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Natural copaiba oil as antibacterial agent for bio-based active packaging

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ABSTRACT

This study investigates the antibacterial properties of the natural oil from *Copaifera multijuga* which was embedded in bio-based materials, to be used in active packaging applications. For this purpose, the naturally extracted copaiba oil was first characterized by gas chromatography and thermogravimetry. Its antibacterial properties were evaluated and confirmed against gram-positive bacteria (*Bacillus subtilis*). The copaiba oil was incorporated into two bio-based materials: (i) paper and (ii) poly(lactic acid), PLA, films. This incorporation was made following two strategies: either at the surface, thanks to a coating process, or in the bulk, through impregnation (paper samples) or by film casting (PLA films). The antibacterial tests confirmed activity against *B. subtilis* in paper with a copaiba oil content of approximately 20 wt%. For the PLA films, the same amount showed satisfactory results against the same bacteria. To the best of our knowledge, it is the first time that copaiba oil has been incorporated on paper sheets and plastic films, with the confirmation of the maintenance of the effective antibacterial properties. The materials developed in this work through simple procedures have great perspective to be used as biodegradable packaging with bactericide effect, to improve the shelf life of food products.

1. Introduction

Good quality and freshness of food can be compromised by possible contamination with microorganisms such as bacteria and fungi, which cause waste and also can affect human health. A recent study (FAO, 2013), highlighted the environmental impact of the global food wastage. It showed that the volume of food produced and not eaten is responsible for adding 3.3 billion tons of greenhouse gases to the planet's atmosphere. The producers are also subjected to a direct economic loss (about $750 billion annually). Furthermore, the risk of food poisoning is still present with new cases registered every year affecting both developed and developing countries (Burke, 2013).

Solutions are thus, required to enhance the food safety and the protection of these valued nutritional substances. Several strategies have been developed and are usually gathered in the range of active packaging (as described by the Regulation (EC) no. 1935/2004), which consists mainly in: (i) antimicrobial or (ii) scavenging solutions (Catalá et al., 2008). Regarding antimicrobial solution, bio-based agents like essential oil can be envisaged. Indeed some natural antimicrobial agents are incorporated today directly into the food (Tajkarimi et al., 2010; Baydar et al., 2004). However, these agents tend to alter the food flavor, which may be a problem. The application of edible coatings has been studied, in order to extend the food shelf life (Campos et al., 2011; Sánchez-González et al., 2011). Nonetheless, generally they have low mechanical strength and low resistance to water vapor. With packaging, foods can be better protected, even during their transport, and tend to have their shelf lives increased. Aiming to improve the packaging efficiency against the action of microbes, antimicrobial agents can be incorporated (Lagarón et al., 2011; Kuorwel et al., 2011; Vermeiren et al., 2002).

Due to their light weights, versatility and satisfactory properties, paper and plastic are widely used as packaging materials. They represent 34% and 37%, respectively, of the total amount of packaging materials and are available in the form of trays, boxes, pots, bags or films, depending on the material and application. Bio-based issues and biodegradability are also important requirements in this field, because packaging has a relatively short life time. Therefore, in this work, two bio-based and biodegradable materials were

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chosen: (i) paper and (ii) poly(lactic acid), PLA. PLA is one of the most industrially available bioplastics, having also the advantage of being biocompatible and bioabsorbable, being used in human prostheses, sutures and delivery systems (Avérous, 2008). The antimicrobial property is also interesting in this kind of application, because it can reduce the risk of infection after a surgical procedure.

Such antimicrobial properties can be obtained by addition of several molecules or macromolecules as detailed in recent reviews (Balasubramanian et al., 2009; Cha and Chinnan, 2004; Joerger, 2007; Khwaldia et al., 2010). By focusing on the use of essential oil, some researchers already have tried to encapsulate it (Abreu et al., 2012; Hosseini et al., 2013) or to add it directly in polymer films (Pelissari et al. (2009), with starch films and oregano essential oil; or Mastromatteo et al. (2009), incorporating thymol in zein based-films). However, in each case, classic essential oils were used and either the flavor was too strong or the antimicrobial activity was not efficient enough. That is why new essential oils with less flavor and higher efficiency must be studied.

Copaiba oil could be one good alternative. It is extracted directly from the trunk of its tree and its correct denomination is oleoresin. The copaiba tree is native from tropical regions of Latin America and West Africa. In Brazil, it can be easily found in the Amazonian and Central regions (Veiga et al., 2002). In the Brazilian Amazon there are numerous traditional families living from the sustainable exploitation of the forest. The extraction of oils and seeds is one of these sustainable activities and helps to avoid the deforestation of the trees related to these activities (Morais and Gutjahr, 2009). A copaiba tree can survive up to 400 years and the sustainable oil extraction is made through a hole drilled in the trunk. After drilling and extracting the oil, the hole in the tree trunk can be closed with clay or a wood stopper until the next oil collection, without damage to the tree. About 4–5 L of oil can be extracted from an adult copaiba tree in each collection, although this quantity depends on the species, the age of the tree and the environment (Veiga et al., 2002; Morais and Gutjahr, 2009).

