Innovative Food Science and Emerging Technologies 36 (2016) 120–127

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

# Antimicrobial and antioxidant activity of essential oil from pink pepper tree (*Schinus terebinthifolius* Raddi) *in vitro* and in cheese experimentally contaminated with *Listeria monocytogenes*

Guilherme da Silva Dannenberg *, Graciele Daiana Funck, Fábio José Mattei, Wladimir Padilha da Silva, Ângela Maria Fiorentini

*Department of Agroindustrial Science and Technology, Federal University of Pelotas, 96010-900, Pelotas, Brazil*

ARTICLE INFO

*Article history:*
Received 10 March 2016
Received in revised form 8 June 2016
Accepted 13 June 2016
Available online 15 June 2016

*Keywords:*
Antimicrobial activity
Antioxidant activity
Biopreservation
Pink pepper tree
Essential oil
Cheese

ABSTRACT

Natural compounds with preservative activity have gained prominence in the field of food science as an alternative to traditional additives. To be effective, biopreservatives must have antioxidant and/or antimicrobial activities, characteristics often found in the essential oils (EO). This study aimed to verify the antimicrobial and antioxidant activity of EO from pink pepper tree fruit. Antimicrobial activity was evaluated *in vitro* on 18 bacteria, and *in situ* (Minas-type fresh cheese) against *Listeria monocytogenes* during storage (30 days/4 °C). The EO from ripe fruit showed the greatest activity in *in vitro* tests (MBC of 6.8 mg/mL for *L. monocytogenes*) and exhibited biopreservative activity *in situ*, having reduced the development of *L. monocytogenes* by 1.3 log CFU/g in 30 days. The values of peroxides and malonaldehydes were reduced by 3 Meq $O_2$/Kg and 0.15 mg MDA/Kg, respectively, in 30 days. Results demonstrate that this EO has the potential to act as a preservative in food.
*Industrial relevance:* The pink pepper tree (*Schinus terebinthifolius* Raddi) is a plant with favorable features for industrial use, but little exploited by the food industry so far. In this work, the essential oil (EO) of the pink pepper tree was presented as an alternative to us of preservatives traditionally applied in food. For this, antimicrobial and antioxidant activities of the EO were evaluated and discussed, analyzing its effects initially *in vitro* and after *in situ*, in order to infer the technological potential for application this extract may have use as a food biopreservative.


## 1. Introduction

In all sectors of the food industry, preservation of food products is extremely important (Sokmen et al., 2004) and principally involves controlling the growth of microbes (Kulisic, Radonic, Katalinic, & Milos, 2004), which are responsible for generating risks to consumer health as well as food spoilage. Microbial contamination has a significant influence on the quality of food, and can compromise both safety, due to the presence of pathogenic bacteria, and preservation status, via the multiplication of spoilage bacteria that reduce product shelf-life.

In industry, synthetic preservatives are typically used to control these undesirable microbiological and chemical alterations in food (Bajpai, Baek, & Kang, 2012). However, these additives are not pleasing to many consumers, who demand foods without what they perceive as artificial and harmful chemicals (Calo, Crandall, O'Bryan, & Ricke, 2015), generating a strong demand for products with fewer synthetic additives as well as natural substitutes (Alzoreky & Nakahara, 2003). This increased demand illustrates the relevance of this topic beyond the fields of food technology and public health to the disciplines of economics and marketing. In this context, natural substances that demonstrate biopreservative activity with similar or even greater capacity than synthetic preservatives are gaining prominence (Al-fatimi, Wurster, Schr, & Lindequist, 2007).

Essential oils (EO) are secondary metabolites produced by plants, which confer resistance to adverse conditions such as climatic variation and insect and microorganism attack, and they are among the class of natural extracts used as an alternative to synthetic preservatives. EO are composed of a large number of biologically active molecules (Kavoosi & Rowshan, 2013), which confer various properties including antimicrobial and antioxidant activity (Salgueiro, Martins, & Correia, 2010). Besides these properties of interest, the availability of raw material as well as the ease of cultivation of the plant are important factors that may restrict or encourage the industrial use of certain EO.

