JOURNAL OF MEDICINAL FOOD
J Med Food 14 (1/2) 2010, 1–6

DOI: 10.1089/jmf.2010.0001

Inhibitory Effects of Some Plant Essential Oils Against *Arcobacter butzleri* and Potential for Rosemary Oil as a Natural Food Preservative

Reyhan Irkin,[1] Secil Abay,[2] and Fuat Aydin[2]

[1]*Susurluk College, Balikesir University, Susurluk, Balikesir; and* [2]*Microbiology Department, Veterinary Faculty, Erciyes University, Kayseri, Turkey*

ABSTRACT We investigated the inhibitory activity of commercially marketed essential oils of mint, rosemary, orange, sage, cinnamon, bay, clove, and cumin against *Arcobacter butzleri* and *Arcobacter skirrowii* and the effects of the essential oil of rosemary against *A. butzleri* in a cooked minced beef system. Using the disc diffusion method to determine the inhibitory activities of these plant essential oils against strains of *Arcobacter*, we found that those of rosemary, bay, cinnamon, and clove had strong inhibitory activity against these organisms, whereas the essential oils of cumin, mint, and sage failed to show inhibitory activity against most of the *Arcobacter* strains tested. The 0.5% (vol/wt) essential oil of rosemary was completely inhibitory against *A. butzleri* in the cooked minced beef system at 4°C. These essential oils may be further investigated as a natural solution to the food industry by creating an additional barrier (hurdle technology) to inhibit the growth of *Arcobacter* strains.

KEY WORDS: • ***antimicrobial*** • **Arcobacter** ***spp.*** • ***essential oils*** • ***minced beef plant*** • ***rosemary essential oil***

INTRODUCTION

THE GENUS *ARCOBACTER* consists of Gram-negative, aerotolerant, spiral-shaped bacteria that are closely related to and were formerly designated as belonging to the genus *Campylobacter*. The key features used to differentiate the members of *Arcobacter* from those of *Campylobacter* are as follows: the ability to grow at 15°C and to grow optimally under aerobic conditions at 30°C; a guanine-plus-cytosine content of 27–30 mol%; and methyl-substituted menaquinone-6 not present as a major isoprenoid quinine.[1] *Arcobacter* spp. can be pathogens, opportunistic pathogens, and commensals. Their toxins or other virulence factors have not been demonstrated, but they have apparent adhesive or invasive properties or both. Moreover, strains of *Arcobacter* have shown significant antibiotic resistance.[2]

Eight species of *Arcobacter* have been identified to date: *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter skirrowii*, *Arcobacter nitrofigilis*, *Arcobacter cibarius*, *Arcobacter halophilus*, *Arcobacter thereuis*, and *Arcobacter mytili*. Strains of *Arcobacter* have been detected mainly in drinking water and meats, including pork, beef, and poultry, as well as in poultry processing plants and in mussels.[3,4]

A recent study in Ireland reported a wide range (5–96%) of contamination by *Arcobacter* spp. of raw animal products available on a retail basis, including 62% of poultry, 35% of pork, and 34% of beef products, as well as 46% of milk products.[5] Vintigni *et al*. [6] found contamination by *A. butzleri* of fresh chicken meat in supermarkets in Thailand.

Essential oils are volatile natural mixtures characterized by a strong odor and consist of organic compounds generated as secondary metabolites by aromatic plants. They are highly complex natural mixtures and can contain from about 20–60 components at quite different concentrations.[7,8]

Essential oils are known for their antiseptic (*i.e*., bactericidal, virucidal, and fungicidal) medicinal properties, for preservation of foods, and as antimicrobial, analgesic, sedative, anti-inflammatory, and local anesthetic remedies.[9,10] The antimicrobial properties of essential oils have been recognized for centuries and, with growing demand through changes in legislation, consumer trends, and increasing isolation of antibiotic-resistant pathogens, may be useful as alternatives to chemically obtained bactericidal agents in animal feeds and foodstuffs.[11]

Very limited research has been done on the inhibitory activities of plant-derived essential oils against strains of *Arcobacter*. This research has included study of the inhibitory activity of the essential oils of mint, rosemary, orange, sage, cinnamon, bay, clove, and cumin and of cefuroxime sodium and gentamicin against several strains of *A. butzleri* and *A. skirrowii* isolated at the Veterinary Faculty of Erciyes University (Kayseri, Turkey). As part of this research, the inhibitory activity of the essential oil of rosemary (*Rosmarinus officinalis* L.) was tested against *A. butzleri* inoculated into a cooked minced beef system.

