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Short Communication

Essential Oils Against Foodborne Pathogens and Spoilage Bacteria in Minced Meat

Lidiane Nunes Barbosa,[1] Vera Lucia Mores Rall,[1] Ana Angélica Henrique Fernandes,[2]
Priscila Ikeda Ushimaru,[1] Isabella da Silva Probst,[1] and Ary Fernandes Jr.[1]

Abstract

The antimicrobial activity of essential oils of oregano, thyme, basil, marjoram, lemongrass, ginger, and clove was investigated *in vitro* by agar dilution method and minimal inhibitory concentration (MIC) determination against Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative strains (*Escherichia coli* and *Salmonella* Enteritidis). $MIC_{90\%}$ values were tested against bacterial strains inoculated experimentally in irradiated minced meat and against natural microbiota (aerobic or facultative, mesophilic, and psychrotrophic bacteria) found in minced meat samples. $MIC_{90\%}$ values ranged from 0.05%v/v (lemongrass oil) to 0.46%v/v (marjoram oil) to Gram-positive bacteria and from 0.10%v/v (clove oil) to 0.56%v/v (ginger oil) to Gram-negative strains. However, the $MIC_{90\%}$ assessed on minced meat inoculated experimentally with foodborne pathogen strains and against natural microbiota of meat did not show the same effectiveness, and 1.3 and 1.0 were the highest log CFU/g reduction values obtained against tested microorganisms.

Introduction

The new technologies of food preservation include nonthermal inactivation, such as ionization radiation, high hydrostatic pressure, and pulsed electric fields; modified atmosphere and active packaging; biopreservation; and natural antimicrobial compounds (Devlieghere *et al.*, 2004). Plants are a source of bioactive molecules and have been widely used both traditionally and commercially to increase the shelf-life and safety of foods (Sasidharan *et al.*, 2008).

Biological properties of essential oils and their antimicrobial activity have been attributed to phenolic compounds, such as the carvacrol, eugenol (2-methoxy-4-(2-propenyl) phenol), and thymol (Seydim and Sarikus, 2006). These compounds have hydrophobic characteristics and interact with different sites of microbial cell (e.g., cell wall and cytoplasmic membrane), causing loss of cellular constituents, collapse of membrane structure, and cell death (Burt, 2004). Bactericidal or bacteriostatic activity of essential oils, *in vitro* and in food assays, against *Salmonella enterica*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Candida albicans* strains has been reported (Lambert *et al.*, 2001; Chorianopoulos *et al.*, 2004; Friedman *et al.*, 2004; Kim *et al.*, 2004). Studies *in vitro* have used spices as antimicrobials in laboratory media although the levels of spices and their essential oils to inhibit microorganisms in food have been found to be higher than those assays performed using culture media (Burt and Reinders, 2003; Uhart *et al.*, 2006).

Thus, we aimed to determine *in vitro* the minimal inhibitory concentration (MIC) of essential oils from *Thymus vulgaris* (thyme), *Origanum majorana* (marjoram), *Origanum vulgare* (oregano), *Ocimum basilicum* (basil), *Zingiber officinale* (ginger), *Cymbopogon citratus* (lemon grass), and *Caryophyllus aromaticus* (clove) against *E. coli*, *S. aureus*, *L. monocytogenes*, and *Salmonella* Enteritidis strains. $MIC_{90\%}$ values were evaluated in minced meat irradiated and experimentally inoculated with these pathogenic bacteria and against natural microbiota of minced meat (mesophiles and psycrotrophs).

Departments of [1]Microbiology and Immunology and [2]Chemistry and Biochemistry, Biosciences Institute, São Paulo State University, Sao Paulo, Brazil.

Materials and Methods

Plants and essential oils

Fresh leaves of oregano, thyme, basil, marjoram, and lemon grass; rhizome of ginger; and dried inflorescence clove were purchased from the local market. Steam distillation using the Clevenger system was used to obtain the essential oils, which were stored at 5°C in sealed glass vials before use.

Bacterial strains

Five strains of *L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella* Enteritidis isolated from food samples were used. ATTC strains of each bacterial species (Lm ATTC 7644, Sa ATCC 25923, SE ATCC 13076, and Ec ATCC 25922) were also tested.

In vitro *antibacterial assay: MIC test*

Mueller–Hinton agar (Difco, Sparks, NV) plates containing essential oils (0.025%v/v, 0.05%v/v, 0.1%v/v, 0.3%v/v, 0.5%v/v, 0.7%v/v, 1.0%v/v, 1.5%v/v, 2.0%v/v, and 3.0%v/v) and Tween 80 (0.2%) were inoculated by Sterr's inoculator with bacterial strains incubated overnight at 36°C in brain heart infusion (Difco) and standardized in sterile saline (10^4–10^5 CFU/mL). The bacterial growth was indicated as the presence and/or absence of colonies on media after at 37°C/24 h, and the MIC was the lowest concentration showing no growth at Mueller–Hinton agar (NCCLS, 2004). Afterward, $MIC_{90\%}$ values were calculated.

