Antimicrobial Effect of *Malpighia Punicifolia* and Extension of Water Buffalo Steak Shelf-Life

Patrizio Tremonte, Elena Sorrentino, Mariantonietta Succi, Luca Tipaldi, Gianfranco Pannella, Eléna Ibañez, Jose Antonio Mendiola, Tiziana Di Renzo, Anna Reale, and Raffaele Coppola

Abstract: In the present study, a multiple approach was used to characterize *Malpighia punicifolia* extract and to evaluate its inhibitory activity against several meat spoilage bacteria. First, volatile fraction, vitamins and phenolic compounds of the extract obtained by supercritical fluid extraction were determined by GC-MS and HPLC. Then, the antimicrobial action of the extract was *in vitro* evaluated against *Pseudomonas putida* DSMZ 291^T, *Pseudomonas fluorescens* DSMZ 50009^T, *Pseudomonas fragi* DSMZ 3456^T, and *Brochothrix thermosphacta* DSMZ 20171^T by the agar well diffusion assay and by the agar dilution test. Based on the results of the minimum inhibitory concentration (MIC) against the assayed bacteria, 4 different concentrations of the extract were used in a challenge test on water buffalo steaks stored for 21 d at 4 °C. Results of chemical analyses showed that *M. punicifolia* extract is characterized by the presence of several compounds, already described for their antimicrobial (phenolic acids, flavonones, and furanes) and antioxidant (ascorbic acid) properties. The *in vitro* detection of antimicrobial activities highlighted that the extract, used at 8% concentration, was able to inhibit all the target bacteria. Moreover, very low MIC values (up to 0.025%) were detected. *In situ* tests, performed on water buffalo steaks treated with the extract in the concentration range 0.025% to 0.05%, showed a strong inhibition of both intentionally inoculated bacteria and naturally occurring microorganisms. Positive results, in terms of color and odor, were also observed during the entire storage of steaks preserved with the extract.

Keywords: *Brochothrix thermosphacta*, *Malpighia punicifolia*, meat spoilers, *Pseudomonas* spp., water buffalo meat

Practical Application: Extract from *Malpighia punicifolia* (Acerola) could play an important role in the preservation of fresh red meat. Our study demonstrated the efficacy of the extract in the shelf-life extension of refrigerated water buffalo meat, characterized by high iron content. In fact, the extract was able to inhibit several meat spoilage microorganisms, prolonging the shelf-life of the product. Our study also showed the role of the extract in the preservation of important sensory parameters of meat, such as color and odor. In conclusion, *M. punicifolia* extract can be used as bio-preservative for fresh red meat, allowing its shelf -life extension.

Introduction

Species belonging to *Pseudomonas* spp. and *Brochothrix thermosphacta* are considered the main contaminant bacteria of fresh meats stored under refrigerated temperature (Tremonte and others 2005; Nychas and others 2008; Nowak and others 2012b). Several studies (Nychas and others 2008; Nowak and others 2012a,b) described *B. thermosphacta* as an important spoiler of meat stored under both reduced and high oxygen atmospheres. On the other hand, *Pseudomonas* species are defined as psychrotrophic bacteria having a very fast growth rate and high affinity for the oxygen, thus able to dominate the microbiota of refrigerated meats stored aerobically or under modified atmosphere packaging (Ercolini and others 2006; Argyri and others 2011; Doulgeraki and Nychas 2013). Among *Pseudomonas* species, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas fragi* are capable of producing extracellular proteases and lipases, generating off-odors and a partial or a complete meat degradation (Liao 2006).

In the last years, different bio-protective strategies were developed to inhibit the growth of spoilage bacteria on both pork and bovine fresh meats (Tremonte and others 2005; Soultos and others 2008; Turgis and others 2008). Many studies were addressed to the search for natural alternatives to chemical additives in foods (Iturriaga and others 2012; Kim and others 2013; Casaburi and others 2014). In this field, different scientific reports highlighted the antimicrobial effect of natural compounds on pathogenic or spoilage bacteria (Holley and Patel 2005; Tipaldi and others 2011; Settanni and others 2012). Among natural substances, extracts obtained by supercritical fluid extraction (SFE) showed promising results in the control of food spoilage and foodborne pathogenic bacteria (Muñoz and others 2009; Herrero and others 2010).

