Université Mouloud Mammeri de Tizi Ouzou
Faculté des Sciences Biologiques et des Sciences Agronomiques
Concours d'entrée en 1ère Année de Doctorat 3ème Cycle (LMD)
Epreuve de Biochimie Fondamentale et Appliquée ; durée : 2h

Questions :

1/ Enumérer les nouvelles caractéristiques (d'ordre physico-chimique, structural, fonctionnel…) des protéines induites par les modifications post-traductionnelles suivantes :

1.1 phosphorylation ;
1.2 glycosylation ;
1.3 formation de ponts disulfures.

2/ Quelle est la nature et l'importance des composés **A, B** et **C** représentés ci-dessous ?

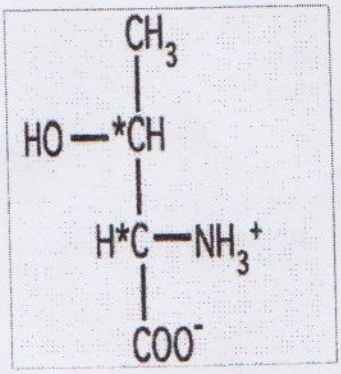
3/ De nos jours, la vocation nutritionnelle des protéines constitue encore un besoin primordial des populations de part le monde. Néanmoins, au cours de ces dernières décennies, la demande sur les protéines se trouve démultipliée par la mise en évidence d'autres champs d'utilisations.

3.1/ énumérer les différentes applications industrielles qui apportent de la valeur ajoutée aux produits alimentaires et non alimentaires par le biais de l'utilisation de certaines propriétés des protéines ;

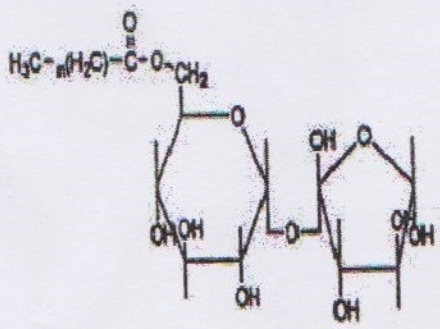
3.2/ quels sont les traitements qui sont susceptibles de réduire l'impact de ces propriétés ?
3.3/ quels sont les traitements qui sont susceptibles d'améliorer ces propriétés ?

4/ Quels sont les éléments fondamentaux que vous pouvez citer qui mettent en évidence l'importance nutritionnelle et technologique des gliadines et des gluténines de la farine de blé ?

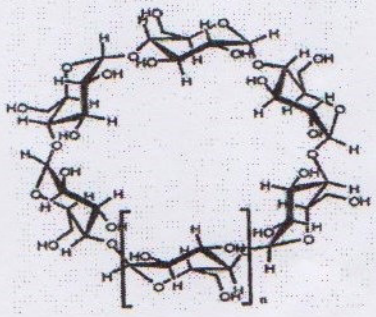
5/ En évoquant les polysaccharides et, tout en citant des exemples de votre choix, dites en quoi les modifications opérées volontairement sur ces produits peuvent présenter un réel intérêt technologique.



A



B



C

Barème :
Quest 1= 4,5 pts ; Quest 2= 3 pts ; Quest 3 = 6 pts ; Quest 4 = 3,5 pts ; Quest 5 = 3 pts

Université Mouloud MAMMERI de Tizi-Ouzou
Faculté des Sciences Biologiques et des Sciences Agronomiques

Epreuve de Biologie moléculaire et génie génétique
Concours de Doctorat Biochimie Microbiologie et Sciences Alimentaires

Le diagnostic génétique du cancer repose tout d'abord sur l'identification d'un gène spécifique de la pathologie étudiée. Cependant, afin de prouver l'implication de ce dernier, on a recours soit à la recherche de l'ARNm du gène soit du produit d'expression de ce même gène. Vous disposez pour votre étude, d'échantillons de sérums et de biopsies.

1- Qu'allez vous rechercher dans les biopsies (ARNm ou produits du gène) ? Justifiez votre réponse. (1 point)
2- De quelle nature chimique est le produit de votre gène ? (1 point)
3- Si vous n'aviez que le sérum comme échantillon qu'allez vous rechercher ? Justifiez votre réponse. (1 point)
4- Vous décidez de cloner le gène d'intérêt afin de l'exprimer dans une cellule hôte, vous ne disposez pour cela que de l'ARNm de ce gène. Quelles sont les étapes par lesquelles vous devriez passer afin de réaliser ce clonage ? (3 points)
5- On vous propose comme cellule hôte pour votre vecteur d'expression, la bactérie *Escherichia coli*, quels sont les problèmes qui peuvent survenir lors de l'expression de votre gène ? (3 points)
6- On désire étudier les mutations au niveau de ce gène, on prélève alors l'ADN de patients atteints de la pathologie et l'ADN de sujet sains, on dispose uniquement de la technique de séquençage. On obtient les profils suivants :

Profil sujet atteint

Profil sujet sain

De quelle mutation s'agit-il ? (1 points) et comment appelle-t-on cette technique de séquençage ? (1 points)

République Algérienne Démocratique et Populaire
Université Mouloud MAMMERI de Tizi-Ouzou
Faculté des Sciences Biologiques et des Sciences Agronomiques

Concours d'accès aux études doctorales 3ème cycle (LMD)

(07/11/2013)

Intitulé: Biochimie-Microbiologie et Sciences Alimentaires

Variante 2

L'article présenté est publié dans la revue ***Food Science and Technology International*** et rentre dans un cadre de projet de recherche internationale.

