International Journal of Food Microbiology 125 (2008) 242–251

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

# Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat

El Akrem Hayouni [a,d,*], Imed Chraief [b], Manaf Abedrabba [c], Marielle Bouix [e], Jean-Yves Leveau [e], Hammami Mohammed [b], Moktar Hamdi [a]

[a] Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de la Technologie (INSAT), BP. 676, 1080 Tunis, Tunisia
[b] USCR Spectrométrie de Masse, Faculté de Médecine, 5019 Monastir, Tunisia
[c] Unité de Recherche: Physico-chimie et Moléculaire: IPEST, La Marsa, Tunisia
[d] Département des Industries Agro-alimentaires, Institut Supérieur des Etudes Technologiques de Zaghouan, 1121 Mograne, Tunisia
[e] AgroParisTech, INRA, UMR 782 Génie et Microbiologie des Procédés Alimentaires, CBAI, 78850 Thiverval-Grignon, France

ARTICLE INFO

*Article history:*
Received 7 January 2008
Received in revised form 10 April 2008
Accepted 11 April 2008

*Keywords:*
Essential oils
*Salvia officinalis* L.
*Schinus molle* L.
Antimicrobial activity
GC–MS
Minced beef meat
*Salmonella*
Sodium chloride

ABSTRACT

The essential oils (EOs) extracted from the aerial parts of cultivated *Salvia officinalis* L. and the berries of *Schinus molle* L. were analysed by gas chromatography–mass spectrometry (GC–MS) and 68 and 67 constituents were identified, respectively. The major constituents were 1,8-cineole (33.27%), β-thujone (18.40%), α-thujone (13.45%), borneol (7.39%) in *S. officinalis* oil and α-phellandrene (35.86%), β-phellandrene (29.3%), β-pinene (15.68%), p-cymene (5.43%) and α-pinene (5.22%) in *S. molle* oil.

In its second part, the present study was conducted to evaluate the *in vitro* antimicrobial activity of both studied EOs. For this purpose, paper disc-diffusion method and broth microdilution test were used. The disc-diffusion method showed significant zone of lysis against all the pathogens studied (gram-negative and gram-positive bacteria, yeast). These activities remained stable after six months, and decreased approximately by 20% after one year of storage of the EOs at 4 to 7 °C. On comparing the efficiency of both EOs, *S. officinalis* EO exhibited higher antibacterial activity against the majority of strains and especially against *Candida albicans* (two fold more active according to the inhibition zones values). The minimal inhibitory concentrations (MICs) were reported between 4.5 mg/ml and 72 mg/ml on nutrient broth. The particular chemotype of each EO may be involved in its specific antimicrobial behaviour.

Furthermore, the inhibitory effect of these EOs were evaluated against two foodborne pathogens belonging to *Salmonella* genus, experimentally inoculated ($10^3$ CFU/g) in minced beef meat, which was mixed with different concentrations of the EO and stored at 4 to 7 °C for 15 days. Although the antibacterial activities of both EOs in minced beef meat were clearly evident, their addition had notable effects on the flavour and taste of the meat at concentrations more than 2% for *S. molle* and 1.5% for *S. officinalis*. One solution to the above-mentioned problem may be the use of combinations of different food preservation systems. In this context, each of the EOs has been used along with low water activity (addition of NaCl) in addition to low refrigeration temperatures. Results on the *Salmonella* growth showed that some combinations could be recommended to eliminate germs from minced raw beef. By using this method, a stable and, from a microbiological point of view, safe meat can be produced without substantial loss in sensory quality.

Results obtained herein, may suggest that the EOs of *S. officinalis* and *S. molle* possess antimicrobial activity, and therefore, they can be used in biotechnological fields as natural preservative ingredients in food and/or pharmaceutical industry.



## 1. Introduction

Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation. Recently, there has been a considerable interest in extracts and essential oils (EOs) from common culinary herbs, spices and aromatic plants characterized by a notable antimicrobial activity. Such substances can be used to delay or

* Corresponding author. Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de la Technologie (INSAT), BP. 676, 1080 Tunis, Tunisia.
*E-mail address:* a.hayouni@gmail.com (E.A. Hayouni).

0168-1605/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2008.04.005

inhibit the growth of pathogenic and/or toxin producing microorganisms in foods (Marino et al., 2001). This growing interest is emphasized by the fact that foodborne diseases are still a major problem in the world, even in well developed countries (Mead et al., 1999). Moreover, commercial antimicrobial drugs used haphazardly in the treatment of many infectious diseases had inevitably led multiple drug/chemical resistance in both human and plant pathogens (Davis, 1994). In such context, plant-based essential oils or organic extracts are well known to exhibit a wide range of biological activities as well as they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Paranagama et al., 2003). That's why; they had formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Deans and Sbodova, 1990).

Despite the ranges of preservation techniques available, the microbiological safety of foods continues to be a major concern to consumers, regulatory agencies and food industries throughout the world. *Salmonella*, from food origin, remains a primary cause of food poisoning worldwide, and massive outbreaks have been witnessed in recent years (Mead et al., 1999). The use of antibiotics is a major strategy for the eradication of these bacteria, and antimicrobial agents are commonly used therapeutically and prophylactically in human and animal Salmonellosis. However, increased resistance to these drugs is an inevitable side effect of antibiotic use and recent studies have shown that the prevalence of antibiotic resistant *Salmonella* in humans and animals is increasing (O'Brien, 2002). Thus, novel efficient and safe remedies for Salmonellosis are necessary.

