Poster exhibition parallel session 6: New and emerging food safety risks

PE6.01 Chemical Composition of Some Essential Oils and Antibacterial Activity in Minced Beef Stored at 4ºC 3.00

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Algerian Essential oils extracted by hydrodistilation from leave parts of Eucalyptus globulus, Myrtus communis L., and Satureja hortensis L. were analyzed by gas chromatography/mass spectrometry (GC/MS). The major components of essential oils were 1,8-Cinéole, -Pinène and Carvacrol respectively for Eucalyptus globulus, Myrtus communis L. and Satureja hortensens L. Essential oils evaluated for their single antibacterial activities against common Gram-positive and Gram-negative pathogenic bacteria Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. The 'in vitro' antimicrobial activity of the essential oils was evaluated. The agar diffusion technique was used to establish, in a rapid way, the susceptibility of the foodborne pathogen to the different essential oils and to choose those with the greatest antimicrobial activity. Minimal inhibitory concentrations (MICs) were also determined. Plant essential oils with potent antimicrobial activities were tested in minced beef stored at 4ºC, experimentally inoculated with various foodborne pathogens at level of 2 to 3×105 cfu/g. Results showed that the essential oils revealed promising antibacterial activities against most pathogens tested. Sensory evaluation revealed that the aroma of minced beef meat treated with essential oils were acceptable by the panelists at the antibacterial levels.

Keywords: Algerian essential oils, pathogens, antimicrobial activity, meat.

I. INTRODUCTION

Foodborne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health in Algeria. The majority of health infections are caused by the consumption of contaminated meat products. Some of the major foodborne disease outbreaks may take on massive proportions. Today, different strategies are applied in order to control pathogens in meats, and particular interest has been focused on the application of essential oils (Burt, 2004; Holley & Dhaval, 2005; Solomakosa, Govarisa, Koidisb & Botsoglouc, 2008). Because of greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become popular. The modulating influence of food composition upon the antimicrobial effectiveness of essential oils is an important area of study. Eucalyptus globulus (E. globulus), Myrtus communis L. (M. communis L.) and Satureja hortensis L (S. hortensis L.), grow wildly in the coastal regions, the internal hill and the forest areas of Kabylie (Algeria). Even though several studies have been conducted regarding the in vitro antibacterial and antifungal properties of plant essential oils (Sari, Biondi , Kaâbeche, Mandalari, D'Arrigo & Bisignano, 2006; Souza, Stamford, Lima & Trajano, 2007; Bendahou, Muselli, Grignon-Dubois, Benyoucef, Desjobert,

Bernardini & Costa, 2008), only a few studies on the activity of essential oils in food systems have been reported in the literature. The aim of the present work was to evaluate the antimicrobial activity of various Algerian essential oils obtained by hydrodistillationin both vitro and in food. Minimum inhibitory concentrations (MICs) values were determined for selected foodborne pathogens. The potency of the essential oils was tested in fresh minced beef held at 4±1°C and sensory analyses were conducted on the resulting meats treated with different oils.

II. MATERIALS AND METHODS

II. 1. Plant material and essential oils extraction. The aerial parts of E. globulus, M. communis L. and S. hortensis L. were collected at Bouzeguène and Freha city (Tizi-Ouzou province, Algeria), in March-July 2008. The essential oils were obtained from dried plant parts by hydrodistillation in a Clevenger-type apparatus for 3 h (Groupe Pharmaceutique SAIDAL, Filiale Biotic, Algiers). The essential oils obtained were separated from water and dried over anhydrous sodium sulphate (Na2SO4) and preserved in darkness in a sealed vial at 2 1°C until use.

II.2. Gas Chromatography-Mass Spectrometry Analysis (GC-MS). GC analyses of essential oils obtained from dried plant parts were performed using a Chrompack 9002 gas chromatograph (Centre de Recherche en Analyses Physico-Chimiques, Université de Bab Ezzouar, Algeria) equipped with a flame ionization detector (FID) and a Stabilwax (PEG) column (30 m 0.32 mm i.d., 1 m film thickness). The GC-MS analysis was performed using a Hewlett-Packard 6890 series GC systems (Agilent Technologies) coupled to a quadrupole mass spectrometer (model HP 5973) equipped with a HP5 MS capillary column (5% phenyl methylsiloxane, 30 m 0.25 mm, 0.25 m film thickness). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used over a scan range of 30-550 atomic mass units. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and detector MS transfer line temperatures were set at 250 and 280 C, respectively; the temperature of the ion source was 175 C. Column temperature was initially kept at 60 C for 8 min, then gradually increased to 280 C at 2 C/min, and finally held isothermal for 30 min. The volume of injections was 1 L of a hexane-oil solution, injected in the splitless factors.

