water.

Fourier transform infrared (*FTIR*) spectroscopy

A spectrophotometer (Perkin Elmer, Paragon 1000 PC, UK) was used to measure FTIR spectra, in the range of (600-4000) cm^{-1}, with a resolution of 32 cm^{-1}.

Results and Discussion

Biodegradable films produced were homogeneous, transparent and flexible, and their surfaces were smooth, continuous and homogeneous, without pores and cracks, or insoluble particles (Figure 1). After impregnation with cinnamaldehyde, using *SSI* technique, films presented the same characteristics (Figure 2a), except for films impregnated in the condition of 15 h of duration time and 10 bar·min^{-1} of depressuration rate, which presented white color (Figure 2b).

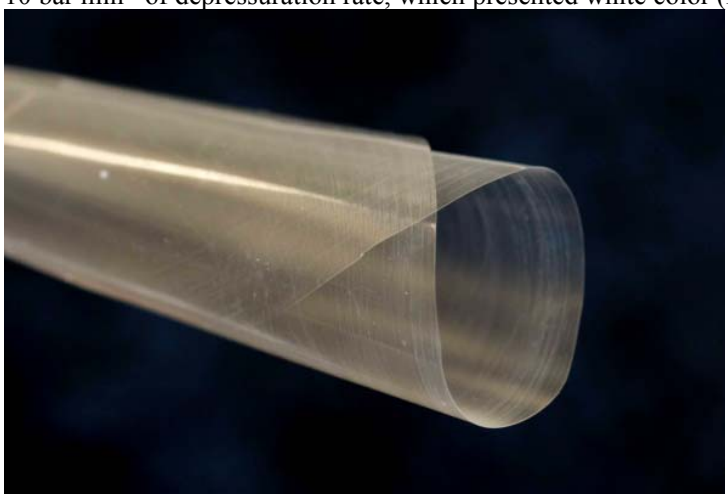

Figure 1. Biodegradable film based on cassava starch, glycerol and clay nanoparticles (Picture of Eduardo Cesar Soares Faria de Oliveira).

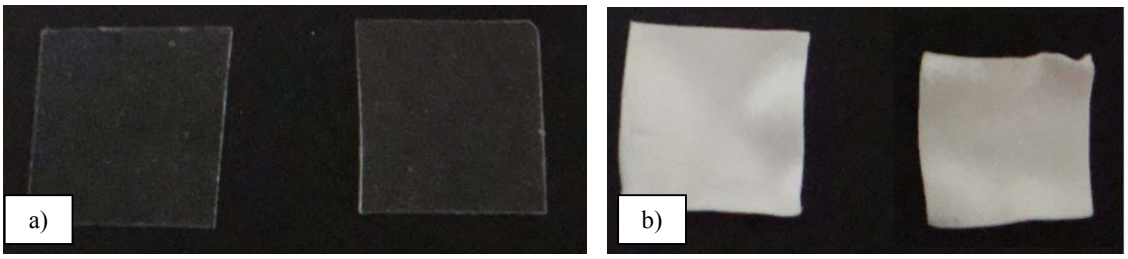

Figure 2. Biodegradable film based on cassava starch, glycerol and clay nanoparticles impregnated with cinnamaldehyde by *SSI* technique in two different conditions: a) t_i: 3 h and d_r: 10 bar·min^{-1}; b) t_i: 15 h and d_r: 10 bar·min^{-1}.

In Table 1, the positive influence caused by the cinnamaldehyde incorporation on water vapor permeability can be noticed. Moreover, impregnation duration times (t_i) and depressurization rates (d_r) significantly affect *WVP* results. The applied technique caused a decrease on *WVP* results due to the loss of glycerol during the system depressurization (probably because its high solubility in CO_2) and also to the incorporation of a new component that served as a barrier against water vapor. Despite the loss of glycerol, films keep their flexibility because sc-CO_2 also acts as a plasticizing agent.