Oils' antimicrobial activity in minced meat assay

Two antimicrobial tests were carried out on minced meat: (1) antimicrobial activity of essential oils against pathogenic bacterial strains (*L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella* Enteritidis) experimentally inoculated on irradiated meat and (2) antimicrobial activity against the natural microbiota (mesophilic and psychrotrophic bacteria) of meat. $MIC_{90\%}$ values achieved *in vitro* tests according to the Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *Salmonella* Enteritidis) were tested in meat because the susceptibility profile was similar among the essential oils.

Minced meat samples (25 g) were packaged in individual portions, frozen (−20°C), and irradiated with 10.2 kGy, aiming to eliminate natural microbial populations. The irradiated meat samples were experimentally inoculated (10^4–10^5 CFU/g) with bacterial strains, and essential oils (%v/g) were added to meat and maintained at 5°C/3 h. Serial dilutions and pour plate method were performed with plate count agar (PCA) (Difco), and after 35°C/24–48 h the CFU/g was recorded. Tests were performed in duplicate.

The mesophilic and psychrotrophic enumeration were performed on minced meat, and individual samples (25 g) were tested with or without essential oils according to the $MIC_{90\%}$ values from *in vitro* sensibility tests. The meat samples with oils and the controls were kept at 5°C, and serial dilutions were performed at 0 h (sample without oil) and 6 and 24 h (samples with and without oil) counting CFU/g in PCA. The psychrotrophics were recorded at 0 and 24 h after contact with essential oils using serial dilution and inoculation on PCA and incubation at 5°C/7 days.

Statistical analysis

The nonparametric test of Kruskal–Wallis was employed to compare independent treatments. Student–Newman–Keuls test was applied to multiple comparisons. To meat assays, nonparametric test of Mann–Whitney was used for groups (with or without oil), while the nonparametric variance analysis and Kruskal–Wallis was performed to bacteria.

Results and Discussion

All strains were susceptible to essential oils, and clove showed the highest antimicrobial activity ($MIC_{90\%}$ = 0.09%v/v), followed by lemongrass (0.25%v/v) and thyme (0.26%v/v). Lemon grass was the most effective against Gram-positive (0.05%v/v), followed by clove, ginger (0.09%v/v), and thyme (0.10%v/v)

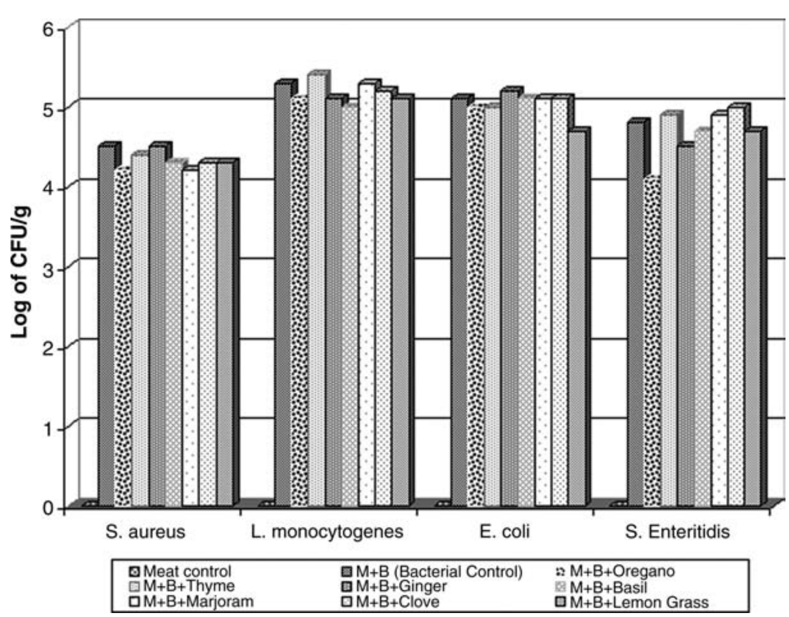

FIG. 1. Log of CFU/g values from *Staphylococcus aures*, *Escherichia coli*, *Listeria Monocytogenes*, and *Salmonella* Enteritidis experimentally inoculated in irradiated minced meat added with essential oils and maintained at 5°C/3 h.

