Vapor phase antibacterial effect of ROSEMARY (*ROSMARINUS OFFICINALIS*) ESSENTIAL OIL and its major component at selected pH's and temperatures

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ABSTRACT

Consumer demand for natural preservatives, such as essential oils (EOS) from plants has increased over the years. Therefore, the aim of this study was to evaluate the vapor phase antibacterial effect of rosemary essential oil (EO) and its major component (1,8-cineole), against *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, or *Pseudomonas fluorescens* at selected pHs and temperatures. The minimum inhibitory concentrations (MICS) of the EO and 1,8-cineole were determined at different pH's and temperatures in culture mediums; resulting that in most of the cases *L. monocytogenes* exhibited less resistance to tested natural antimicrobials when compared to *S. enterica* and *P. fluorescens*.

KEY WORDS:

Antibacterial effect · Vapor phase · Rosemary · 1,8-cineole · Essential oils.

INTRODUCTION

Bacteria are the main cause of foodborne illnesses and difficult to detect and control by the food industry (USDA, 2012). Foodborne illnesses are important health concerns for governments, nowadays commonly attributed to minimally (or not appropriately) processed (or stored) food products.

Chemical preservatives have been used as food antimicrobials for a long time; however, consumer search for food products with «clean» labels has increased over recent years. Therefore, there is an increasing need to find natural preservatives such as herbs and spices, and/or their extracts or essential oils (Hyldgaard *et al*., 2012). Essential oils are defined as mixtures of volatile and aromatic compounds. EOS could be extracted from different parts of plants, where 85-95% of the main volume of EOS is constituted by their major components (Burt, 2004). Among several alternative natural preservatives are EOS extracted from rosemary (Rosmarinus officinalis), which have shown antimicrobial activity against different microbial strains, when applied either in liquid or vapor phase.

Though, when EOS are applied in the liquid phase (directly to the food) they usually have a significant impact on food sensory attributes, because of their strong aromas and flavors. In contrast, vapor phase application (indirectly) of EOS typically requires lower concentrations for their use as antimicrobials; thus, vapor phase application could be a solution to the adverse effects of the intense aroma and flavor of applying EOS directly to foods. Several culture medium (*in vitro*) studies have helped to simulate the antibacterial behavior of selected EOs in food systems (Mejía-Garibay *et al*., 2015). Thus, the aim of this study was to evaluate the vapor phase antibacterial effect of rosemary essential oil and its major component (1,8-cineole) against three different bacteria at selected pHs and temperatures.

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METHODOLOGY

Rosemary essential oil was obtained from Hersol® laboratories (Hersol, Mexico City, Mexico) while its major component (1,8-cineole also known as eucalyptol) was obtained from Sigma-Aldrich® (Sigma, St. Louis, MO, USA). Tested essential oil was analyzed by a gas chromatographer coupled to a mass selective detector (GC/MSD, Thermo Fisher Scientific Inc., Waltham, MA, USA); compounds were identified by comparing their retention indices with the US National Institute of Standard Technology Library and with Shimadzu retention index (RI) isothermal equation.

WHEN EOS ARE APPLIED IN THE LIQUID PHASE THEY USUALLY HAVE A SIGNIFICANT IMPACT ON FOOD SENSORY ATTRIBUTES, BECAUSE OF THEIR STRONG AROMAS AND FLAVORS.

Salmonella enterica serovar Typhimurium ATCC 14028, *Listeria monocytogenes* Scott A, and *Pseudomonas fluorescens* were obtained from the Universidad de las Américas Puebla Food Microbiology Laboratory strain collection. They were maintained on Trypticase soy agar (TSA, Merck, Germany) slants at 5 °C. Cultures were prepared by inoculating the bacteria strains into 10 mL of Trypticase soy broth (TSB, Merck, Germany), incubated at 35 °C for 24 h, and adjusted to a cell concentration to 107 CFU mL^{-1}.