À partir des faits saillants qui découlent de cet article:

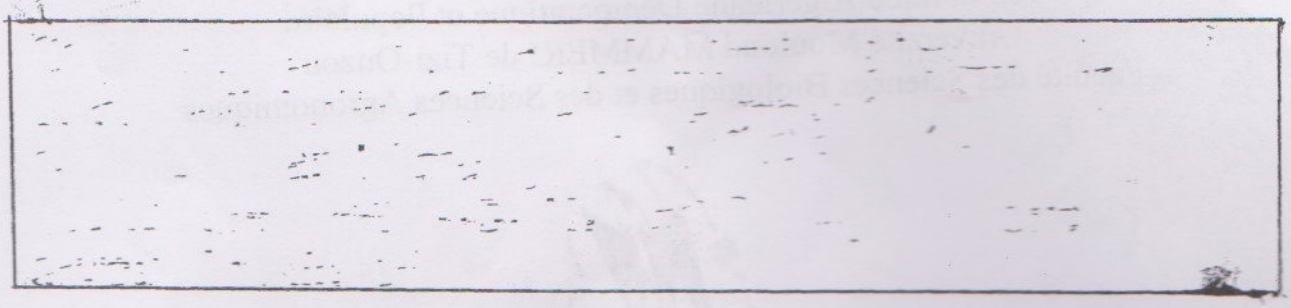
1.- Proposez un intitulé succinct pour cet article;
2.- Elaborez un résumé de 300 à 400 mots au maximum;
3.- Proposez six (06) mots clés (Keywords) les plus significatifs;
4.- Quelle est votre réflexion personnelle sur les aboutissements de ce travail en relation avec les pratiques algériennes concernant la commercialisation et la consommation des viandes hachées.

Bon courage

.../...

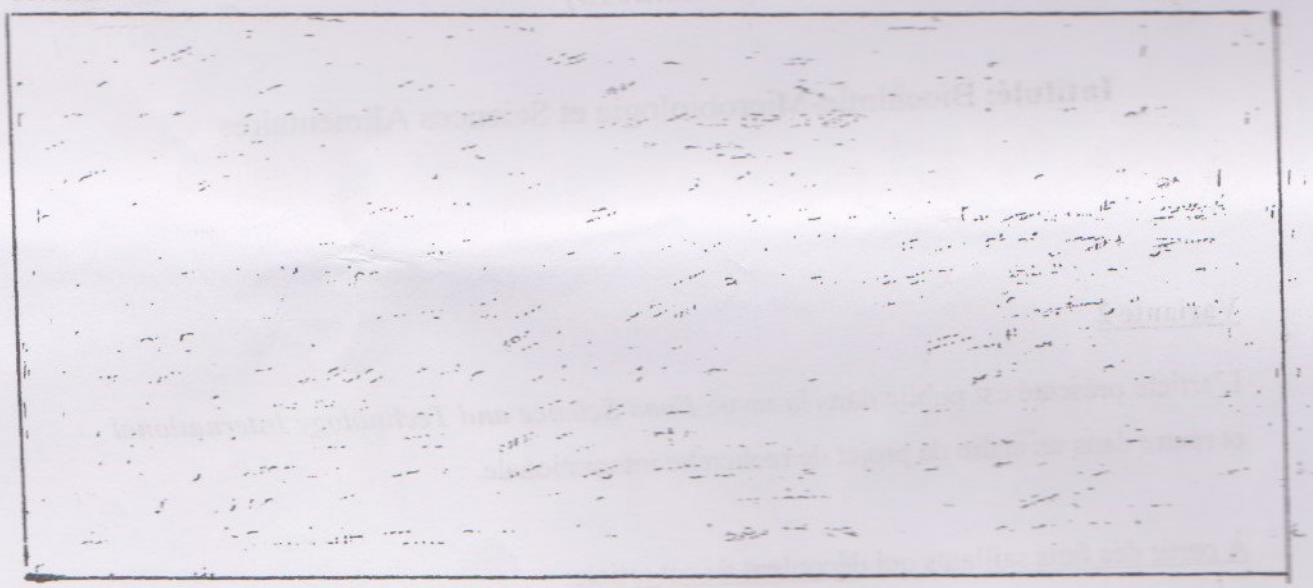
Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

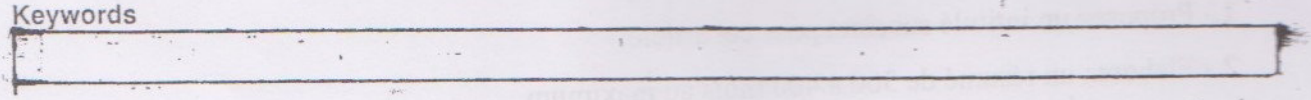


D. Djenane[1], J. Yangüela[2], T. Amrouche[1], S. Boubrit[1], N. Boussad[1] and P. Roncalés[2]

Abstract



Keywords



Date received: 18 August 2010; revised: 13 December 2010

INTRODUCTION

Foodborne illness resulting from consumption of food contaminated with pathogenic bacteria is of vital concern to public health in the world. Those infections are

Food Science and Technology International 17(6) 505–515

DOI: 10.1177/1082013211398803
fst.sagepub.com
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caused by the consumption of contaminated food products. In Algeria, almost 6000 cases have been recorded, for example, in 2008 at a national level and the risk is higher with the arrival of summer season. Collective intoxications take place mainly in collective food-serving because of the lack of hygienic conditions during food preparation.

The origins of staphylococcal food poisoning differ widely among countries; this may be due to differences in the consumption and food habits in each of the countries (Le Loir et al., 2003). The presence of *Staphylococcus aureus* in foods is often related to improper handling by personnel, who are frequently contaminated with these microorganisms (Hatakka et al., 2000). Nevertheless, in foods such as fresh meat, contaminations from animal origins are more frequent (Le Loir et al., 2003). The typical scenario for staphylococcal food poisoning is by contamination of a heat-treated food, through handling by personnel, followed by a temperature abuse. Heating will destroy most of the competing bacteria, which together with cooling failure will provide ideal conditions for growth of staphylococci, should the food be contaminated by accident or malpractice.

E. coli is a widespread pathogen, whose strain O157:H7 is capable of producing an enterohaemorrhagic toxin and additional pathogenic factors. Infections are characterized by diarrheas that vary from mild to severe, bloody and painful. In about 10% of the cases, patients develop severe complications. A total of 5000 cases were reported in 2006 from the European Union (EU) member states (European Food Safety Authority, 2007).