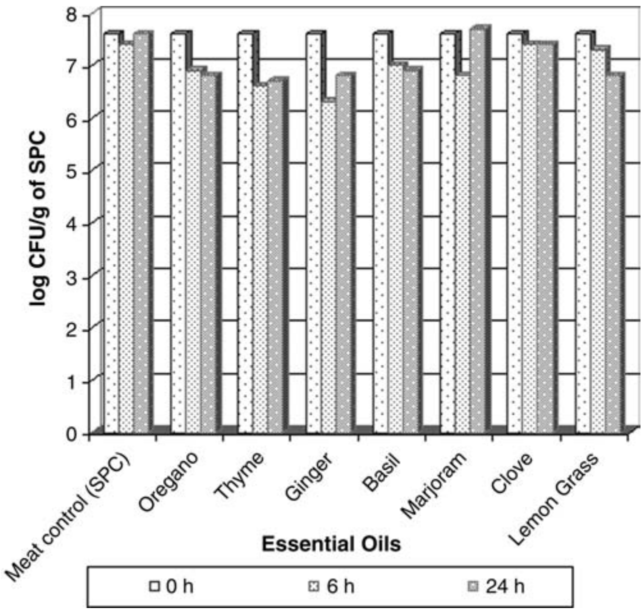

FIG. 2. Log of CFU/g of standard plate count (SPC) values recorded on minced meat samples after 5°C/6 and 24 h of essential oil addition.

oils and clove oil for Gram-negative strains (0.10%v/v). *In vitro* studies have demonstrated the antibacterial activity of essential oils against *L. monocytogenes*, *Salmonella* Typhimurium, *E. coli* O157:H7, *Shigella dysenteria*, *Bacillus cereus*, and *S. aureus*, and Gram-negative bacteria were less susceptible than Gram-positive bacteria (Burt, 2004).

The CFU/g (log) values of *S. aureus*, *L. monocytogenes*, *E. coli*, and *Salmonella* Enteritidis assays with or without essential oil zacontact in meat experimentally inoculated are shown in

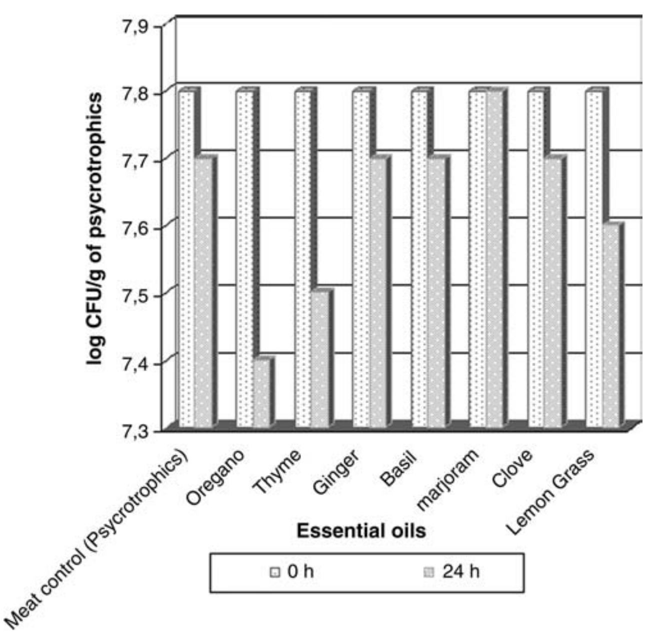

FIG. 3. Log of CFU/g for psychrotrophic microorganism values recorded on minced meat samples after 5°C/24 h of essential oil addition.

Fig. 1. The reduction between tests and control treatments was not significant, and the oils were able to reduce 1 log compared with the control. Although no significant differences were found, the bacteriostatic effect of oils was verified, and no bacterial developments were recorded at 5°C/3 h for all bacterial strains.

The log CFU/g values for mesophilic aerobic bacteria from minced meat recorded at 0 h (positive control) and 6 and 24 h after adding oil to meat are presented in Fig. 2. No significant differences were verified, and 1.3 and 1.0 were log CFU/g reduction values to ginger and thyme oils, respectively. The psychrotrophic reduction tests (Fig. 3) after 24 h of oils and meat contact produced the highest log reduction with oregano oil (0.4).

Although the antimicrobial activity *in vitro* of oils has been moderately effective on meat model, the potential use of these oils in food preservation technologies should be found in optimal concentrations to ensure the safety of the food, appropriated organoleptical characteristics, and accepted by consumers. Studies aiming to elucidate the interaction between essential oils and components of food matrices or additives, stability of oils during food processing, and the standardization of antibacterial methods are still needed.

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Address correspondence to:
Ary Fernandes Jr., Ph.D.
Department of Microbiology and Immunology
Biosciences Institute
São Paulo State University
IBB/UNESP/Botucatu
Sao Paulo 18618-000
Brazil

E-mail: ary@ibb.unesp.br