Raw and/or processed foods are open to contamination during their production, sale and distribution (Gould, 1996). Thus, at present, it is a necessity to use preservatives to prevent the growth of food spoilage microbes in the food industry (Davidson, 1997). Although, a small number of food preservatives containing EOs are commercially available, until the early 1990s very few studies of the activity of EOs in foods had been published (Board and Gould, 1991). Generally, the susceptibility of bacteria to the antimicrobial effect of EOs also appears to increase with a decrease in the pH of the food, the storage temperature and the amount of oxygen within the packaging. The physical structure of a food may limit the antibacterial activity of EO (Skandamis et al., 2000). Moreover, it has been established that, certain EOs stand out as better antibacterial agents than the commonly used preservatives for meat applications (Tassou et al., 1995).

A number of potential synergists have been suggested for use with EOs: low pH, low water activity, chelators, low oxygen tension, mild heat and raised pressure (Tassou et al., 2000). Sodium chloride has been shown to work as a synergist and an antagonist under different circumstances with EOs and/or their components. For example, synergism between NaCl and mint oil (*Mentha piperita*) against *Salmonella enteritidis* and *Listeria monocytogenes* has been recorded in a Greek taramosalata (fish roe salad) (Tassou et al., 1995).

*Salvia*, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. Some members of this genus are of economic importance since they have been used as flavouring agents in perfumery and cosmetics. Sage (*Salvia officinalis* L.) has been credited with a long list of medicinal uses: e.g. spasmolytic, antiseptic, astringent (Ahmadi and Mirza, 1999; Perry et al., 1999). Some of the phenolic compounds of plants belonging to this genus have also shown excellent antimicrobial activity as well as antioxidant capacity (Tepe et al., 2005; Matsingou et al., 2003). Consequently, the corresponding extracts have been widely used to stabilize fat and fat-containing foods (Skandamis et al., 2000).

*Schinus molle* L. commonly called as peppertree is belonging to the Anacardiaceae family. All parts of this shrubby tree have high oil and essential oil content that produce a spicy scent (Marongiu et al., 2004). The berries-like fruits, which have a pepper flavour, are used in beverages in some countries in South America and they are dried and ground up for a pepper substitute in the Tropics. Throughout South and Central America, peppertree is reported to be an astringent, antibacterial, diuretic, digestive stimulant, tonic, antiviral and wound healer (Murray et al., 2005). In Tunisia, *S. molle* L. is rather used as urban greening tree throughout the country and no real exploitation as medicinal plant was being noted.

Despite the medicinal potential of plants in Tunisia being considerable, knowledge of this area and studies on the biological activities of these plants remained scarce. Furthermore, as far as our literature survey could ascertain, antimicrobial of the Tunisian cultivated herb *S. officinalis* L. and the tree *S. molle* L. have not previously been published, although, there are many reports concerning essential oils from these species in other countries (Marongiu et al., 2004; Murray et al., 2005; Tepe et al., 2005; Delamare Longaray et al., 2007). Moreover, knowing that the activity of EOs from aromatic plants depends on several factors such as the geographical origin, the genetic background of the plant from which the EO was obtained or the insolubility of oil in aqueous media and strain to strain variation in susceptibility to oils (Pattnaik et al., 1997), the aims of this study were (i) to determine the antimicrobial activities of EOs extracted from the aerial parts of *S. officinalis* L. and the berries of *S. molle* L., respectively as related to their chemical composition (ii) to assess the efficacy of these EOs, as antimicrobial, after their conservation, at 4 to 7 °C, for a long period (iii) to test the antibacterial activity of these EOs against two foodborne pathogens, belonging to *Salmonella* genus, inoculated in raw beef meat as well as the impact of their addition on the sensory characteristics of this product; (iv) As an example of a hurdle technology, to check the combined effect of these EOs and NaCl on stabilizing this processed food model, when refrigerated at 4–7 °C.

## 2. Materials and methods

### *2.1. Collection of plant materials*

The herbal parts of *S. officinalis* L. were collected from Tunis suburbs (La Marsa), when flowering (late July, 2006). Berries of *S. molle* L. were gathered, from trees at Tunis, in December and January, 2006. Specimens were identified by Dr. Nadia Ben Brahim at the Department of Botany, National Institute of Agronomic Research (INRAT, Tunis) and voucher specimens were deposited at the Herbarium of the Department of Botany in the cited institute.

### *2.2. Isolation of the essential oils*

The air-dried and finely ground raw materials were submitted to water distillation for 3 h, using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and after filtration, stored at 4 to 7 °C until use. Yields of 0.58% and 0.75% (v/w) were recorded from *S. officinalis* aerial parts and *S. molle* L. berries, respectively.

### *2.3. Gas chromatography analysis/mass spectrometry analysis conditions*

#### *2.3.1. Gas chromatography analysis*

The essential oils were analysed using a Hewlett Packard 5890 II GC equipped with Flame Ionization Detector (FID) and HP-5 MS capillary column (5% phenyl/95% dimethylpolysiloxane: 30 m×0.25 mm id, film thickness 0.25 μm). Injector and detector temperature were set at 250 °C and 280 °C, respectively. Oven temperature was kept at 50 °C for 1 min then gradually raised to 250 °C at 5 °C/min and subsequently, held isothermal for 4 min. Nitrogen was the carrier gas at a flow rate of 1.2 ml/min. Diluted samples (1/100 in hexane, v/v) of 1.0 μl were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.