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Biological activities and major components determination in essential oils intended for a biodegradable food packaging

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ABSTRACT

Since ancient times, aromatic plants have been used in food and in folk medicine. Currently, essential oils and their compounds are attracting great interest due to their proven action to preserve food quality, to extend foodsí shelf-life and due to be natural. Essential oils from *Ocimum basilicum* (basil), *Cinnamomun cassia* (cinnamon), *Cinnamomun zeylanicum* (cinnamon) and *Rosmarinus officinalis* (rosemary) were analyzed by Gas Chromatography coupled with Flame Ionization Detector and with Mass Spectroscopy and by Ultra High Performance Liquid Chromatography with a Diode Array Detector. The antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Penicillium* spp and antioxidant activities by FRAP, DPPH•, $ABTS^{\bullet+}$ and β-carotene bleaching assays were evaluated. *C. cassia* essential oils presented a strong antimicrobial activity, with a range of minimum inhibitory concentrations between 0.04–0.07 mg mL^{-1}, depending on the microorganism tested. The main compound of *C. cassia* essential oil, cinnamaldehyde, showed antimicrobial effectiveness. In general, *C. zeylanicum* essential oil and its major compound, eugenol, presented the highest antioxidant activity. Ultra High Performance Liquid Chromatography confirmed the results of the essential oils main constituents, analyzed firstly by Gas Chromatography. Ultra High Performance Liquid Chromatography technique showed to be a valuable alternative tool for the identification and quantification of the major compounds of essential oils, especially when these are incorporated into food packaging and there is the need of carrying out migration studies in food or food simulants. Cinnamon essential oils exhibited the highest biological activity directly related to its major compounds, eugenol and cinnamaldehyde. Essential oils showed to have high antioxidant and antimicrobial activity and therefore, they have great potential as natural additives of food packaging.

1. Introduction

Since ancient times, aromatic plants have been used in folk medicine, like traditional Chinese medicine and Ayurvedic (the Indian traditional medicine) and also to extend the shelf-life of some foods (Sartoratto et al., 2004; Ulbricht et al., 2008). These plants naturally produce essential oils (EOs) as secondary metabolites that provide several properties to the plant, like antimicrobial and antioxidant properties (Sartoratto et al., 2004).

EOs are natural aromatic volatile liquids extracted from different parts of plants such as flowers, buds, seeds, leaves, stems and bark (Burt, 2004; Dvaranauskaité et al., 2009; Aidi Wannes et al., 2010; Lv et al., 2012; Hill et al., 2013). The composition and physicochemical properties of EOs are greatly influenced by the species, part of plant used, geographic origin, time of harvest, stage of development, age of plants and extraction method (Khajeh et al., 2005; Elzaawely

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Table 1
Description of essential oils according to the supplier.[a]

Plant used to prepare the essential oil	Scientific name of the plant	Part of the plant	Origin
Basil	*Ocimum basilicum* L.	Leaves	India
Cinnamon cassia	*Cinnamomum cassia* (L.) J. Presl	Stem, bark, leaves	China
Cinnamon	*Cinnamomum zeylanicum* Blume	Leaves	China
Rosemary	*Rosmarinus officinalis* L.	Leaves	Tunisia

[a] Ferquima®.

et al., 2007; Negi, 2012; Riahi et al., 2013; Costa et al., 2015; Ribeiro-Santos et al., 2015).

Many EOs are considered GRAS (Generally Recognized as Safe) food additives by the Food and Drug Administration (Food And Drug Administration (FDA), 2015). Therefore, they can be used as potential preservatives or flavoring agents in order to prolong the shelf-life of foods, reducing or eliminating pathogenic microorganisms and increasing the overall quality of food products (Keshvari et al., 2013; Dussault et al., 2014; Szczepanski and Lipski, 2014; Echegoyen and Nerín, 2015).

Lipid oxidation is one of the major causes of the deterioration in the quality of natural and processed foods, which originates huge losses to the food industry (McClements and Decker, 2000; Sanches Silva et al., 2004; Sanches-Silva et al., 2004).

The products of the lipid oxidation reactions are proved to be harmful for human health and they are associated with mutagenesis, cancer, cardiovascular and chronic inflammatory diseases (Márquez-Ruiz et al., 2008). Foods with high lipid content, are more susceptible to lipid deterioration. In fact, oxidation, mediated by free radical reactions, is responsible for the rancidity of unpreserved food rich in unsaturated fatty acids and the natural antioxidants are suggested as a good alternative to synthetic such as Butylated hydroxyanisole (BHA) or Butylated hydroxytoluene (BHT) (Sanches-Silva et al., 2007; André et al., 2010).