II.3. In vitro tests of antimicrobial activity. Two bacterial strains were employed. They included Gram-positive Staphylococcus aureus, and Gram-negative Escherichia coli. Screening of essential oils for antibacterial activity was done by the disk diffusion method, which is normally used as a preliminary check and to select between efficient essential oils. Petri plates were prepared by pouring 20 ml of Mueller Hinton agar medium and allowed to solidify and 0.1 ml of standardized inoculum suspension was poured and uniformly extend. The inocula were allowed to dry for 5 min. To prepare the stock solution of the samples, the pure essential oils were dissolved in 0.5% (v/v) Tween 80. Whatman sterile filter paper disk (6 mm diameter) was impregnated with 05 ?l essential oil. The plates were left 15 min at room temperature to allow the diffusion of the essential oil, and then they were incubated at 37°C for 24 h. At the end of the period, the diameter of the clear zone around the disc was measured with a caliber and expressed in millimeters (mm) as antimicrobial activity. Negative controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotics, Kanamycine and Ticarcilline (05 g/disc), were used as positive controls in order to control the sensitivity of the tested microorganisms. Each assay in this experiment was duplicated twice.

II.4. Determination of minimal inhibitory concentration. The essential oils that previously showed antimicrobial activity were screened for determination of minimum inhibitory concentration (MIC: l/ml) by the disc diffusion method against the same microorganisms. The oils were dispersed at room temperature in sterile 0.5% (v/v) Tween 80 solution. Serial dilutions (1/2, 1/4, 1/8, 1/16, 1/20, 1/32, 1/40, 1/50, 1/64, 1/128 and 1/300) of the essential oil solution were deposited on sterile paper disks (6 mm diameter) which were subsequently placed in the centre of the inoculated Petri dishes with 05 l of adjusted inoculums. The Petri dishes were then incubated at 37°C for 18 h and the (bacterial growth) inhibition zone diameter was measured to the nearest mm. The lowest concentration of each essential oil solution deposited on the sterile paper disk showing a clear zone of inhibition was taken as the MIC. Controls were set up with Tween 80 in amounts

corresponding to the highest quantity present in the test solution. All analyses were applied in duplicate.

II.5. Antimicrobial activity of essential oils in meat system. Lean beef muscles were obtained from beef carcasses at 48 h post-slaughter from the Boucherie et Volaille Khatir Amar, DBK, Tizi-Ouzou, Algeria. Six hundred g (4) of minced beef were placed in sterile bag and inoculated with single strain of pathogen. The inoculated samples were homogenized for 2 min at room temperature to ensure proper distribution of the pathogen. Following homogenization, essential oils were added to the inoculated samples. Six beef samples (100±2 g) were performed. All the bags containing the samples of meat were refrigerated (4°C) and examined after 2, 5 and 7 days of storage for each microorganism. The untreated samples (controls) were added sterile water (instead of essential oil), inoculated with the test bacteria, and stored under the same conditions as the other samples. Two individual duplicates of each experiment were performed, in all cases.

II.6. Sensory evaluation. Meat samples were evaluated by a five-member expert panel. Three open-discussion sessions were held to familiarise panellists with the attributes and the scale to be used. This method makes it possible to appreciate differences among various samples, based on the relative intensity of taste and aroma of untreated and treated samples. The concentrations of antibacterial in the samples were those determined to be effective in the previous assays of antimicrobial activity.

II.7. Microbiological analysis At each sampling time, samples (25 g) of minced beef in the stomacher bags were aseptically added with 225 ml of 0.1% peptone water. The contents were macerated in the stomacher. Resulting slurries were serially diluted (1:10) in 0.1% sterile peptone water. Sample dilutions (0.1 ml) of minced beef were spread plated on appropriate media in duplicate and incubated at appropriate temperature for 24 to 48 h. Counts were expressed as the log10 of colony forming units (CFU) per gram.