Table 1. Water vapor permeability (WVP) of biodegradable films based on cassava starch, glycerol and clay nanoparticles, before and after cinnamaldehyde impregnation according different impregnation duration times (t_i) and depressurization rates (d_r).

	t_i [h]	d_r [bar·min^{-1}]	*WVP** [g·mm·m^{-2}·day^{-1}·KPa^{-1}]
Before impregnation	-	-	10,09 ± 0,35 ^{a}
After impregnation	3	1	7,27 ± 1,01 ^{bc}
	3	10	7,36 ± 1,43 ^{bc}
	15	1	5,64 ± 0,69 ^{c}
	15	10	4,07 ± 0,82 ^{c}

* Means with the same letter are not significantly different ($P > 0.05$).

Regarding cinnamaldehyde release experiments, Figure 3 compares the released amounts of antimicrobial agent, for a monitoring period of 3 h, from starch based films processed at the experimental conditions studied in this work (250 bar, 35 °C). It can be realized an increase of cinnamaldehyde content impregnated with depressuration rate elevation from 1 bar·min^{-1} to 10 bar·min^{-1}.
In previous work, authors concluded that an amount of (0.86 ± 0.07) mg of cinnamaldehyde per g of film could inhibited selected fungi (*Pennicilium commune* and *Eurotium amstelodami*), commonly found in bread products. In this way, it can be concluded that all conditions studied in this work was able to supply active films against the selected microorganisms.

Cinnamaldehyde higher affinity for starch based films may justify the observed higher impregnated amounts. Despite its lower solubility in water (1 g·L^{-1}), the release during the first hour during release experiments was fast.

With the presented results, it can be concluded that the best condition of supercritical impregnation was 15 hours of duration time and 10 bar·min^{-1} of depressuration rate since this condition yielded films with the largest content of cinnamaldehyde impregnated and the smallest result of *WVP*.

FTIR spectra of impregnated and of non-impregnated starch based membranes, presented in Figure 4, shows significant differences in the range of (1200 and 1400) cm^{-1} and of (1600 and 1700) cm^{-1}, which correspond to the aromatic ring found in the chemical structure of cinnamaldehyde, suggesting that there is cinnamaldehyde present inside the polymeric matrix.

