Evaluation of the bacteriostatic properties of essential oils and their potential applications for food microbiology

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INTRODUCTION

The recent multiplication of food scandals has made food safety a major concern for food producers. More than ever, consumers are asking for products that are microbiologically safe and, in the same time, containing less artificial preservatives. The use of small concentrations of essential oils could bring a solution to this dilemma, as some of those "natural additives" already have proven bacteriostatic properties. Essential oils also beneficiate from a very good image, thanks to their numerous properties (antioxidant, antiviral, anti-inflammatory) and the fact they have been used by humans for thousands of years.

OBJECTIVES

The purpose of this study was to evaluate *in vitro* the bacteriostatic properties of 7 food-compatible essential oils on food pathogens or spoilage bacteria.

The bacteriostatic activity of a food preservative is characterized by its Minimal Inhibitory Concentration (MIC), which is the minimal concentration at which no bacterial growth is observed. The evaluation of the MIC of food-compatible essential oils is the first step to select the most interesting ones for potential applications in the food industry.

ESSENTIAL OILS

Essential oils are aromatic liquids extracted from plants by steam distillation. Their numerous health properties are widely used in aromatherapy, flavouring, cosmetics and fragrances industries.

The antibacterial properties of several essential oils have been demonstrated by *in vitro* studies. However, seen the diversity of essential oils, the bacteriostatic potential of many of them remains unknown.

Table 1: Description of the 7 essential oils tested in this study.

Essential oils	Scientific name	Origin	Main components
Cinnamon bark	*Cinnamomum zeylanicum*	Ceylan	Cinnamaldehyde
Cinnamon leaf	*Cinnamomum zeylanicum*	Madagascar	Eugenol
Saro	*Cinnamosma fragrans*	Madagascar	1,8-Cineole, monoterperes
Thymol thyme	*Thymus vulgaris Ct. Thymol*	France	Thymol
Oregano, moroccan	*Origanum compactum*	Morocco	Carvacrol, thymol
Ravensare	*Ravensara aromatica*	Madagascar	Limonene, Sabinene
Helichryse	*Helychrisum gymnocephalum*	Madagascar	1,8-Cineole, pinenes

MATERIALS AND METHODS

Table 2: The 8 bacterial strains used in this study

Gram	Species	Strain	Origin
+	*S. aureus*	SI 0148	Food
+	*L. Innocua*	IHE2	Food
+	*B. cereus*	ATCC 13061	Reference strain
+	*B. cereus*	ATCC 10876	Reference strain
-	*E. coli*	ATCC 18739	Reference strain
-	*E. coli*	SI 0059	Food
-	*Salmonella abaetetuba*	ATCC 35640	Reference strain
-	*Salmonella enterica*	SI 0022	Food

The laboratory experiments were conducted on 8 strains of 5 bacterial species relevant in food microbiology (either pathogens or spoilage species). After preliminary "spot-on" experiments, aiming to delimit the panel of concentrations to be tested, Agar Dilution Assays were performed, in double, for each bacterial strain and for each essential oil.

In this experiment, 300 bacteria were plated on PCA plates containing different concentrations (0% - 1%) of essential oil. After 48h incubation at 37°C, the colonies were enumerated and the MIC were determined as the minimal concentrations of essential oil at which no colonies were observed.

RESULTS AND DISCUSSION

As illustrated in Fig.1, the results of the Agar Dilution Assays showed a clear bacteriostatic potential for some essential oils. A general observation was that essential oils were more effective on Gram-positive than on Gram-negative bacteria, a tendency already mentioned in the literature.

The results indicated that the 7 essential oils tested could be classified into 3 groups:
- The **strong bacteriostatics**, whose MIC are below or equal to 0,1% : **thyme, cinnamon bark**;
- The **middle bacteriostatics**, having MIC comprised between 0,1% and 1% : **oregano, cinnamon leaf**;
- The **weak bacteriostatic**, whose MIC are superior to 1% : **saro, helichryse, ravensare**.

Table 3: Summary of the MIC (%) measured for the essential oils on the 8 strains

	Thyme	Cinn. bark	Cinn. leaf	Oregano	Saro	Helichryse	Ravensare
S. aureus	0,1	<0,05	0,2	0,2	>1	>1	>1
L. innocua	0,1	<0,05	0,2	0,1	>1	>1	>1
B. cereus	<0,05	<0,05	<0,05	0,1	>1	>1	>1
B. cereus	<0,05	<0,05	0,1	0,1	>1	>1	>1
S. abaetetuba	<0,05	<0,05	0,2	0,2	>1	>1	>1
S. enterica	0,1	<0,05	0,2	0,2	>1	>1	>1
E. coli	0,1	<0,05	0,2	0,2	>1	>1	>1
E. coli	0,1	<0,05	0,2	0,2	>1	>1	>1

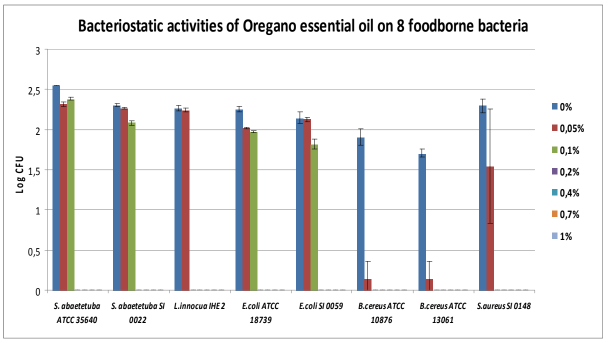

Fig. 1: Results of the Agar Dilution Assays on Oregano essential oil

Essential oils can inhibit microbial growth by degrading the cytoplasmic membrane, inactivating enzymes and depleting the proton gradient.

The chemical composition of the essential oils appeared to be of major importance, since the phenolic (thymol, carvacrol, eugenol) and aldehyde (cinnamaldehyde) compounds were more effective antibacterials than the other compounds (1,8-Cineole, limonene, pinene).

CONCLUSIONS AND PERSPECTIVES

This work enabled to classify the 7 essential oils following their MIC. Only the "strong" essential oils have potential applications in food microbiology, since the concentrations needed for preservation could be sufficiently low not to alter the organoleptic properties of the foods.

As a perspective, the bacteriostatic effect of thyme and cinnamon bark should be tested in food matrices compatible with their strong aromatic properties. These essential oils could also be used in combination with other conservation techniques and enable to significantly reduce the levels of artificial additives in foods.