Besides antimicrobial activities, natural extracts were also evaluated for their anti-oxidant effects on stored meat. Interesting results were obtained by Naveena and others (2006), which showed the effect of clove oil plus lactic acid and vitamin C on the improvement of color in water buffalo meat. This topic is particularly interesting, taking into account that the fast meat darkening due to the formation of met-myoglobin (Bekhit and others 2003) negatively affects the shelf-life of meat, as well as the consumer acceptability.

MS 20150672 Submitted 4/22/2015, Accepted 10/5/2015. *Authors Tremonte, Sorrentino, Succi, Tipaldi, Pannella, and Coppola are with the Dept. of Agricultural, Environmental and Food Sciences (DiAAA), Univ. of Molise, via De Sanctis, 86100 Campobasso, Italy. Authors Ibañez and Mendiola are with the Inst. of Food Science Research, CIAL (CSIC-UAM), c/Nicolás Cabrera, 9, 28049, Madrid, Spain. Authors Di Renzo and Reale are with the Inst. of Food Science of the National Research Council (ISA-CNR), via Roma, 64, 83100 Avellino, Italy. Direct inquiries to author Succi (E-mail: succi@unimol.it).*

Journal of Food Science A Publication of the Institute of Food Technologists

doi: 10.1111/1750-3841.13141

The extract of *Malpighia punicifolia* (Acerola), among other natural compounds, shows promising results, thanks to a series of active components, which could positively affect the overall quality of fresh meat (Schreckilger and others 2010). This extract, having a long history of use in folk medicine, was screened *in vitro* for its antibacterial activity (Schelz and others 2010). However, the literature is poor in scientific reports on the effect of the extract in the preservation of fresh meat. In the light of previous findings, this study used a multiple approach to characterize a SFE *M. punicifolia* extract and to evaluate its inhibitory activity against several meat spoilage microorganisms. The effect was also tested *in situ* on water buffalo steaks, characterized by high perishability, during the storage at 4 °C for 21 d.

Materials and Methods

Natural extract

A fruit extract of *M. punicifolia* (Bioma Life Science, Quartino, Switzerland) was tested in this study. As reported by the supplier, the extract was obtained by SFE at 42.2 °C and 70 atm. In short, the extract was obtained by treatment of *M. punicifolia* dried powder (300 g) with liquid CO_2 (1 L). The dried extract was dissolved in hydroalcoholic solution (ethanol:water 30:70, v/v) to obtain a 8% (w/v) stock solution, and it was stored at −20 °C until its use.

Chemicals

Folin–Ciocalteu phenol reagent and sodium carbonate (Na_2CO_3) were acquired from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) was purchased from Scharlau Chemie (Barcelona, Spain). All vitamins, fat-soluble (α-tocopherol, retinol acetate, and cholecalciferol) and water-soluble vitamins (ascorbic acid, thiamine, folic acid, pyridoxine, nicotinamide, and cobalamin) were purchased from Accustandard (New Haven, Conn., U.S.A.). All standard phenolic compounds (caffeic acid, coumaric acid, catechin, catechol, cyanidine chloride, gallic acid, genistein, hesperidin, and quercetin) were purchased from Extrasynthese (Genay, France) and chlorophylls and zeaxanthin from DHI (Hørsholm, Denmark). Milli-Q water was obtained using a Millipore purification system (Millipore, Bedford, Mass., U.S.A.). Methanol [high-performance liquid chromatography (HPLC)-grade] was purchased from LabScan (Dublin, Ireland).

Standard solutions (3 mg/mL) were prepared using solutions of 0.01% TFA in water (polar vitamins, caffeic acid, and gallic acid), methanol:water (the rest of phenolic compounds) or methanol (pigments and fat-soluble vitamins) and stored in darkness under freezing conditions (−20 °C) until use.