In Brazilian popular traditional medicine, copaiba oil has been used as a healing agent, anti-inflammatory and antibiotic for a long time (Veiga et al., 2001; Santos et al., 2008), being swallowed or applied directly on the skin surface. A recent work, studied the use of copaiba oleoresine applied in electrospun mats for wound care applications (Millas et al., 2014). Nonetheless, no study was found about the incorporation of copaiba oil into bulk materials as paper and plastics for packaging applications.

Copaiba oil is approved by the Food and Drug Administration (FDA) since 1972, being suitable for food contact (Veiga et al., 2002). It is also widely used by the cosmetic and varnish industries. Today it is one of the most important natural commercial products in the North of Brazil. According to the Brazilian Institute of Geography and Statistics, in 2010, 580 t of copaiba oil were produced, mainly in the state of Amazonas (IBGE, 2013). This oil is also exported to the United States, France, Germany and England (Veiga et al., 2002).

Aiming to incorporate antimicrobial properties to paper and PLA and to preserve their characteristics of biodegradability and non-toxicity, the copaiba oil was incorporated for the first time on paper sheets and plastic films, and their antibacterial properties were evaluated against gram-positive bacteria, *Bacillus subtilis*.

2. Materials and methods

2.1. Materials

In this study, the copaiba oleoresin was used, supplied by *Naturais da Amazônia*, naturally extracted from the species *Copaifera multijuga* from the Amazon rainforest, in the state of Pará, Brazil. The oil was submitted only to a simple filtration process by the supplier. It is a yellow to light-brown liquid and it was used as received in this study.

The poly(lactic acid), PLA 2002D, was supplied by NatureWorks. A paper from Papyrus Plano Superior, extra-white, with a basis weight of 80 g m^{-2} was also used. Chloroform (purity ≥ 99.5%) and heptane (99.5%) were acquired from Sigma–Aldrich and used as received.

A suspension of *B. Subtilis* (10^7 spores mL^{-1}) was purchased from Humeau (France) and used as test microorganism to evaluate the antibacterial activity. A nutrient agar adapted to the development of the spores was also purchased from Humeau (France). The agar powder was dissolved in deionized water to reach a final concentration of 28 g L^{-1} and boiled to obtain a clear nutrient agar solution. Petri dishes with a diameter of 90 mm were purchased from Humeau (France) and used for the antibacterial tests.

2.2. Characterization of the copaiba oil

Gas chromatography coupled with mass spectrometry (GC/MS) was performed with an equipment Agilent 6850/5975C, operated in the electronic impact (EI) mode at 70 eV of electron energy. The analyses were performed with a heating program from 50 to 300 °C, during 15 min, in a HP-5MS 5% phenylmethyl siloxane column. For this analysis, copaiba oil was completely dissolved in acetone, in the proportion of 5 mg cm^{-1}.

The rheological characterization of the pure copaiba oil was performed with a rheometer Physica MCR 301 from Anton Paar at air atmosphere. Shear viscosity versus shear rate curves were obtained at three different temperatures: 25, 50 and 70 °C. A 50 mm diameter parallel-plate fixture and a gap of 0.05 mm were used.

Thermogravimetric analysis was done with equipment STA 6000 from PerkinElmer, from 30 °C to 600 °C, at a heating rate of 20 °C min^{-1}, under air atmosphere (50 mL min^{-1}).

2.3. Impregnation and coating of paper with copaiba oil

For the impregnation process, discs of 16 mm of 80 g m^{-2} porous paper were immersed into the pure copaiba oil for 1 h, at 25 °C, and dried for 4 days at the same temperature and 50% humidity.

For the coating process, the copaiba oil was applied directly onto the paper surface using a universal coating machine (Endupap) with a velocity of 5 cm cm^{-1} and a Meyer bar of 0.7 mm. The same paper described above was used. The coated paper was also dried at room temperature (25 °C) and 50% humidity during 4 days.

Just before the antibacterial assay, the final content of the copaiba oil into the paper samples was determined weighting the samples before and after the incorporation processes.

2.4. Casting and coating of PLA with copaiba oil

The poly(lactic acid) was dissolved in chloroform after magnetic stirring for 2 h at room temperature (around 25 °C). It was then mixed with a solution of the copaiba oil (also in chloroform). Different nominal contents of copaiba oil in the PLA matrix were studied: 1, 5, 10, 20, 30, 40 and 50 wt%. The mixture was casted into a polytetrafluoroethylene (PTFE) mold with a diameter of 10 cm and dried at room temperature under controlled evaporation system.

For the coating process, films of PLA were obtained by extrusion in a DSM Xplorer 15 mL micro-extruder, at 190 °C, with a screw speed of 80 rpm and residence time of 2 min. The coating of PLA film surface by copaiba oil was performed as described previously for paper coating.

The final contents of copaiba oil into the PLA samples submitted to the antibacterial assay were determined weighting the samples before and after the incorporation processes. In the case of the films obtained by casting, the quantity of PLA added to the process was