Control of the bacterial cells in foods is an important factor to reduce outbreaks of the foodborne diseases (Kim et al., 2004). Today, different strategies are applied in order to control pathogens in foods, and particular interest has been focused on the application of EOs. Because of greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural ingredients have become popular. This is an important new approach that could solve many problems associated with food alteration and safety. With demands from consumers to find alternatives to chemical-based antimicrobials for food application, further studies are required to assess the changes of sensory properties of foodstuffs after the application of the EOs (Djenane et al., 2011; Holley and Patel, 2005).

The EOs are volatile, natural, complex molecule mixes characterized by a strong odor, which are formed by aromatic plants as secondary metabolites. They are usually obtained by steam or hydrodistillation, first developed in Middle Age by Arabs (Bakkali et al., 2008).

Eucalyptus globulus, *Myrtus communis* and *Satureja hortensis* grow wildly in the Mediterranean basin and particularly in the coastal regions, the internal hills and the forest areas of Kabilya (Algeria) and have been traditionally used as antiseptic, disinfectant agents and for other medical purposes. Even though several studies have been conducted regarding the in vitro antibacterial and antifungal properties of plant EOs, they are poorly soluble in water, and this causes many problems for studying their bacteriological properties. In order to overcome these problems, many authors have recommended the use of various solvents in the dilution of EOs (Burt, 2004).

The assessment of EOs in system models is crucial 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 (Fisher and Phillips, 2006). One of the most novel and promising approaches is to use an active, antimicrobial packaging material for preservation of foods (Camo et al., 2008).

Several studies have shown the in vitro antibacterial properties of many EOs. From this, it was inferred that they could be beneficial for human health in-line with the fact that many diseases are due to an overload of foodborne infection resulting from consumption of food contaminated with pathogenic bacteria. Based on the traditional application of wild-growing *Eucalyptus*, *Myrtus* and *Satureja* species in Algeria as a culinary herb and in folk medicine, the purpose of the present work was to evaluate the antimicrobial activities of their EOs both in vitro and in meat and relate them with their chemical composition, for their application in meat as natural antibacterial ingredients.

MATERIALS AND METHODS

Materials

The aerial parts of *E. globulus*, *M. communis* and *S. hortensis* were collected at Tizi-Ouzou province (Algeria), in March–July 2008, and authenticated by the Department of Biology, University of Tizi-Ouzou (Algeria). Plant specimens were deposited at the herbarium of the Biology Department of the same University. The whole fresh plants were then extensively washed with distilled water (20 °C) to remove epiphytic hosts normally found on the surface and were dried in the darkness at 25 °C. Only the leaves were recuperated for subsequent extraction.

Methods

Essential oil extractions. The EOs were obtained from dried leaves plant parts by hydrodistillation in a Clevenger-type apparatus for 3 h (Groupe Pharmaceutique SAIDAL, Filiale Biotic, Algiers,

Algeria). The EOs obtained were separated from water and dried over anhydrous sodium sulfate (Na_2SO_4) and preserved in darkness in a sealed vial at 2 ± 1 °C until use.

Analysis of essential oils

Gas chromatography analysis. Gas chromatography (GC) analyses of EOs obtained from dried material were performed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and a Stabilwax (PEG) column (30 m × 0.32 mm i.d., 1 µm film thickness; Centre de Recherche en Analyses Physico-Chimiques-CRAPC; USTHB, Algiers, Algeria).

The operating conditions were as follows: injector and detector temperatures, 250 and 280 °C, respectively; carrier gas, N_2 at a flow rate of 1 mL/min; oven temperature program, 3 min isothermal at 50 °C, raised at 2 °C/min to 220 °C and finally held isothermal for 15 min. The identities of the separated components on the polar column were determined by comparing their retention indices relative to aliphatic hydrocarbons injected under the above temperature program with literature values measured on columns with identical polarities.

GC-MS analysis. The GC-MS analysis was performed using a Hewlett-Packard 6890 series GC systems (Agilent Technologies) coupled to a quadruple mass spectrometer (model HP 5973) equipped with a HP5 MS capillary column (5% phenyl methylsiloxane, 30 m × 0.25 mm, 0.25 µm film thickness; CRAPC, USTHB, Algiers, Algeria). 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 0.5 mL/min. Injector and detector MS transfer line temperatures were set at 250 and 280 °C, respectively; the temperature of the ion source was 230 °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 0.20 µL of a hexane-oil solution, injected in the splitless mode. The identity of the components was assigned by matching their spectral data with those detailed in the Wiley 7N, NIST 02 and NIST 98 libraries. The results were also confirmed by the comparison of their retention indices, relative to C7-C29 n-alkanes assayed under GC-MS in the same conditions as the oils. Some structures were further confirmed by available authentic standards analyzed under the same conditions described above. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as the mean value of two injections from each EO.

In vitro antibacterial activity assays

Bacterial strain and culture conditions. The bacterial strains of gram-positive *S. aureus* and gram-negative *E. coli* were provided by the Spanish Type Culture Collection (STCC). Strains used were *S. aureus* CECT 4459, corresponding to STCC type strain for production of enterotoxin B and *E. coli* O157:H7 (CECT 4267). Bacterial strains were cultured overnight at 37 °C on Mueller Hinton agar (MHA, Oxoid, Basingstoke, UK). A total of 1 mL of stock culture was standardized through two successive 24 h growth cycles at 37 ± 1 °C in 9 mL of Brain–Heart Infusion Broth (BHIB; Oxoid, Basingstoke, UK). After 48 h, 100 µL of the suspension were then inoculated in fresh BHIB and incubated at 37 ± 1 °C for 12 h to obtain a working fresh culture containing about 5×10^8 cfu/mL, determined by measuring transmittance at 600 nm (spectrophotometer: Spectronic 20 Bausch & Lomb). These strains were maintained frozen (−80 °C) in cryovials containing an antifreezing agent (Difco Laboratories, Detroit, MI) to preserve the viability of the cells during storage and were subcultured for every antibacterial test.