II.8. Statistical analysis The significance of differences among samples at each day of storage was determined by analysis of variance (ANOVA) using the least square difference method of the General Linear Model procedure of SPSS (1995). Differences were considered significant at the $P<0.05$.

III. RESULTS AND DISCUSSION

Chemical composition of the essential oils: The chemical composition of the essential oils was analyzed using a GC-MS technique. Various components were determined and identified by GC and combined GC-MS, representing about 97,45%, 94,37% and 98,34% of the oils of E. globulus, M. communis L and S. hortensis, respectively. The main constituents of the essential oils are 1,8-Cineol, -Pinene, -Terpinene, p-Cymene for E. globulus; -Pinene, Cinéole 1-8, Limonene, p-Cymene, Linalol, -Caryophyllene for M. communis and Carvacrol, p-Cymene, -Terpinene, Thymol, -Caryophyllene, Borneol, -Terpinene, -Bisabolene, Terpinen-4-ol, Myrcene for S. hortensis, repectively. Antimicrobial activity (assay disk): Preliminary screening of the antimicrobial activity in vitro of the three essential oils from E. globulus, M. communis L. and S. hortensis species was studied against three common pathogens associated with foodborne illness using the paper disc agar diffusion technique (Fig. 1).

The results showed variation in the antimicrobial properties of plant essential oils (Table 1).

Table 1: Antibacterial activity of the essential oils from *E. globulus*, *M. communis* and *S. hortensis*, using paper disc-diffusion method

	ϕ (mm)[a]				
	E. globulus	*M. communis L.*	*S. hortensis*	*Kanamycine*	*Ticarcilline*
Escherichia coli (food isolate)	12.84	10.69	23.32	17.25	11.07
Pseudomonas aeruginosa (clinical isolate)	NA[b]	NA	NA	ND[c]	ND
Staphyloccus aureus (food isolate)	29.00	14.79	14.23	16.50	12.30

All tests were performed in duplicate.
[a]ϕ: Inhibition zone in diameter around the discs impregnated with essential oils. The diameter (6mm) of the disc is included.
[b]NA: *Not active*
[c]ND: *Not determined*

The essential oils showed strong activity: inhibition zone >13 mm, moderate activity: inhibition zone 6 mm < < 13 mm and no inhibition: zone <06 mm (de Billerbeck, 2001). According to the results given in table 1, all essential oils exhibited antimicrobial activity against E. coli and S. aureus. However, the essential oils failed to show antibacterial activity against P. aeruginosa. Results obtained from disc-diffusion method, followed by measurements of Minimal inhibition concentration (MIC), indicated that S. hortesis is the most active essential oil tested, with the lowest MIC value against E. coli and S. aureus (10.1 l/ml). Antimicrobial activity of essential oil on pathogens species inoculated in minced meat: Use of natural preservatives to inhibit growth of serious pathogens such as E. coli and S. aureus is of great interest to the meat industry. The assessment of essential oils in food models is essential to establish if they will be effective antimicrobials within the food matrix. It has been found that higher MICs are often required when applied to food (Burt, 2004).

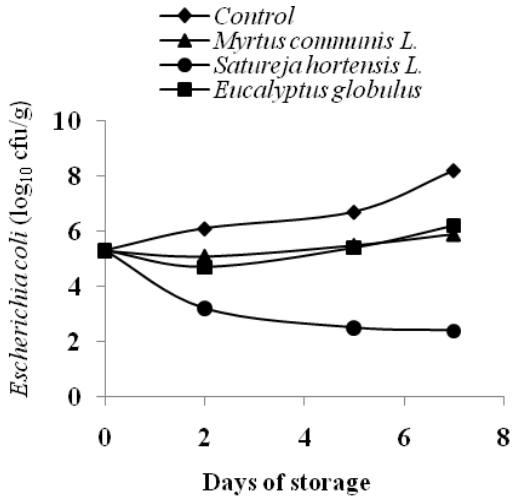

Fig. 2. Inhibition of *E.coli* added in concentration of 2-3 $\times 10^5$ cfu/g by various essential oils in minced beef stored at 4ºC