Manuscript received 6 January 2010. Revision accepted 15 May 2010.

Address correspondence to: Dr. Reyhan Irkin, Susurluk College, Balikesir University, TR10600, Susurluk, Balikesir, Turkey, E-mail: rirkin@hotmail.com *or* reyhan@balikesir.edu.tr

MATERIALS AND METHODS

Arcobacter *strains*

All *Arcobacter* isolates used in the study were obtained during the previous research of Aydin *et al.*[12] (they examined isolates from a total of 806 samples collected from January to June 2005 in the city of Kayseri). *Arcobacter* isolates were identified at the species level using multiplex polymerase chain reaction analysis in the Veterinary Faculty of Erciyes University. A reference strain of *A. butzleri* (LMG 10828; LMG Bacteria Collection, Ghent University, Ghent, Belgium) was included as a positive control throughout the study.

Essential oils and antibiotic discs

Essential oil of rosemary was purchased from the Royal Botanic Gardens Kew (Richmond, UK), and all other essential oils were obtained from the Sefer Yasemin Spice Food Botanic Company (Manisa, Turkey). All the essential oils were pure (100%) concentrations, and the parameters used in assessing the quality of the oils (appearance, color, purity, odor, density at -20°C, and refraction index at -20°C) were described in a technical report that accompanied the oils. Discs containing cefuroxime sodium (30 μg) and gentamicin (10 μg) were purchased from Oxoid Ltd. (Basingstoke, UK).

Disc diffusion procedure

All *Arcobacter* isolates were cultured in *Arcobacter* Enrichment Broth (catalog number CM 965, Oxoid) at 37°C for 24 hours under microaerobic conditions, and 0.1-mL aliquots of these cultures (containing approximately 10^7–10^8 colony-forming units [cfu]/mL) were inoculated on blood agar (BA) plates. The BA was prepared by adding 5% (vol/vol) defibrinated sheep blood to BA base No. 2 (catalog number CM271, Oxoid). Sterile paper discs (6 mm in diameter; catalog number 2668, Schleicher & Schuell, Dassel, Germany) were placed on the BA and impregnated with 50 μL of the plant essential oils. The plates were then incubated at 37°C for 24 hours under microaerobic conditions. Gas-generating kits (catalog number 1.16275.0001, Anaerocult® C, Merck, Darmstadt, Germany) were used to provide the microaerobic atmosphere for culture. The zones of inhibition surrounding the discs on each BA plate were measured in millimeters around the discs. Positive activity was defined as a zone of inhibition of $\geq$10 mm surrounding a disc.[8]

Treatment of minced beef

Psoas major muscles were obtained from beef carcasses at 1 hour after slaughter at a local abattoir and were transported within 30 minutes and under refrigerated conditions to our laboratory, where they were diced into approximately 1-cm cubes and ground through a 3-mm-diameter orifice. The minced beef was sterilized by autoclaving at 121°C for 15 minutes, and three portions, of 250 ± 0.1 g each, were put into separate, sterilized, high-density polyethylene bags. All three portions of minced beef were inoculated with 10^{10} cfu/g *A. butzleri* (minced beef sample 7, Table 1); rosemary essential oil was added to two portions of the minced beef at concentrations of 0.25% and 0.5%, while the third portion served as a control (vol/wt, final concentration), and mixed thoroughly for 1 minute to achieve uniform dispersal of the essential oil throughout the minced beef. Control samples were prepared with cooked minced beef alone, without rosemary essential oil. All samples were stored under refrigeration at 4°C for 7 days. Three specimens from each sample group were analyzed.

Determining counts of A. butzleri *in the samples*

Specimens of cooked minced beef weighing 10 g were transferred aseptically into a medium containing 90 mL of sterile buffered peptone water solution (0.1% wt/vol) and were homogenized in a laboratory blender (model MP80, Braun, Kronberg, Germany). For each sample, appropriate serial decimal dilutions were prepared in buffered peptone water solution (0.1%). Counts of *A. butzleri* were made in *Campylobacter* Blood Free Selective Agar Base (catalog number CM-0739, Oxoid) after incubation at 37°C for 48 hours under microaerobic conditions produced with gas-generating kits as described above.