In vitro tests of antimicrobial activity. Screening of EOs for antibacterial activity was carried out by the agar diffusion method as previously described (Hazzit et al., 2009), which is normally used as a preliminary check and to select among effective EOs. Petri plates were prepared by pouring 20 mL of MHA medium and allowed to solidify. Plates were dried for 30 min in a biological safety cabinet with vertical laminar flow and 0.1 mL of standardized inoculum suspension was poured and uniformly spread over the plate. The inocula were allowed to dry for 5 min. To prepare the stock solution of the samples, the pure EOs were dissolved in 5% (v/v) Tween 80 (Sigma Aldrich®-Química, S.A.). Then sterile filter paper disks (6 mm diameter, Filter LAB ANOIA, testing paper, Barcelona, Spain) were impregnated with 5 µL EO, using a capillary micropipette (Finnpipette®, Thermo Fischer Scientific Inc.). The plates were left for 15 min at room temperature, to allow the diffusion of the EO, 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 caliper (Wiha dialMax® ESD-Uhrmessschieber, CH) and expressed in millimeters (mm: disk diameter included) as its antimicrobial activity. The sensitivity to the different oils was classified by the diameter of the inhibition halos as follows: not sensitive (–) for diameter less than 8 mm; sensitive (+) for diameter 9–14 mm; very sensitive (++) for diameter 15–19 mm and extremely sensitive (+++) for diameter larger than 20 mm (Ponce et al., 2003). Negative

controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotic, chloramphenicol (10 µg/disc), obtained from Sigma Aldrich® was used as positive control in order to test the sensitivity of the tested microorganisms. Each assay in this experiment was replicated three times.

Determination of MIC. The EOs were screened for determination of MIC by the tube dilution method against the same microorganisms. The oils were dispersed at room temperature for 1 min using a homogenizator Ultra-Turrax TP18/1059 (Janke and Kunkel, Staufen, Germany) at 20 000 rpm in sterile 0.5% (v/v) Tween 80 solution to obtain a colloidal suspension (0.50%, v/v). Serial dilutions (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256 and 1/512) of the Tween 80/EO solution were deposited on sterile paper disks, which were subsequently placed in the center of the Petri dishes inoculated with 10 µL of inocula adjusted to approximately 10^6 cfu/mL. 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 Tween 80/EO 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 triplicate.

Inhibitory effect of the EOs against foodborne pathogens inoculated in minced beef meat

Preparation of meat. The *semimembranosus* muscle (initial pH 5.70–5.80) was excised from three beef carcasses 48 h *postslaughter* from a local supplier (Boucherie Khatir, Draâ Ben Khedda, Algeria) and transported to the laboratory under refrigerated conditions within 30 min. After the aseptic removal of the outer surface, meat was aseptically minced by means of a sterile steel meat grinder.

Antimicrobial activity of EO in a meat system. In order to evaluate the antimicrobial activity of EOs in a meat system, a sufficient amount of fresh minced beef was prepared following good practices, and was tested using twice the MIC value (optimal concentration) found for all EOs and bacteria tested. The pieces (600 g) of meat were minced in a sterile grinder; portions of 100 ± 2 g were placed into polystyrene trays (15.50 × 21.50 × 2.50 cm) and overwrapped in polyethylene film (Sidlaw Packaging-Soplaril, Barcelona, Spain). A total of 48 meat samples were obtained. In all, 18 samples were inoculated with approximately 5×10^5 cfu of *E. coli*/g of meat, another 18 of the samples with approximately 5×10^5 cfu of *S. aureus*/g of meat, and 12 of the samples served as controls. Prior to meat inoculation, the samples were added to different concentrations of either *E. globulus*, *M. communis* or *S. hortensis* EOs. Added concentrations of EOs ranged between 0.18 and 0.40, 0.24 and 0.44 and 0.10 and 0.20 % for *E. globulus*, *M. communis* and *S. hortensis*, respectively.

The samples were thoroughly mixed following good practices. All the bags containing the samples of meat were refrigerated at 5 ± 2°C and examined after 2, 5 and 7 days of storage for each microorganism and EO. The untreated controls were added Tween-80 dissolved in sterile water (instead of EO), inoculated with the test bacteria, and stored under the same conditions as the other samples. Two individual duplicate of each experiment were performed in all cases.

Measurement of pH. The pH of meat samples was measured using a micro pH-meter model 2001 (Crison Instruments, Barcelona, Spain) after homogenizing 3 g of sample in 27 mL distilled water for 10 s at 1300 rpm with an Ultra-Turrax T25 (Janke and Kunkel, Staufen, Germany). Each value was the mean of three replicates.

Sensory analysis. Samples of minced beef were evaluated for off-odor by an eight-member trained panel. Panelists were selected among students and staff of the department and trained according to the method described by Djenane et al. (2001). Though already skilled in this kind of evaluation, panelists received further training prior to analysis. Three open-discussion sessions were held to familiarize the individuals with the attributes and the scale to use. The attribute off-odor was evaluated using a 5-point scale, according to Sørheim et al. (1996). Odor scores referred to the intensity of off-odors associated to meat spoilage: 1 = none; 2 = slight; 3 = small; 4 = moderate and 5 = extreme. Results were expressed as the predominant score given by panelists.

Bacterial enumeration. A microbiological check on the meat before inoculation with the target bacteria was performed, with the aim to assess quantitatively and qualitatively the background microflora (results not shown). Microbiological analyses of samples for populations of *E. coli* O157:H7 and *S. aureus* were carried out at 2 or 3 days intervals up to the 7th day of refrigerated storage (5 ± 2°C). At each sampling time, samples (25 g) of minced beef in the stomacher bags were aseptically added with 225 mL of 0.10% sterile peptone water. The contents were macerated in the stomacher (Stomacher 400-Circulator. Seward. Worthing, U.K.) for 1 min at room temperature. Resulting slurries were serially diluted (1:10) in 0.10% sterile peptone water. Sample dilutions (0.10 mL) were spread plated on appropriate media in duplicate. The selective media used for isolation of *S. aureus* was Baird-Parker agar

(Oxoid; CM275) supplemented with Egg Yolk–Tellurite emulsion (Oxoid; SR054C). The plates were incubated aerobically 37 °C for 48 h. Populations of *E. coli* were determined on Cefixime–Tellurite Sorbitol MacConkey (CT-SMAC) agar (DIFCO Lab, Detroit, MI, USA) plates, incubated for 24 h at 37 °C. Counts were expressed as the $\log_{10}$ of cfu per g.