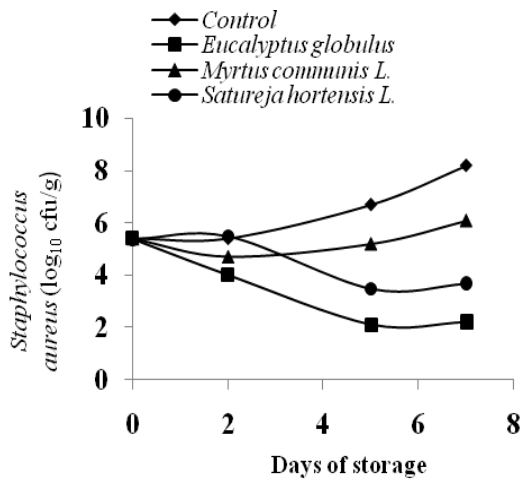

Fig. 3. Inhibition of *S. aureus* added in concentration of 2-3 $\times 10^5$ cfu/g by various essential oils in minced beef stored at 4ºC

Both figures (2, 3) shows the results of microbial counts throughout the storage of minced beef at 4ºC inoculated with pathogens species without essential oils and those with essential oils of E. globulus, M. communis and S. hortensis. Results demonstrated that the essential oils effectively delayed count of bacteria. In both cases, the number of bacteria in unsupplemented meat from the first day to reach, one week later, 8.2 log cfu/g and 8 log cfu/g for E. coli and S. aureus, respectively. When applied, at determined concentration in samples inoculated with microorganisms, the essential oils extracted from E. globulus, M. communis L. and S. hortensis species exerted an antibacterial effect against pathogens species tested. This effect was evident from day 2 of storage onwards, showing significant ($P<0.05$) differences with untreated samples. Concerning the effects of essential oils against E. coli inoculated in minced beef (fig. 2), it appears that S. hortensis essential oil remained more effective. Starting E. globulus and M. communis essential oils displayed moderate antibacterial effect against the same microorganism. Indeed, a reduction of 2.9 log cfu/g (47.54% of reduction) was recorded in 2 days of storage. Five days later (at day 7), the same effects were observed with reduced levels of E. coli during storage by 5.8 log ufc/g (70.74% of reduction). E. globulus essential oil exhibited a similar behavior as seen against S. aureus (fig. 3). Sensory evaluation (results not shown) revealed that the organoleptic properties of minced beef meat treated with essential oils were

acceptable by the panelists at the supplementation levels.

IV. CONCLUSION

According to the gas chromatography/mass spectrometry analysis, the major compounds of the essential oils were 1, 8-Cineol and -Pinene for Eucaliptus. globulus, -Pinene and 1,8-Cineol for Myrtus communis and Carvacrol and p-Cymene for Satureja hortensis. All three essential oils significantly inhibited in vitro the growth of foodborne pathogens E. coli and S. aureus, but failed to inhibit P. aeruginosa. MICs for essential oils of E. globulus and S. hortensis were significantly lower (10.1 ?l/ml) than for M. communis. All three essential oils inhibited growth of E. coli and S. aureus inoculated in minced beef; S. hortensis was the most effective against E. coli, while both E. globulus and S. hortensis were highly effective against S. aureus.

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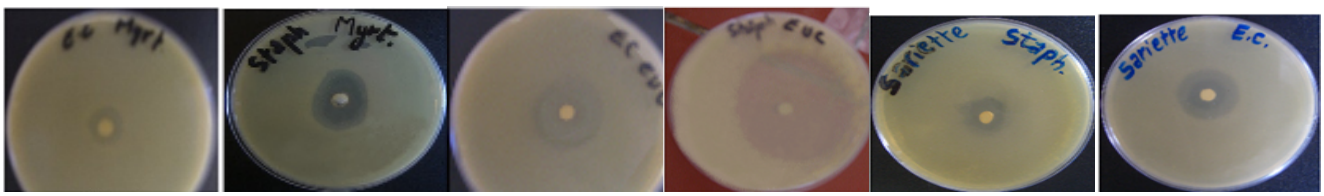

Fig. 1. Inhibition zone of EEO—*E. globulus* essential oil; MEO—*M. communis* essential oil and SEO—*S. hortensis* essential oil against foodborne pathogens: EC—*Escherichia coli*; SA—*Staphylococcus aureus*.