Chemical analysis

The pH values of the cooked minced beef were recorded with a pH meter (model HI221 microprocessor, Hanna Instruments, Woonsocket, RI, USA). The initial pH was 6.17, and no significant differences ($P > .05$) were observed in the pH values of the control and treated samples during storage at 4°C. The fat and moisture contents of the minced beef preparations were determined according to guidelines published by the Association of Official Agricultural Chemists[13] and the International Organization for Standardization,[14] respectively. Chemical analysis of the beef preparations showed a fat content $2.6 \pm 0.1\%$ (wt/wt) and moisture content of $73.5 \pm 0.2\%$ (wt/wt).

Statistical analysis

Analysis of variance of the results of the disc diffusion method was done with SPSS version 10.0 for Windows (SPSS, Inc., Chicago, IL). Significance was set at $P = .05$. The data for the numbers of *A. butzleri* in cooked minced beef were analyzed with the two-tailed Student's t test. Results were considered significant at $P = .05$.[15]

RESULTS

The data presented in Table 1 show the inhibition zones of the plant essential oils, gentamicin, and cefuroxime sodium against the *Arcobacter* strains examined in our study. The differences in activities of the essential oils against the same strains of *Arcobacter* were significant at $P < .05$ and $P < .01$, indicating that the resistance of these strains to the essential oils was significantly different, again at $P < .05$ and $P < .01$.

TABLE 1. AVERAGE INHIBITION BY ESSENTIAL OILS, GENTAMICIN, AND CEFUROXIME SODIUM OF ISOLATES OF TWO SPECIES OF *ARCOBACTER*

Antimicrobial agent	*Inhibition zone (mm)*[†]											
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
CN	16.5 ± 0.7^{b}	14.3 ± 2.0^{b}	14 ± 1.7^{b}	10 ± 1.0^{a}	15.6 ± 1.5^{b}	14.3 ± 2.0^{b}	9.3 ± 0.6^{a}	14.7 ± 2.5^{b}	14 ± 1.0^{b}	11 ± 1.0^{a}	12.3 ± 0.6^{a}	16.7 ± 1.5^{b}
CXM	0	0	0	0	0	0	0	0	0	0	0	0
Rosemary	—^{A**}	0	24.3 ± 1.5^{b}	16.7 ± 1.5^{b}	$55.3 \pm 3.0^{bA**}$	15.3 ± 0.6^{b}	—^{A**}	16 ± 1.0^{b}	—^{A**}	$55 \pm 1.0^{bA*}$	—^{A**}	$36 \pm 1.7^{bA*}$
Clove	16 ± 0.0^{b}	21.7 ± 1.5^{b}	20.7 ± 1.1^{b}	$41 \pm 1.7^{bA*}$	25.6 ± 2.5^{b}	$36 \pm 1.0^{bB*}$	$75 \pm 3.0^{bA**}$	9.0 ± 1.0^{a}	11.7 ± 0.6^{a}	$37 \pm 2.6^{bB*}$	22 ± 0.6^{b}	18 ± 1.5^{b}
Mint	0	0	0	0	0	0	9.0 ± 1.1^{a}	0	0	0	10 ± 0.0^{a}	0
Bay	—^{A**}	$38.3 \pm 1.5^{bA*}$	25.3 ± 2.5^{b}	0	$44.7 \pm 2.0^{bA*}$	—^{A**}	$62.7 \pm 2.5^{bA**}$	0	$44.7 \pm 0.6^{aA*}$	14 ± 1.0^{b}	≥^{A**}	30.1 ± 1.1^{b}
Orange	17.5 ± 0.7^{b}	18.3 ± 1.5^{b}	9.0 ± 1.0^{a}	$46 \pm 1.0^{bA*}$	24.7 ± 1.5^{a}	17.3 ± 0.6^{b}	13.7 ± 1.5^{b}	0	8.7 ± 0.6^{a}	0	21.7 ± 1.5^{b}	23 ± 1.0^{b}
Cumin	8.0 ± 0.0^{a}	9.0 ± 1.0^{a}	0	0	0	0	0	0	0	0	7.6 ± 0.6^{a}	0
Cinnamon	$35 \pm 1.4^{bB*}$	27 ± 1.0^{b}	$36.7 \pm 3.0^{bB*}$	$32 \pm 2.0^{bB*}$	$51.3 \pm 1.1^{bA*}$	0	$45.3 \pm 1.5^{bA*}$	0	23.7 ± 1.1^{b}	25.7 ± 1.1^{b}	$39.7 \pm 1.5^{aA*}$	$37.7 \pm 2.5^{bA*}$
Sage	0	0	0	$32 \pm 1.0^{bB*}$	0	0	23.3 ± 0.6^{b}	0	0	0	0	0