Statistical analysis. Variance analyses were used to test the significant difference among the results from the antibacterial assays (SPSS, 1995). Differences between means were tested through LSD and values of $p < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Yields and chemical constituents of EOs

The average values of hydrodistillation extraction yields of plant EOs from *E globulus*, *M communis* and *S hortensis* were found to be 3.50, 0.05 and 0.06% (volume by weight [v/w]), respectively. In recent years, the supercritical fluid extraction using supercritical carbon dioxide (CO_2) has become an alternative to more conventional extraction procedures. The highest extraction yield was obtained with the supercritical fluid extraction process (Glišić et al., 2007).

To optimize EO yields, several studies were performed on the fresh plant, in particular variation of water content, but the yields obtained were very poor. This can be explained by the high level of water content in the plant. Yields were optimum after 8 days of dehydration and decreased after this drying time. The decline is certainly due to the evaporation of the volatile compounds during long drying times (Bendimerad et al., 2005).

The identified EOs components by GC and combined GC-MS accounted for about 98.12%, 95.98% and 95.57% of the oils of *E. globulus*, *M. communis* and *S. hortensis*, respectively. The main constituents (Tables 1–3) of the EOs were γ-terpinene (94.48%), 1,8-cineole (3.20%) for *E. globulus*; 1,8 cineole (46.98%), cis-geraniol (25.18%), α-terpinol (5.16%), linalylacetate (5.13%), 2-methylbuterate (3.36%), methyleugenol (2.22%), α-terpinolene (1.79%), cis-β-ocimene (1.33%) for *M. communis*; and carvacrol (46.10%), p-cymene (12.04%), γ-terpinene (11.43%), carvacrolacetate (9.57%), α-caryophyllene (5.06%), α-terpinene (3.70%), β-bisabolene (2.65%) and camphene (2.06%) for *S. hortensis*. Because EOs are natural products, all environment, genetics, geographical origin and harvest period affect their chemical composition. The effect of the method of extraction on the resulting chemical composition has been also reported (Bocevska and Sovová, 2007).

Tuberoso et al. (2006) and Batish et al. (2008) reported that 1, 8-cineole, α-pinene and carvacrol were the main constituents of *E. globulus*, *M. communis* and *S. hortensis* EOs, which is in good agreement with our results, except for γ-terpinene, which was found to be 94.48% in *E. globulus*. Differences in EO composition were observed within three different *Eucalyptus* ecotypes, *E. citriodora*, with citronellal (73.30–74.50%) and β-citronellol (5.40–5.70%); *E. globulus* (Australia), characterized by 1,8-cineole (81.20–83.70%) and limonene

Table 1. Percentage of the essential oil obtained from leaves of *Eucalyptus globulus* (only components at percentage ≥0.05 are given)

	Compounds	%
1	α-pinene	0.05
2	α-phellandrene	0.06
3	α-terpinene	0.05
4	p-cymene	0.07
5	1,8-cineole	3.20
6	γ-terpinene	94.48
7	Terpinen-4-ol	0.07
8	α-terpenyl acetate	0.08
9	β-caryophyllene	0.06
	Total identified	**98.12**

Table 2. Chemical composition (%) of the essential oil obtained from leaves of *Myrtus communis* (only components at percentage ≥0.05% are given)

	Compounds	%
1	α-pinene	0.33
2	Camphene	0.05
3	Sabinene	0.07
4	1,8-cineole	46.98
5	Cis-β-ocimene	1.33
6	Trans-β-ocimene	0.07
7	γ-terpinene	1.37
8	α-terpinolene	1.79
9	2-methylbuterate	3.36
10	Terpinen-4-ol	0.54
11	α-terpineol	5.16
12	Linalylacetate	5.13
13	Hydroxycineole acetate	0.11
14	α-terpinyl acetate	2.11
15	Cis-geraniol	25.18
16	Methyleugenol	2.22
17	10-nonadecanone	0.18
	Total identified	**95.98**

Table 3. Chemical composition (%) of the essential oil obtained from leaves of *Satureja hortensis* (only components at percentage ≥0.05% are given)

	Compounds	%
1	α-pinene	0.07
2	Camphene	2.06
3	Sabinene	0.08
4	β-pinene	0.06
5	α-phellandrene	0.20
6	α-terpinene	3.70
7	p-cymene	12.04
8	γ-terpinene	11.43
9	α-terpinolene	0.34
10	Borneol	0.37
11	Terpinen-4-ol	0.90
12	Carvacrol acetate	9.57
13	Carvacrol	46.10
14	α-caryophyllene	5.06
15	Aromadandrene-allo	0.08
16	β-cubebene	0.38
17	Ledene	0.09
18	α-cadinene	0.18
19	β-bisabolene	2.65
20	δ-cadinene	0.09
21	Spathulenol	0.07
22	Caryophyllene oxide	0.05
	Total identified	**95.57**

(7.60–11.70%), and *E. globulus* (China), with 1,8-cineole (79.10–80.10%) and limonene (8.50–8.60%) (Baranska et al., 2006). On the other hand, Sacchetti et al. (2005) found that *E. globulus* was characterized by 1,8-cineole (56.60%), α-pinene (20%), α-phellandrene (6.18%) and α-terpinyl acetate (3.68%).

Tuberoso et al. (2006) reported that the chemical composition of individual samples of *Myrtus* species exhibited small qualitative differences. Nevertheless, large variations depending on the origin of the samples were observed in the concentration of the main constituents. Generally, α-pinene was 30% of each sample except for one of them, in which the content was two-fold higher (59.50%); limonene ranged from 5.20 to 29.80%; 1,8-cineole ranged from 15.90 to 41.70%; linalool ranged from 0.20 to 16.70%; α-terpineol ranged from 1.30 to 4.80% and geranyl acetate ranged from 0.40 to 7.20%.