Culture mediums were prepared with TSA adjusting its pH (6.0 or 6.5) with hydrochloric acid, then sterilized (15 min at 121 °C), and allowed to solidify in sterile Petri dishes. Subsequently, sterilized culture media were inoculated with 50 µL of inoculum of each bacteria strain, using a spiral plater (Spiral Biotech, Inc., Norwood, MA, USA).

Minimum inhibitory concentration (MIC) refers to the minimum concentration necessary to inhibit the visible growth of the studied strain (López-Malo *et al.*, 2005), and it was utilized to evaluate the antibacterial activity of tested EO by means of the inverted Petri dish technique. This method consists in placing a sterile paper disc (Whatman No. 1), on the lid of the Petri dish, impregnated with a known volume of the tested EO or its major component. The volumes tested varied from 5 to 1900 µL, depending on the studied combinations of bacteria, pHs, and temperatures. The culture medium with the paper disc was immediately inverted on top of the lid, sealed with Parafilm® and incubated as follows: 1) at 35 °C for

24 h, 2) at 25 °C for 48 h, 3) at 15 °C for 8 days, or 4) at 10 °C for 9 days. These incubation conditions were selected from previous experiments that corroborated that studied bacteria were able to grow at the studied temperatures after those incubation times. The obtained MICS were expressed as mL of EO (or 1,8-cineole) per L of air.

Quantification of colony forming units (CFU mL^{-1}) was made when growth was observed using a Q-Count counter and its corresponding software (Spiral Biotech, Inc., Norwood, MA, USA). Whereas, no growth was detected, the culture mediums were incubated again for the same corresponding period and temperature (35 °C/24 h, 25 °C/48 h, 15 °C/8 days, or 10 °C/9 days), changing the Petri dish lid with a new sterile one (with non-impregnated paper disc), removing the systems from the generated antibacterial atmosphere (caused by the EO/1,8-cineole) with the aim of proving a bacteriostatic or a bactericidal effect; if the bacteria grew after the lid change, it was reported as bacteriostatic effect, whereas if no growth was found, it was reported as a bactericidal effect. Every test was performed by triplicate.

RESULTS AND DISCUSSION

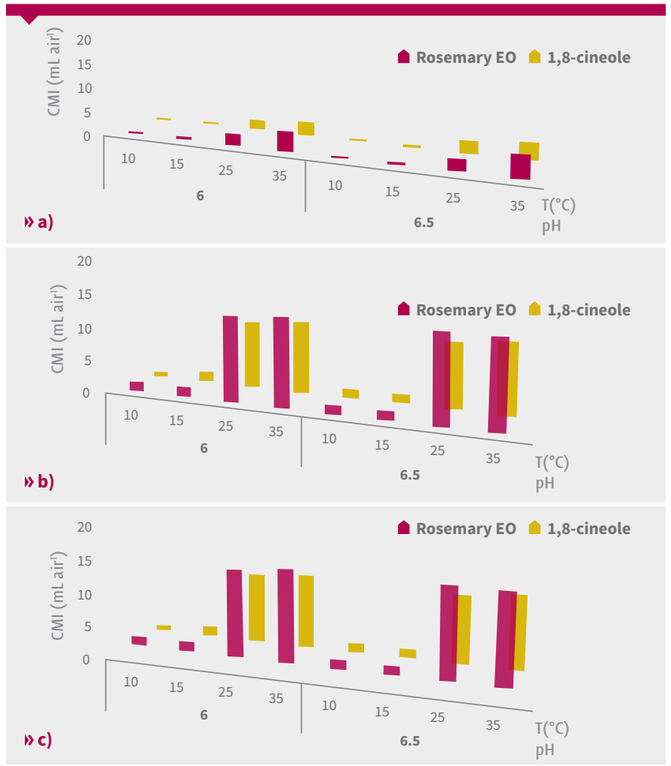
Tested rosemary EO main components identified by GC-MSD, and their calculated retention index, are reported in table 1. Burt (2004) reported that rosemary EO may contain 6-14% of 1,8-cineole and 2-10% of β-pinene, which agrees with the results obtained in our case. On the other hand, Miladi *et al*. (2013) found in rosemary EO α-pinene, 1,8-cineole, β-pinene, and camphene as its main components; also similar to our reported results, except for α-pinene.