Nowadays, even in the developed countries, the foodborne diseases still represent a major concern. When certain microorganisms are present in our foods, in addition to represent great losses to the food industry, they are a threat to the human health (Hussain et al., 2008).

The use of EOs in food products is becoming popular since consumers are more conscious about potential health problems associated with synthetic preservatives. Therefore, there is an increasing interest in the use of natural antioxidants and antimicrobials for food preservation to inhibit the growth of foodborne pathogens and extend shelf-life of food products (Cacho et al., 2016). These natural compounds can be added directly to food or they can be incorporated in food packaging (Sanches-Silva et al., 2001, 2013, 2014).

The aim of this study was to evaluate the chemical composition (by GC and UHPLC) and *in vitro* antimicrobial and antioxidant activity of basil (*Ocimum basilicum* L.), cinnamon (from different species, *Cinnamomum zeylanicum* Blume and *Cinnamomum cassia* (L.) J. Presl) and rosemary (*Rosmarinus officinalis* L.) essential oils to prepare a formulation to be further used in active packaging. Three microorganisms were tested, *Escherichia coli*, *Staphylococcus aureus* and *Penicillium* spp., to evaluate the activity of the selected EOs.

2. Materials and methods

2.1. Essential oils and their main compounds

Four commercial EOs were acquired from Ferquima® (Ferquima Indústria e Comércio Ltda, Vargem Grande paulista, São Paulo). Table 1 compiles information regarding EOs such as their common name, plant variety, and part of the plant used for the extraction. All selected EOs were industrially produced by distillation and kept in an amber glass flask.

The main compounds present in the EOs were also studied. Compounds were acquired from Sigma-Aldrich® (Madrid, Spain), namely cinnamaldehyde, eugenol, methyl chavicol, 1,8-cineole and α-pinene.

The literature shows cinnamon, basil and rosemary EOs as potent antimicrobials and antioxidants. In addition, these plants have great health benefits as anti-tumor effect, anti-diabetic and anti-inflammatory activities (Singh et al., 2007; Hussain et al., 2008; Rao and Gan, 2014; Ribeiro-Santos et al., 2015).

2.2. Chromatography analysis

2.2.1. Gas chromatography flame ionization detector

A Gas chromatograph (Hewlett Packard-5890 series II) coupled with a flame ionization detector (CG-FID) and equipped with a Factor Four-VF-5 ms capillary column (Varian) (30 m × 0.25 mm, 0.25 μm film thickness) was used for quantification of EOs. The injector and detector temperatures were maintained at 220 and 250 °C, respectively. The amount of the injection of the samples was 1 μL in split mode (1:30). Carrier gas was helium at a flow rate of 1 mL min^{-1}. The oven ramp temperature was programmed as follows: 60 °C for 2 min, increased to110 °C at 2 °C min^{-1}, 110–150 °C at 3 °C min^{-1} and to 290 °C at 15 °C min^{-1}. The relative percentage of the oil constituents was expressed as percentage by area normalization.

2.3. Gas chromatography–mass spectrometry

Analyses of volatile oils were carried out on a Gas chromatography coupled with a mass spectrometer (GCMS-QP2010 Plus-Shimadzu) with a Factor Four-VF-5 ms capillary column (Varian) (30 m × 0.25 mm, 0.25 μm film thickness). The injector, GC–MS interface and ion source temperatures were maintained at 220, 310, and 250 °C, respectively, with ionization energy of 70 eV. The oven temperature was programmed as follows: 60 °C at 2 min, 60–110 °C at 2 °C min^{-1}, 110–150 °C at 3 °C min^{-1}, 290 °C at 15 °C min^{-1}. The identity of the components of the EOs was assigned by comparison of their retention indices and mass spectra with published data in the literature (Adams, 2007). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8-C_{20} indices.

2.4. Ultra high performance liquid chromatography

An UHPLC-diode array detector (UHPLC-DAD) method was developed and validated for simultaneously quantification of the major components of the selected EOs. An ACQUITY ™ UPLC® BEH C18 pre-column (2.1 × 5 mm, 1.7 μm particle size) and an ACQUITY™ UPLC® BEH Shield RP 18 column (2.1 × 100 mm, 1.7 μm particle size) were used. The mobile phase was a gradient of acetonitrile with 0.1% acetic acid (v/v) (solvent A) and ultra-pure water with 0.1% acetic acid (v/v) (solvent B). The major components of the EOs were quantified at the wavelengths that correspond to their maximum absorption. The method was validated regarding the parameters: linear range, linearity, limit of detection, limit of quantification and precision. The analytical standards were obtained from Sigma-Aldrich® (Madrid, Spain).

2.5. Determination of total phenolics content

The assay of Total Phenolics Content (TPC) was performed according to Erkan et al. (2008). Briefly, an aliquot of 1 mL of EO (at