Total phenolic content of the extract

Total phenols were estimated as gallic acid equivalents, expressed as mg gallic acid/g extract (Kosar and other 2005). The total volume of reaction mixture was miniaturized to 2.0 mL. In detail, 1.2 mL water and 20 μL of sample were mixed, and 100 μL undiluted Folin–Ciocalteau reagent was subsequently added. After 1 min, 0.3 mL of 2% (w/v) Na_2CO_3 were added and the volume was made up to 2.0 mL with water. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve elaborated in the same manner. Data were presented as the average of triplicate analyses.

Volatile fraction by gas chromatography-mass spectrometry (GC-MS)

The extract was analyzed using an Agilent-6890N GC system with a programmed split/splitless injector coupled to an Agilent-5973N quadruple mass spectrometer (Agilent, Palo Alto, Calif., U.S.A.). The system was controlled by means of an Agilent MSD Chemstation software. The column used in the GC was a 30 min × 0.25 mm i.d. fused silica capillary column coated with a 0.25 μm layer of SE-54 (HP-5MS, Agilent). The injection was carried out at 250 °C in split mode (ratio 1:20). The volume of sample injected was 1 μL. Extract was injected at a concentration of 5 mg/mL. Helium was the carrier gas (7 psi). The oven temperature was programmed as follows: 40 °C as initial temperature (maintained for 2 min) to 150 °C in 24 min, 5 °C/min, and from 150 °C to a final temperature of 300 °C at 15 °C/min. A solvent delay of 4 min was selected before analyzing the compounds reaching the MS. Compounds were tentatively identified by MS in SCAN mode using a mass interval ranging from 35 to 450 m/z. Their spectra were compared with those in a MS library (Wiley Registry of Mass Spectral Data), with data found in the literature and with standards when available.

Determination of vitamins and phenolic compounds

A modified HPLC method, previously developed in CIAL laboratory (Madrid, Spain) was used (Mendiola and others 2008). Shortly, analytes were separated on an ACE-100 Å C18 (150 mm × 4.6 mm, 3 μm particle size; Advanced Chromatographic Technologies, Aberdeen, U.K.) in a single run, using combined isocratic and linear gradient elution with a mobile phase consisting of 0.010% TFA (solvent A, pH 3.9) and methanol (solvent B) at the flow rate of 0.7 mL/min using an Agilent 1100 HPLC apparatus. The gradient profile (A:B) started at 95:5 and was constant in the first 4 min, increased up to 0:100 in 2 min, then constant until a total analysis time of 40 min, and finally linearly increased up to 95:5 to reach initial conditions. The most suitable detection wavelength for simultaneous vitamin-polyphenol determination was 280 nm. Quantification of the different families of compounds was performed by building a calibration curve for each of them, as described by Mendiola and others (2008).

Direct injection of pure vitamin standards was done by dissolving them in TFA 0.01% or 100% methanol depending on the polarity of the vitamin. For polyphenols, all samples were injected dissolved in hydromethanolic solutions.

In vitro detection of *M. punicifolia* antimicrobial activity

The antimicrobial activity of *M. punicifolia* extract was evaluated against 4 bacterial type strains, represented by *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *Brochothrix thermosphacta* DSMZ 20171^T. Strains of *Pseudomonas* and *Brochothrix thermosphacta* were revitalized at 28 °C in PAB (Pseudomonas Agar Base, Oxoid, Milan, Italy) and in STA Agar Base (Oxoid), respectively, and then stored in the same media at 4 °C. Prior to experiments, strains were overnight cultured in the proper conditions. The inhibitory action of *M. punicifolia* was assessed by the agar well diffusion assay (Tremonte and others 2007, 2010). Briefly, 1 mL (inoculum size of 4.0 log CFU/mL) of each bacterial suspension was inoculated into 20 mL of proper soft media (0.7% agar), gently mixed and poured into Petri plates. A well of 4.0 mm in diameter was bored into each agar plate, and 85 μL of the stock hydroalcoholic solution of *M. punicifolia* extract at 8%