Data are mean ± SD values (in mm) ($n = 6$). A value of 0 means that growth was observed and there was no halo around the 6-mm filter paper discs. A dash indicated that values were equal to or greater than the diameter of the Petri dishes. (Note that zones of inhibition were 0 for all controls.)

[†]1, *A. butzleri* (human isolate); 2, *A. skirrowii* (cattle isolate); 3, *A. butzleri* (cattle isolate); 4, *A. butzleri* (minced beef isolate). 5, *A. butzleri* (minced beef isolate); 6, *A. butzleri* (minced beef isolate); 7, *A. butzleri* (minced beef isolate); 8, *A. butzleri* (minced beef isolate); 9, *A. butzleri* (chicken isolate); 10, *A. butzleri* (chicken isolate); 11, *A. butzleri* (chicken isolate);12, *A. butzleri* (standard strain, LMG10828).

[ab]Mean values within the same row with different lowercase letters are in different subsets.

[AB]Mean values within the same column with different capital letters are significantly different: $^{*}P < .05$, $^{**}P < .01$.

CN, gentamicin (10 μg); CXM, cefuroxime sodium (30 μg).

In minced meat model system, the growth of isolates of *A. butzleri* was monitored over a 7-day period, and the results were compared with those for controls (Fig. 1). We selected 0.25% and 0.5% (vol/wt) concentrations of rosemary oil for addition to our minced beef samples because of the unacceptably strong flavor produced by higher concentrations. The counts of *A. butzleri* in our control samples increased after 3 days and reached 8 log cfu/g at the end of the 7-day storage period. The 0.25% (vol/wt) concentration of rosemary oil did not show strong inhibitory activity against the high inoculum of *A. butzleri* in minced beef. The numbers of *A. butzleri* in these samples differed significantly ($P < .05$ and $P < .01$) from those in the controls, and they decreased to 6 log cfu/g during the first 3 days of storage, but thereafter increased sharply, reaching the same 8 log cfu/g as seen in the control samples across the 7-day period of storage.

Addition of 0.5% (vol/wt) rosemary oil to cooked minced beef inhibited viable *A. butzleri* by about 8 log cfu/g after 4 days of storage.

DISCUSSION

All *Arcobacter* strains were resistant to cefuroxime sodium at 30 μg, and it was used as a control agent for the sensitivity of these strains in our study. Atabay and Aydin[16] observed that cephalosporins, including cefuroxime sodium, are commonly used in selective media to isolate *Campylobacter* strains. Compared with some of the essential oils examined in our study, gentamicin at 10 μg had moderately inhibitory activity against the strains of *Arcobacter*.

Rosemary oil

Rosemary (*R. officinalis* L.) has been reported to contain certain compounds including rosmanol, rosmariquinone, rosmaridiphenol, and carnosol. Several authors[17,18] have reported that compounds present in rosemary extracts might have antibacterial activity. Fernandez-Lopez *et al.*[18] reported that the compounds responsible for this antibacterial activity seemed primarily to be phenolic diterpenoids, which are the main constituents of the nonpolar fraction of rosemary extracts. In our study, rosemary oil completely inhibited *A. butzleri* from a human subject, two isolates of *A. butzleri* from minced beef, and two isolates of *A. butzleri* from chicken meat. However, a strain of *A. skirrowii* isolated from cattle was found to be very resistant to rosemary oil.