Regarding *S. hortensis*, Oussalah et al. (2007) reported that the major constituent of the EO from the aerial parts was carvacrol (41%), while Eminagaoglu et al. (2007) found carvacrol, γ-terpinene and p-cymene to be major components of its EO. So far, there have been no attempts to study the chemical composition and biological activities of EOs and extracts from *S. hortensis* plants collected from the Kabilya region of Algeria, although several articles on the antimicrobial and antioxidant properties of this species collected from elsewhere have been published (Güllüce et al., 2003). Recently, Oke et al. (2009) reported that carvacrol (44.99%) and p-cymene (21.61%) were found to be the major compounds of summer savory EO; other important compounds were thymol (9.01%), γ-terpinene (4.35%), borneol (2.51%) and terpinen-4-ol (2.04%). Our study supports the view that carvacrol is a major component of the EO of *Satureja* of Algerian origin.

In vitro antimicrobial activity

Preliminary screening of the in vitro antimicrobial activity of the EOs from *E. globulus*, *M. communis* and *S. hortensis* against two common foodborne pathogens using the paper disc agar diffusion technique is summarized in Table 4. The antimicrobial activities of the EOs were compared to those of chloramphenicol, used as positive controls.

Both *E. coli* O157:H7 and *S. aureus* were significantly inhibited by the three EOs. In fact, *E. globulus* EO gave rise to an inhibition zone of 29 mm against *S. aureus* (extremely sensitive) and 12.80 mm against *E. coli* (sensitive). *S. hortensis* caused an inhibition zone of 23.30 mm against *S. aureus* (extremely sensitive) and of 14.23 mm against *E. coli* (sensitive). Finally, *M. communis* showed inhibition zones of 14.80 mm and 10.70 mm against *S. aureus* (very sensitive) and *E. coli* (sensitive), respectively.

Oussalah et al. (2007), showed that two species of Satureja (S. *hortensis* and S. *montana*), with a concentration of carvacrol of 41% and 43%, respectively, had a strong antibacterial activity against all pathogenic bacteria tested. However, *S. aureus* was four times more sensitive than *E. coli* O157:H7 to these oils, which was in good agreement with our results.

The MIC is cited by most researchers as a measure of the antibacterial performance of EOs (Burt, 2004). The MIC values (Table 5) for all three EOs against *E. coli* and *S. aureus* were in the range 0.05–0.22% (v/v). Oke et al. (2009) reported that inhibition zones of the *Satureja* EO against foodborne spoilage bacteria showed a significant correlation with MIC values. This can be explained by the fact that the sensitivity depends on the type of target microorganism, the type, composition and concentration of the EO, insolubility in aqueous media, seasonal and intraspecific variation of EOs composition (Marino et al., 2001).

Our results demonstrated that gram-positive *S. aureus* was more sensitive to the EOs than

Table 4. Antibacterial activity of the essential oils from *Eucalyptus globulus*, *Myrtus communis* and *Satureja hortensis* using paper disc-diffusion method, expressed by diameter (ϕ) of inhibition zone (mean ± SD) including the disc diameter (6 mm)

	ϕ* (mm)			
	E. globulus	*M. communis*	*S. hortensis*	Chloramphenicol
E. coli O157:H7 (CECT 4267)	12.84 ± 1.2 x	10.69 ± 1.1 x	14.23 ± 1.7 x	17.25 ± 1.4 x
S. aureus (CECT 4459)	29.10 ± 2.3 y	14.79 ± 0.5 y	23.32 ± 3.2 y	22.50 ± 1.3 y

*Values followed by the same letter under the same column are not significantly different ($p > 0.05$). All tests were performed in duplicate.

Table 5. Results of minimum inhibitory concentrations (MICs) for plant essential oils

Essential oil	Bacteria	Gram type	MIC % (v/v)
E. globulus	*E. coli*	–	0.20
	S. aureus	+	0.09
M. communis	*E. coli*	–	0.22
	S. aureus	+	0.12
S. hortensis	*E. coli*	–	0.10
	S. aureus	+	0.05

gram-negative *E. coli*. Delaquis et al. (2002) also found that gram-positive bacteria were more sensitive than gram-negative to the EO of *Eucalyptus*. In general, the antibacterial activity of EOs is mostly due to the presence of phenols, aldehydes and alcohols (Fitzgerald et al., 2003). gram-negative bacteria have been shown to be generally more resistant than gram-positive ones to the antagonistic effects of EOs because of the lipopolysaccharide present in the outer membrane (Russel, 1991). Because of the large number of constituents, EOs seems to have no specific cellular targets. In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool. The EOs can coagulate the cytoplasm and damage lipids and proteins. Damage to the cell wall and membrane can lead to the leakage of macromolecules and thus to lysis (Bakkali et al., 2008).

Carvacrol, p-cymene, γ-terpinene, 1,8-cineole and cis-geraniol are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Marino et al., 2001). Synergism has been observed between carvacrol and its precursor p-cymene (Burt, 2004). Some studies have concluded that whole EOs have a greater antibacterial activity than the major components individually (Gill et al., 2002), which suggests that minor components are critical to the activity and may have a synergistic effect. Mourey and Canillac (2002) demonstrated that whole EOs had a greater antibacterial activity than a mixture of their major components.

Farag et al. (1998) examined the antimicrobial activity of the oils of rosemary, sage and thyme leaves against three gram-negative bacteria (*E. coli*, *Pseudomonas fluorescens* and *Serratia marcescens*) and four gram-positive bacteria (*Bacillus subtilis*, *Micrococcus* spp., *Sarcina* spp. and *S. aureus*). They found that the EOs had no or very little effect against gram-negative bacteria. According to Oussalah et al. (2007), *S. hortensis* and *S. montana* (41% and 43% carvacrol, respectively) exerted a four-fold greater inhibitory on *S. aureus* (gram positive) than on *E. coli* O157:H7 or *S.* Typhimurium (both gram negative). These results were in agreement with ours, which showed that the active compounds present in *S. hortensis* had a stronger and a broader spectrum of antimicrobial activity.