The antibacterial activity of tested rosemary EO and its major component (1,8-cineole) against *L. monocytogenes*, *S. enterica*, and *P. fluorescens* is shown in figure 1. It can be observed that the EO and its major component exhibited strong antibacterial effects (at different concentrations) against the three studied bacteria.

In both cases (rosemary EO and 1,8–cineole) the MICS against *L. monocytogenes* were the lowest, followed by the ones against *S. enterica,* and then by the ones against *P. fluorescens*. In general, the smallest concentrations needed to inhibit studied bacteria were observed when incubated at 10 or 15 °C for both tested pH's. However, when the temperature was increased up to 25 or 35 °C (at both pHs) the MIC for the three studied bacteria, augmented considerably.

It is important to note that microorganisms have an optimum pH and temperature for

Figure 1. Minimum inhibitory concentrations (MICS) of tested rosemary (*Rosmarinus officinalis*) essential oil and its major component (1,8-cineole) against a) *L. monocytogenes*, b) *S. enterica*, or c) *P. flourescens*, at studied pHs and temperatures.



Compound	Percentage of essential oil	Retention index
α-Pinene	27.39	930
1,8-cineole	20.89	1026
Camphor	20.64	1141
Camphene	7.16	946
β-Pinene	6.41	974
α-Terpineol	4.25	1186
Borneol	3.69	1165

Table 1. Main components of tested rosemary (*Rosmarinus officinalis*) essential oil determined by gas chromatography-mass spectrometry.

growth, and when these values are changed microbial growth could be deferred. Furthermore, when a microorganism is exposed to different preservation factors, such as temperature, pH, and/or preservatives, such as EOS and its major components, there is an interaction amongst factors that could affect microbial growth. In this study, the temperature only showed a lower inhibition effect when increased to 25 or 35 °C, which could be related to the fact that the optimum growth temperatures are between 25 and 37 °C for the studied bacteria. In some of the tested combinations, when the pH increased the MIC also increased; however, the impact of pH was much lower than the one presented by temperature. Summarizing, in most cases when temperature and pH increased, the observed MIC was higher.

When comparing the MICS of tested rosemary EO and of its major component (1,8 cineol), it can be deduced that the antibacterial effects of tested EO cannot be correlated solely to the presence of 1,8 cineol, since in many of the evaluated conditions the concentrations needed to inhibit studied bacteria were higher for the major component than those observed for the EO.

It has been reported that Gram-positive bacteria are more susceptible to EOS than Gram-negative bacteria as can be observed in figure 1. *L. monocytogenes* exhibited less resistance to tested natural antimicrobials when compared to *S. enterica* and *P. fluorescens*. The resistance of Gram-negative bacteria can be related to their hydrophilic cell wall, which helps them against penetration of hydrophobic compounds of the EOS.

Finally, a bacteriostatic effect refers to inhibition of cell growth while a bactericidal effect denotes cell death (Reyes-Jurado *et al*., 2016). In our case, only five of studied combinations were bacteriostatic, most of them against L. monocytogenes: 1) 1,8-cineole MIC at pH 6 and 25 °C, 2) rosemary EO MIC at pH 6.5 and 25 °C, or 3) rosemary EO MIC at pH 6.5 and 35 °C. The only bacteriostatic effect observed against S. enterica was for the rosemary EO MIC at pH 6.5 and 25 °C, while the only one against P. fluorescens was for 1,8-cineole MIC at pH 6.5 and 35 °C.

Tested rosemary EO and its major component (1,8-cineole), when applied in vapor phase, could be considered a good alternative to traditional chemical antimicrobials. Further studies regarding their sensory compatibility with selected foods can lead to the development of new and distinct products.

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