Clove oil

Clove oil inhibited all of the strains of *Arcobacter* examined in our study with varying efficacy. An isolate of *A. butzleri* from minced meat was the *Arcobacter* strain least sensitive to clove oil, with a 9 mm zone of inhibition. That eugenol is the main component of clove oil has been reported by Goni *et al.*,[19] and inhibitory activity of clove oil is explained by eugenol.[20]

Mint oil

Mint oil exhibited minimal effects against the *Arcobacter* isolates examined in our study; two isolates of *A. butzleri* from minced meat and chicken samples were affected, with inhibition zones of 9 and 10 mm, respectively. Mint (*Mentha villosa*) is an endemic plant, and the composition and antimicrobial efficacy of its essential oil vary throughout the world. However, research to date has clearly shown that *Arcobacter* strains are moderately susceptible to mint oil.[17]

Bay oil

The essential oil of bay (*Laurus nobilis*) contains 1, 8-cineole, sabinene, α- and β-pinenes, and linalool as its major constituents, all of which have been reported to be very useful in preservation of foods.[21] In our study, bay oil was the second most effective essential oil after rosemary oil against the *Arcobacter* strains examined except for two isolates from minced beef. This can be explained by the sensitivity of some *Arcobacter* isolates to 1,8-cineole, which is the main component of bay oil. Among the essential oils that we tested, bay oil showed the greatest inhibitory activity against *A. skirrowii*, with a 38 mm zone of inhibition. Inhibitory activity of bay oil against *Arcobacter* spp. has not previously been reported, and we could therefore not compare our findings for the efficacy of this essential oil against *Arcobacter* strains with those of other studies.

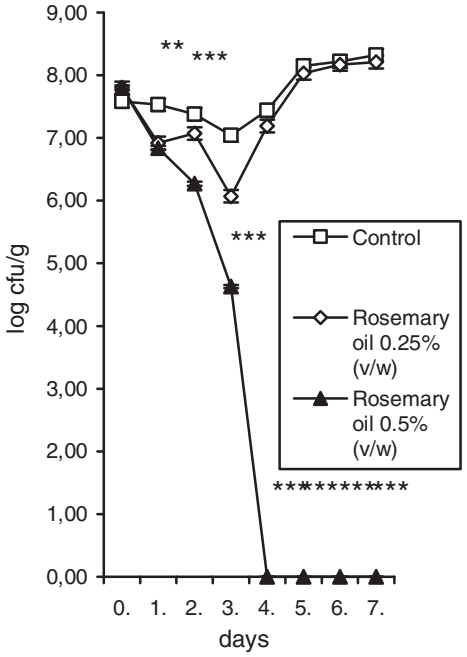

FIG. 1. Effect of essential oil of rosemary on viable cell numbers of *A. butzleri* in cooked minced beef during storage at 4°C. Data represent mean values of triplicate measurements, and error bars are indicated. Significant differences between samples with 0.5% rosemary oil and controls: $^{**}P < .05$, $^{***}P < .01$. cfu, colony-forming units.

Orange oil

A single minced beef isolate and a single chicken isolate of the *A. butzleri* examined in our study were resistant to orange oil, and other isolates were moderately affected by this essential oil. An isolate of *A. butzleri* from cattle was the *Arcobacter* most sensitive to orange oil, with a 46 mm zone of inhibition. Fisher *et al.*[22] similarly found a weak effect of orange oil against a chicken isolate, the standard strain NCTC 12148, and water isolates of *A. butzleri*, with 0, 14, and 30 mm zones of inhibition, respectively.

Cumin oil

Like the essential oils of mint and sage, the essential oil of cumin demonstrated weak activity against *Arcobacter* strains. Cervenka *et al.*[17] found zones of inhibition of 7.9–13.3 mm, 9.3–9.4 mm, and 11.6–15.1 mm with methanol and chloroform extracts of cumin against *A. butzleri* CCUG 30484, *A. cryaerophilus* CCM 3934, and *A. skirrowii* CCUG 10375, respectively.

Cuminal (36.31%), cuminic alcohol (16.92%), γ-terpinene (11.14%), safranal (10.87%), *p*-cymene (9.85%), and β-pinene (7.75%) have been reported as the major components of cumin oil.[23] It is known that the composition of botanical decoctions depends on the plant species from which they come and that their antimicrobial effects also depend on the plant species of their origin and the regional conditions under which these species are grown.