Antimicrobial activity of EOs on pathogens inoculated in minced meat

pH measurements. The initial meat pH of 5.70–5.80 decreased to about 5.60 after treatment with EOs (data not shown). The values of pH did not differ significantly ($p > 0.05$) within treatments throughout storage. The fact that initial meat pH decreased slowly in the presence of EOs and that there were no significant differences ($p > 0.05$) within treatments may be explained

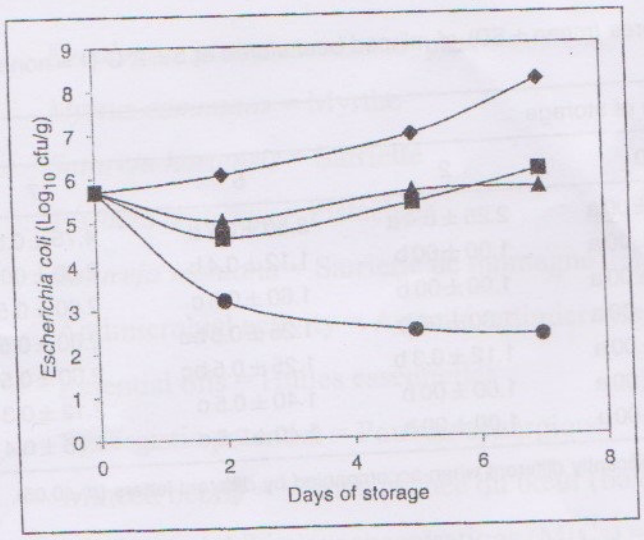

Figure 1. Inhibition of *Escherichia coli* added in a concentration of approximately 5×10^5 colony forming units (cfu)/g by various essential oils (EOs) in minced beef stored at 5 ± 2°C: (♦) Control; (•) *Satureja hortensis*; (■) *Eucalyptus globulus*; (▲) *Myrtus communis*.

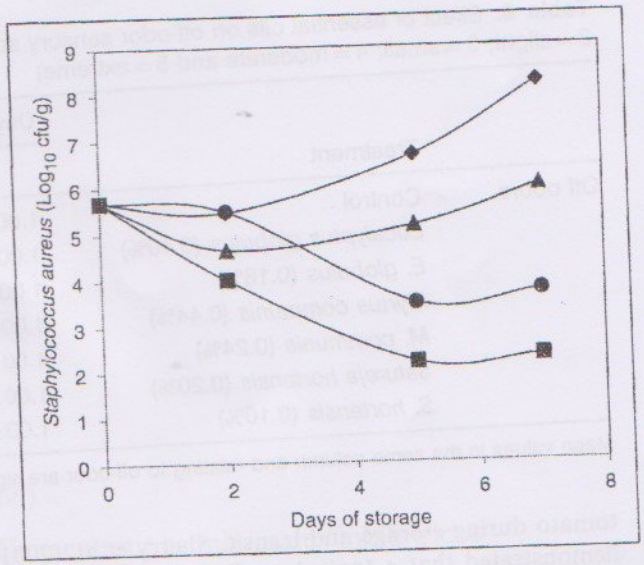

Figure 2. Inhibition of *Staphylococcus aureus* added in a concentration of approximately 5×10^5 colony forming units (cfu)/g by various essential oils (EOs) in minced beef stored at 5 ± 2°C: (♦) Control; (•) *Satureja hortensis*; (■) *Eucalyptus globulus*; (▲) *Myrtus communis*.

by the buffering capacity of meat (Djenane et al., 2003; Smulders, 1995).

Gutierrez et al. (2009) found that the antimicrobial activity of EOs against foodborne pathogens and spoilage bacteria was increased at acidic pH conditions (pH = 5). Previously, it was also observed that the inhibitory effect of plant extracts was greater at acidic pH values. Synergism between EO and pH in antimicrobial action must be therefore considered.

Microbial Analysis. Figures 1 and 2 show the results of microbial counts throughout the storage of minced beef at 5 ± 2°C for 7 days inoculated with *E. coli* O157:H7 and *S. aureus*, respectively, either with or without EOs added.

Results demonstrated that the EOs from *E. globulus*, *M. communis* and *S. hortensis* effectively inhibited bacterial growth or reduced numbers of viable bacteria. In both cases, the numbers of bacteria in unsupplemented meat (without added EOs) reached after 1 week of storage 8.20 log cfu/g and 8 log cfu/g for *E. coli* and *S. aureus*, respectively. This effect was evident from Day 2 of storage onwards, showing significant ($p < 0.05$) differences with untreated samples. Concerning the effects against *E. coli* (Figure 1), it appeared that *S. hortensis* EO was by far the most effective ($p < 0.05$). Indeed, a reduction of 2.90 log cfu/g (47.54% of reduction) was recorded after 2 days of storage. A total of 5 days later (at Day 7), the same effects were observed; levels of *E. coli* were reduced by 5.80 log cfu/g (70.74% of reduction). *E. globulus* and *M. communis* EOs had a moderate inhibitory effect against this microorganism.

Regarding *S. aureus* (Figure 2), both *S. hortensis* and *E. globulus* caused a highly significant decrease of microbial counts, most evident after 5 days of storage; *S. aureus* numbers were 3.50 and 2.50 cfu/g, respectively, after 1 week of storage. The effect of *M. communis* was much lower. These results for the inhibition of both *S. aureus* and *E. coli* in a meat system were in very good agreement with prior in vitro results, as well as with the MIC calculated for each EO. *S. hortensis* EO was most effective in inhibiting both gram-positive and gram-negative bacteria, while *E. globulus* exerted a higher inhibition on gram-positive *S. aureus*.