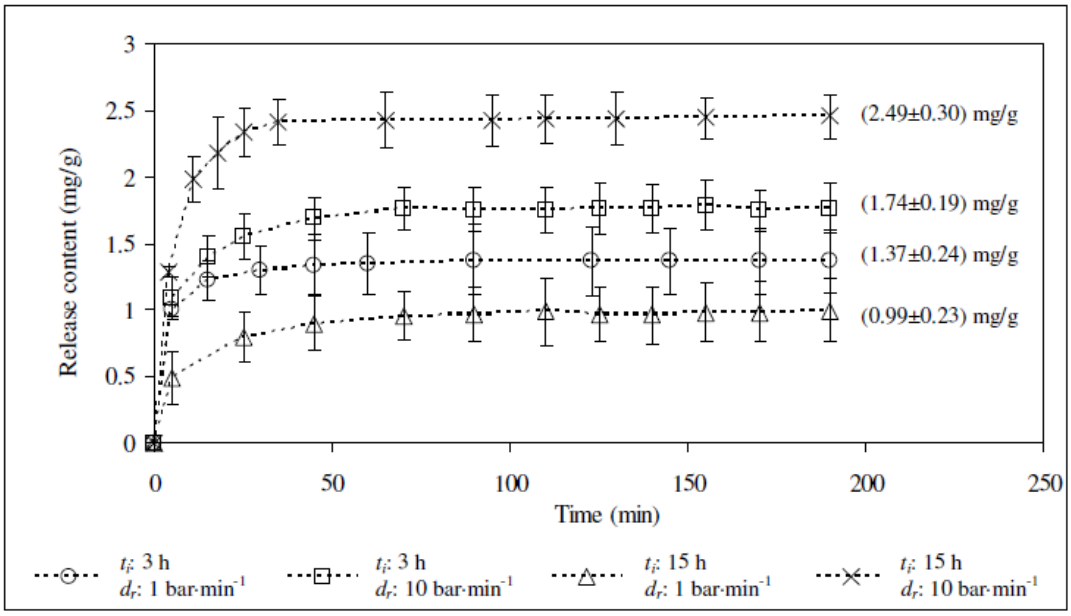

Figure 3. Released content of cinnamaldehyde [mg $_{cinnamaldehyde}$/g $_{film}$] in samples of biodegradable film based on cassava starch, glycerol and clay nanoparticles impregnated by supercritical fluid technique.

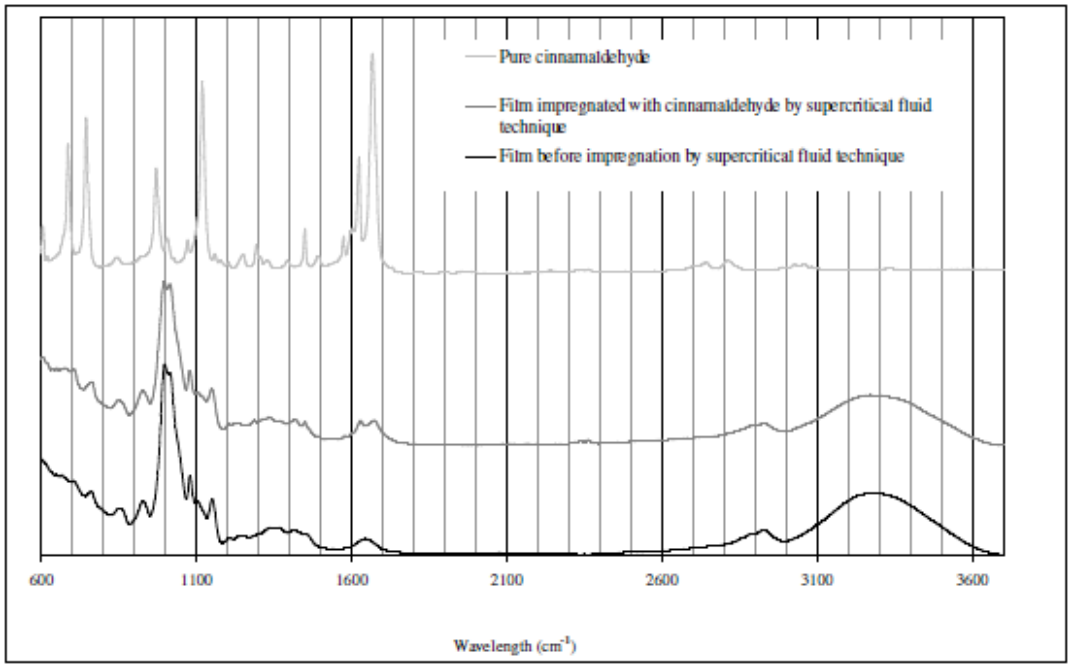

Figure 4. FTIR spectra of biodegradable film before and after cinnamaldehyde impregnation by supercritical fluid technique and FTIR spectra of pure cinnamaldehyde.

Conclusions

The main objective of this work was achieved since films based on cassava starch were successfully incorporated with an antimicrobial agent (cinnamaldehyde) by the use of a green method (Supercritical Solvent Impregnation - *SSI*), supplying biodegradable and active films. Obtained results showed that different impregnation conditions was able to supply films with different cinnamaldehyde load and also with different barrier property, concluding that the best condition of supercritical impregnation was 15 hours of duration time and 10 bar·min^{-1} of depressuration rate. Furthermore, aromatic ring present in the chemical structure of cinnamaldehyde was found in the impregnated film,

showed in FTIR spectra, indicating that there is cinnamaldehyde incorporated inside the polymeric matrix.

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