Cinnamon oil

Cinnamon oil exhibited various effects against the *Arcobacter* isolates examined in our study except for two of the isolates of *A. butzleri* from minced beef. Earlier studies had found antimicrobial activity of cinnamon extracts and oil against a wide variety of pathogenic microorganisms, including *Arcobacter* strains and related microorganisms such as *Campylobacter* and *Helicobacter pylori.*[24,25] Similarly, Cervenka *et al.*[17] reported that *A. butzleri* and *A. cryaerophilus* were highly sensitive to a chloroform extract of cinnamon, for which the respective zones of inhibition against the two organisms were 30.9 mm and 41.2 mm, and also reported that methanol and chloroform extracts of cinnamon had similar zones of inhibition of 22.8 and 23.8 mm, respectively, against *A. skirrowii*. The essential oil of cinnamon contains approximately 75% cinnamaldehyde and 8% eugenol, both of which have shown bactericidal effects against foodborne pathogens.[26]

Sage oil

Of the 12 *Arcobacter* isolates examined in our study, only two minced beef isolates of *A. butzleri* were sensitive to sage oil, for which the respective zones of inhibition were 23.3 and 32 mm, and the other 10 isolates were unaffected by sage oil. Tullio *et al.*[27] determined that *cis*-thujone and camphor are the main components of sage (*Salvia officinalis* L.) oil. Menaker *et al.*[28] reported that sage oil exhibited its greatest inhibitory effect against Gram-positive bacteria and had no effect against Gram-negative organisms.

Inhibitory activity of rosemary oil in cooked minced beef

Several studies have reported an antimicrobial effect of rosemary in meat model systems. In addition to its antimicrobial activity, it was indicated that the addition of rosemary extracts or oils improved the organoleptic quality of the meat products examined in these studies because of the antioxidative effect of these rosemary derivatives.[18,29–31]

Research on the susceptibility of *Arcobacter* strains to plant extracts has been limited. Cervenka *et al*.[17] found very strong inhibitory effects of rosemary extracts against *A. butzleri*, *A. cryaerophilus*, and *A. skirrowii*. Our study used minced beef contaminated with a possibly higher inoculum size of *A. butzleri* than should be encountered in naturally contaminated products. Consequently, lower concentrations of essential oils than were used in our study might be sufficient to inhibit *A. butzleri* in naturally contaminated minced beef.

Our study demonstrates the potential of commercial rosemary, bay, cinnamon, and clove oils as natural antimicrobial agents against *Arcobacter* spp.