According to Burt (2004), a higher concentration of EO is needed to achieve the same effect in foods than in vitro. Studies with fresh meat, fish, milk, fruits and vegetables and their products have shown that the concentration needed to achieve a significant antibacterial effect is around 0.50–20 μL/g in foods and about 0.10–10 μL/mL in solutions for washing fruit and vegetables. Tassou et al. (1995) examined the antimicrobial activity of *Mentha piperita* EO in three food systems with different composition; the food system containing beef required a higher concentration of EO for microbial inhibition and this was believed to be due to the higher concentration of protein and fat present.

Tzortzakis (2007) demonstrated that EO vapors from *E. globulus* offered a good choice for maintaining postharvest freshness and firmness of strawberry and

Table 6. Effect of essential oils on off-odor sensory scores (mean ± SD) of minced beef stored at 5 ± 2°C (1 = none; 2 = slight; 3 = small; 4 = moderate and 5 = extreme)

	Treatment	Days of storage			
		0	2	5	7
Off odors	Control	1.00 ± 00 a	2.25 ± 0.4 a	3.50 ± 0.7 a	4.75 ± 0.5 a
	Eucalyptus globulus (0.40%)	1.00 ± 00 a	1.00 ± 00 b	1.12 ± 0.4 b	2.00 ± 00 b
	E. globulus (0.18%)	1.00 ± 00 a	1.00 ± 00 b	1.60 ± 0.5 c	2.60 ± 0.5 c
	Myrtus communis (0.44%)	1.00 ± 00 a	1.00 ± 00 b	1.25 ± 0.5 bc	2.00 ± 0.5 b
	M. communis (0.24%)	1.00 ± 00 a	1.12 ± 0.3 b	1.25 ± 0.5 bc	2.60 ± 0.5 c
	Satureja hortensis (0.20%)	1.00 ± 00 a	1.00 ± 00 b	1.40 ± 0.5 c	2.12 ± 0.3 b
	S. hortensis (0.10%)	1.00 ± 00 a	1.00 ± 00 b	1.40 ± 0.5 c	1.75 ± 0.4 b

Mean values in the same column and relating to off-odor are significantly different when accompanied by different letters ($p < 0.05$).

tomato during storage and transit. Sherry et al. (2001) demonstrated that a topical application of *Eucalyptus* oil could effectively remove the methicillin-resistant *S. aureus* infection.

Several studies have reported the effect of a food matrix on microbial resistance to EOs, but no one of them quantified it nor explained the mechanism, although some suggestions have been made. The greater availability of nutrients in foods compared to laboratory media may enable bacteria to repair damaged cells faster (Gill et al., 2002). On the other hand, it is generally accepted that the high levels of fat and/or protein in foodstuffs protect the bacteria from the action of the EO (Juven et al., 1994).

The antibacterial effect of these EOs against *E. coli* and *S. aureus* in minced beef had not yet been reported. Nevertheless, further research is needed to evaluate the effectiveness of combined *E. globulus*, *M. communis* and *S. hortensis* EOs in this and other food systems, as well as by using active packaging, in order to assess their performance as natural antimicrobial agents in food preservation and safety.

Sensory analysis (off-odor). Sensory scores for off-odor are summarized in Table 6. Results showed that off-odor intensity increased throughout storage in all samples, though not at the same rate. Control minced beef reached the highest value, corresponding to extreme off-odor, at Day 7 of storage. The presence of EOs significantly extended fresh meat odor; in fact, minced meats with added EOs were scored 2, which may be considered as acceptable, at Day 7 of storage. These results were in agreement with those reported by Sánchez-Escalante et al. (2003), who showed that meat treated with natural antioxidants/antimicrobials, either alone or in combination, maintained their fresh meat odor at higher scores than controls during the first phase of storage (12 days). It must be also emphasized that, although the results are not shown, panelists did not perceive any odor related to EOs in minced meat; consequently, the sensory properties of minced beef meat treated with EOs were acceptable by the panelists at the supplementation levels. Solomakos et al. (2008) showed that the sensory properties of minced meat treated with EOs were acceptable at the supplementation levels of 0.30 and 0.60%, but unacceptable at the level of 0.90%. However, Ouattara et al. (2001) reported that addition of EO at 0.90% exerted no negative effects on the flavor and appearance of cooked shrimps. Therefore, more work on the acceptability of these ingredients will be necessary. Moreover, other important parameters, such as the discoloration and color of the meat samples should be taken into consideration, when interpreting the overall acceptability of the meat.

Our data support the possible use of EOs of the tested species *Eucalyptus*, *Myrtus* and *Satureja* from the Kabilya region against two important pathogenic microorganisms, *E. coli* O157:H7 and *S. aureus* inoculated in minced meat.

ACKNOWLEDGMENTS

The authors are grateful to Ministerio de Asuntos Exteriores y Cooperación of Spain (AECID) for financial assistance to this work within the Programa de Cooperación Interuniversitaria e Investigación Científica PCI/MED Algeria-Spain (grants ALI A/011170/07 and ALI A/019342/08).

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Glossaire globale (*Food Control* + *Food Science and Technology International*)

Eucalyptus globulus = Eucalyptus

Myrtus communis = Myrthe

Satureja hortensis = Sarriette

Pistacia lentiscus = Pistachier

Satureja montana = Sarriette de montagne

Antimicrobial activity = Activité antimicrobienne

Essential oils = Huiles essentielles

Synergistic potentiel = Pouvoir synergique

Minced beef = Viande hachée du bœuf (bovine)

Minimum inhibitory concentrations (MICs) = Concentrations minimales inhibitrices

Target bacteria = Bactérie cible

Sensory evaluation = Analyse sensorielle

Panellists = Panel de dégustateurs

Off odor = Odeur altérée

Acceptability = Acceptabilité

Disc-diffusion method = Méthode de diffusion sur gélose

Microdillution assays = Méthode de dilution sur milieu liquide

Diameter of the inhibition halos = Halos d'inhibitions

The aerial parts = Les parties aériennes (ex. feuilles)

Foodborne pathogens = Bactéries pathogènes alimentaires

Illness = Maladies

Yields = Rendements

Storage = Stockage