The pink pepper tree (*Schinus terebinthifolius* Raddi), also known as pink pepper, native to Brazil, Paraguay, and Argentina, is widely distributed throughout South America, especially on the Brazilian coast (Agra, Silva, Basílio, Freitas, & Barbosa-Filho, 2008). Studies on the EO from this species are limited; however, previous work determined the chemical composition, identifying predominance of monoterpenes such as α-

* Corresponding author.
*E-mail address:* gui.dannenberg@gmail.com (G. da Silva Dannenberg).

http://dx.doi.org/10.1016/j.ifset.2016.06.009
1466-8564/

pinene, β-Pinene, myrcene and limonene, followed by a lower concentration of sesquiterpenes such as *D*-germacrene (Cavalcanti et al., 2015; Gois et al., 2016). Other studies have verified the *in vitro* antimicrobial activity of the EO from pink pepper tree fruits (Aumeeruddy-elalfi, Gurib-fakim, & Mahomoodally, 2015). The antioxidant activity has been evaluated in EO from pink pepper tree leaves (Uliana, Fronza, Vargas, Andrade, & Scherer, 2016). However, previous studies with EO from pink pepper tree were restricted to evaluation *in vitro*. To our knowledge, there are no studies that have evaluated pink pepper tree EO in food (*in situ*).

As previously reported, the antimicrobial and antioxidant activity of pink pepper tree extracts coupled with the wide availability of plant material justify carrying out research for future industrial applications.

The application of EO in foods may initially be considered in view of the fruit and pink pepper extracts having been used for human consumption without reports of health damage. The fruits have been used as condiments in cooking for many years. Extracts of this plant are also widely used in popular medicine for the treatment of various diseases. The pharmaceutical industry makes use of this EO in cosmetics for its aroma (Uliana et al., 2016), and EO are classified by the FDA as GRAS (generally recognized as safe) (Ghabraie, Vu, Tata, Salmieri, & Lacroix, 2016). These factors justify studies evaluating the pink pepper EO as a food preservative; however, for effective commercial application in the food industry, detailed toxicological studies need to be performed to ensure safety.

The purpose of this study was to verify the antimicrobial and antioxidant activity of EO from pink pepper tree fruit at two ripening stages by qualifying and quantifying *in vitro* antimicrobial activity against 18 food bacterial strains; it also aimed to evaluate the preservative activity of EO *in situ* in Minas-type fresh cheese experimentally contaminated with *Listeria monocytogenes*.

## 2. Material and methods

### 2.1. Pink pepper tree

The fruit samples used in this study were collected from adult trees located on the campus of the Federal University of Pelotas (UFPel) in Capão do Leão, RS, 31°48′0459″ latitude and 52°24′5532″ longitude, and were botanically identified as *Schinus terebinthifolius* Raddi based on similarity to UFPel Department of Botany herbarium specimen 25.131. Both immature (green) and mature (red) fruit were harvested. Fruit color was determined by colorimeter (MINOLTA® CR 300). Green fruit was considered to be those having mean values of L = 37.90; a = −2.28; b = 10.54, and mature fruit with color intensity equal to or higher than mean values of L = 36.42; a = 17.19; b = 6.75. The value "L" refers to luminosity varying from white to black, "a" to the coloration in the interval from red to green, and "b" to the coloration in the interval from yellow to blue (Lorenzo, Gómez, & Fonseca, 2014).

### 2.2. Extraction of essential oils

Pink pepper tree fruit were frozen in liquid nitrogen and ground in a ball mill (MARCONI® MA 350), and EO was extracted by hydrodistillation using clevenger apparatus. The resulting EO was dehydrated by centrifugation at 6600*g* for 60 s in a refrigerated centrifuge (Eppendorf F® Centrifuge 6430R) and subsequently filtered with anhydrous sodium sulfate ($Na_2SO_4$ - SYNTH®). EO was stored in an amber glass bottle, and maintained at −80 °C until analysis.