The results of our study support the promise of rosemary oil and other plant essential oils as natural antibacterial agents in foods that are often contaminated with *Arcobacter* isolates, such as cooked minced beef. However, the complexity of essential oils and their variability in chemical composition indicate the need for more research to better characterize their potential as antimicrobial agents and to identify their effective concentrations in food and feed matrices.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Phillips CA: *Arcobacter* spp in food: isolation, identification and control. *Trends Food Sci Technol* 2001;12:263–275.
2. Ho HTK, Lipman LJA, Gaastra W: *Arcobacter*, what is known and unknown about a potential foodborne zoonotic agent. *Vet Microbiol* 2006;115:1–13.
3. Houf K: *Arcobacter* in a food safety perspective. *Arch Lebensmitt* 2009;60:73–76.
4. Collado L, Inza I, Guarro J, Figueras MJ: Presence of *Arcobacter* spp. in environmental waters correlates with high levels of fecal pollution. *Environ Microbiol* 2008;10:1635–1640.
5. Fisher K, Phillips CA: The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus in vitro* and in food systems. *J Appl Microbiol* 2006;101:1232–1240.
6. Vintigni SM, Srijan A, Wongstitwilairoong B, Marcus R, Meek J, Riley PL, Mason C: Prevalence of foodborne microorganisms in retail foods in Thailand. *Foodborne Pathog Dis* 2007;4:208–215.
7. Irkin R, Korukluoglu M: Growth inhibition of pathogenic bacteria and some yeasts by selected essential oils and survival of *L. monocytogenes* and *C. albicans* in apple–carrot juice. *Foodborne Pathogens Dis* 2009;6:387–394.
8. Irkin R, Korukluoglu M: Effectiveness of *Cymbopogon citratus* L. essential oil to inhibit the growth of some filamentous fungi and yeasts. *J Med Food* 2009;12:193–197.
9. Bakkali F, Averbeck S, Averbeck D, Idaomar M: Biological effects of essential oils—a review. *Food Chem Toxicol* 2008;46: 446–475.
10. Burt S: Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol* 2004;94: 223– 253.
11. Fisher K, Phillips C: Potential antimicrobial uses of essential oils in food: Is citrus the answer? *Trends Food Sci Technol* 2008;19: 156–164.
12. Aydin F, Gumussoy KS, Atabay HI, Ica T, Abay S: Prevalence and distribution of *Arcobacter* species in various sources in Turkey and molecular analysis of isolated strains by ERIC-PCR. *J Appl Microbiol* 2007;103:27–35.
13. Association of Official Agricultural Chemists: Determination of fat content. Method 24.007. In: *Official Methods of Analysis. Meat and Meat Products, Vol. 2* (Horwitz W, ed.), 15th ed. Washington, DC, 1980, 1446 pp.
14. *ISO/1442: Meat and Meat Products. The Determination of Moisture Content Guidelines.* International Organization for Standardization, Geneva, 1973.
15. Ozdamar K: *Statistical Data Analysis with Pocket Programmes*, 5th ed. Kaan Publications, Eskisehir, Turkey, 2004.
16. Atabay HI, Aydin F: Susceptibility of *Arcobacter butzleri* isolates to 23 antimicrobial agents. *Lett Appl Microbiol* 2001;33:430–433.
17. Cervenka L, Peskova I, Foltynova E, Pejchalova M, Brozkova I, Vytrasova J: Inhibitory effects of some spice and herb extracts against *Arcobacter butzleri*, *A. cryaerophilus*, and *A. skirrowii*. *Curr Microbiol* 2006;53:435–439.
18. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Perez-Alvarez JA, Kuri V: Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Sci* 2005;69:371–380.
19. Goni P, Lopez P, Sanchez R, Gomez-Lus R, Becerril R, Nerin C: Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chem* 2009;116:982–989.
20. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AM, Rouabhia M, Mahdouani K, Bakhrouf A: The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): a short review. *Phytother Res* 2007;21:501–506.
21. Simic A, Sokovic MD, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin PD: The chemical composition of some Lauraceae essential oils and their antifungal activities. *Phytother Res* 2004;18: 713–717.
22. Fisher K, Rowe C, Phillips CA: The survival of three strains of *Arcobacter butzleri* in the presence of lemon, orange and bergamot essential oils and their components in vitro and on food. *Lett Appl Microbiol* 2007;44:495–499.
23. Singh G, Marimuthu P, Murali HS, Bawa AS: Antioxidative and antibacterial potentials of essential oils and extracts isolated from various spice materials. *J Food Safety* 2005;25:130–145.
24. Tabak M, Armon R, Neeman I: Cinnamon extracts' inhibitory effect on *Helicobacter pylori*. *J Ethnopharmacol* 1996;67:269–277.
25. Smith-Palmer A, Stewart J, Fyfe L: Antimicrobial properties of plant essential oils and essences against five important foodborne pathogens. *Lett Appl Microbiol* 1998;26:118–122.
26. Valero M, Frances E: Synergistic bactericidal effect of carvacrol, cinnamaldehyde or thymol and refrigeration to inhibit

Bacillus cereus in carrot broth. *Food Microbiol* 2006;23: 68–73.
27. Tullio V, Nostro A, Mandras N, Dugo P, Banche G, Cannatelli MA: Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. *J Appl Microbiol* 2007;102:1544–1550.
28. Menaker A, Kravets M, Koel M, Orav A: Identification and characterization of supercritical fluid extracts from herbs. *C R Chimie* 2004;7:629–633.
29. Lee J-W, Park K-S, Kim J-G, Oh S-H, Lee Y-S, Kim J-H, Byun M-W: Combined effects of gamma irradiation and rosemary extract on the shelf-life of a ready-to-eat hamburger steak. *Radiat Phys Chem* 2005;72:49–56.
30. Djenane D, Sanchez-Escalante A, Beltran JA, Roncales P: Ability of α-tocopherol, taurine and rosemary, in combination with vitamin C, to increase the oxidative stability of beef steaks packaged in modified atmosphere. *Food Chem* 2002;76: 407–415.
31. Djenane D, Sanchez-Escalante A, Beltran JA, Roncales P: Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. *Meat Sci* 2003;64:417–426.