The yield (y) was calculated as the ratio between the mass of the obtained EO (m2) and fruit mass used in the process (m1): y = (m2/m1) × 100. The green fruits and mature fruits presented means EO yield (w/w) of 1.4% and 3.6% respectively. Both EO were slightly yellowish translucent liquid, and presented refractive index appropriate to for pure EO as defined by the Brazilian pharmacopoeia (1.481). The EO chemical composition, performed by chromatographic analysis GC/MS in a parallel work (unpublished data), identified β-myrcene (41%), β-cuvebene (12%) and limonene (9%) as major constituents of these extracts.

### 2.3. Bacterial strains

The microorganisms used in this study (Table 1) were selected based on their importance in food, both as undesirable bacteria (pathogenic and spoilage), or as technologically applicable bacteria (starter and probiotic cultures), and were obtained from the Food Microbiology Laboratory's bacteria collection in the Department of Agroindustrial Science and Technology (DCTA) at UFPel.

### 2.4. Antimicrobial activity in vitro

#### 2.4.1. Disk-diffusion

The antimicrobial activity of the EO was initially evaluated by the disk-diffusion technique (CLSI, 2012b) with adaptations. Bacteria were activated in Brain Heart Infusion broth (BHI-Oxoid®) for 12 h, and bacterial concentration was adjusted to 8.18 log CFU/mL (0.5 McFarland) in peptone water (Acumedia®). Inoculum were spread uniformly with a sterile swab (Absorve®) on the surface of petri plates (Cralplast®, 90 × 15 mm) containing 4 mm of Mueller-Hinton Agar (Oxoid®) with pH 7 ± 0.2. A sterile paper disk (Laborclin®) 6 mm in diameter was added to the center of each plate, upon which 10 μL of EO were placed. The petri plates were incubated at 37 °C, and the presence of inhibition zones around the paper disk were verified after 24 h with a digital pachymeter (King.tools®). The plates of starter cultures and probiotic bacteria were incubated in anaerobic jars (2.5 L - Permution®) with AnaeroGen sachets (Oxoid®), at 37 °C for 48 h. Antimicrobial activity of EO against each bacterial strain was tested in triplicate, and the evaluation was repeated a second time.

#### 2.4.2. Minimum inhibitory concentration (MIC)

The lowest concentration of EO required to inhibit the growth of the tested microorganisms was determined by the broth microdilution technique (CLSI, 2012a) with adaptations. Dilutions of the EO were performed in Tryptone Soy Broth supplemented with 0.6% yeast extract (TSB-YE-Oxoid®) for *L. monocytogenes* and Brain Heart Infusion Broth (BHI-Oxoid®) for all other bacteria. For both types of broth, pH was adjusted to 7.2 to 7.4, and 3% tween 80 (Vetec®) was added.

Essential oils were initially diluted to 25% (m/v) in the cultivation broths, producing concentrations of 217.58 mg/mL and 218.23 mg/mL of EO from green fruits (GEO) and EO from ripe, mature fruits (MEO), respectively, due to the difference in density between them (0.870 and 0.873 g/mL). Serial dilutions were then performed in 96-well microdilution plates until the minimum concentrations of 0.11 mg/mL of GEO and 0.10 mg/mL of MEO were reached.

Bacterial concentrations were adjusted to $1.5 \times 10^8$ CFU/mL (0.5 McFarland) and analyzed by spectrophotometer (Jenway® 6705) at a wavelength of 625 nm until absorbance read between 0.08 and 0.1 (CLSI, 2012b). Ten microliters of the inoculum were added to each well of the microdilution plates that contained 190 μL of the respective mixtures of broth and EO. Inoculum in broth without inhibitors was used as a positive control, and broth without inoculum was used as a negative control. The plates were incubated at 37 °C for 24 h, and readings were performed with a plate reader (Robonik® Readwel plate) at a wavelength of 620 nm (Martins, Arantes, Candeias, Tinoco, & Cruz-Morais, 2014; Ojeda-sana, Baren, Elechosa, Juárez, & Moreno, 2013).

#### 2.4.3. Minimum bactericidal concentration (MBC)

The lowest concentration of GEO and MEO capable of inducing microbial cell death was determined by inoculating Muller-Hinton (Oxoid®) agar plates with 10 μL aliquots from the wells that did not grow in the MIC test. Microdilution plates were incubated at 36 °C,