(w/v) concentration were placed into each well. Hydroalcoholic solution without extract was used as control. Streptomycin (25 μg) and tetracycline (30 μg; both from Oxoid) were used as reference controls for *Pseudomonas* spp. and *B. thermosphacta*, respectively, as well as to compare the inhibitory activity of *M. punicifolia* extract. After incubation at 28 °C for 24 to 48 h, inhibition halos (IH, mm) were accurately measured with a caliper and the ratio between IH (mm) of the proper reference control and IH (mm) of *M. punicifolia* extract was calculated. Thereafter, 4 levels of inhibition were established: very strong (ratio ≤1), strong (ratio between 1 and 1.2), moderate (ratio between 1.2 and 1.4), and low (ratio >1.4). The presence of growth around the well was considered as absence of inhibition. Data were reported as the mean value and standard deviation on 3 analyses.

Minimum inhibitory concentration (MIC) evaluation of *M. punicifolia* extract

The minimum inhibitory concentration (MIC) assay was carried out using the agar dilution method as described in the EUCAST definitive document 3.1 (2000) with some modifications. In detail, the extract of *M. punicifolia* was filter-sterilized (Filter Unit Red rim FP 30/0.2 CA-S, 0.22-μm pore size; Schleicher & Schuell, Dassel, Germany) and added to sterile molten Mueller-Hinton (MH) agar at appropriate volume to produce a concentration range between 0.0002% and 0.4% (w/v), as indicated in Table 1. The resulting MH agar solutions were poured into 90 mm Petri plates immediately after vortexing. The surface of plates was inoculated with the microbial strains at a concentration of 4.0 log CFU/mL and incubated at 28 °C for 48 h. At the end of the incubation period, plates were evaluated for the presence or absence of growth. MIC values were recorded as the lowest concentration of natural extract that completely inhibited the growth. Each test was performed in triplicate.

Challenge test on water buffalo steaks

Water buffalo steaks (*Musculus quadriceps femoris*) were obtained from commercial sources and transported at 4 °C in few hours into the DiAAA laboratories (Campobasso, Italy). Steaks (each about 200 g and approximately 1 cm thick) were intentionally inoculated with a 4-strain cocktail of *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T. The inoculation of each steak was performed by disposing 100 μL of the mixed cocktail with a micropipette on the surface and spreading over the surface with a sterile disposable plastic spatula in order to obtain an inoculum of about 4.0 log CFU/g. The meat steaks were then divided into 5 batches: batches M1, M2, M3, and M4, steaks dipped for a few seconds in a presterilized container with 0.0063%, 0.0125%, 0.025%, and 0.05% (w/v) *M. punicifolia* extract, respectively, and batch C (control), steaks treated with the hydroalcoholic solution without extract. Removing excess solution was done by gently pressing each piece against the inside walls of containers. Then, each steak was individually packaged in polypropylene tray wrapped in air-permeable film, and stored at 4.0 ± 0.5 °C for 21 d. Three biological replicates were performed for each batch.

A 2nd test was performed as described previously, except that after inoculation with the 4-strain cocktail, steaks were incubated at 4 °C for 2 h to allow the bacterial adhesion to the meat surface (Rhoades and others 2013). Then steaks were divided into 5 batches and treated as described above.

Microbiological and sensory analyses on water buffalo steaks

Microbiological analyses were performed at 0, 4, 8, 12, 15, 18, and 21 d of storage at 4 ± 0.5 °C to evaluate the microbial load in water buffalo steaks. For this purpose, about 10 g of meat from each batch were aseptically withdrawn and decimal diluted in a sterile solution of 0.1% peptone water. After homogenization in a Lab-blender (Stomacher Seward Medical, London, SE1 1PP, U.K.), subsequent serial dilutions were inoculated in appropriate media as described below:

- *B. thermosphacta* was enumerated on STA Agar base (Oxoid) with STA selective supplement (Oxoid) after incubation at 28 °C for 48 h.
- *Pseudomonas* spp. was counted on PAB (Oxoid) with SR102E supplement (Oxoid) after incubation at 28 °C for 24 to 48 h.
- Total and fecal coliforms were counted on Violet Red Bile Agar (Oxoid) after incubation for 48 h at 37 °C and 44 °C, respectively.
- *Eumycetes* (yeasts and molds) were counted on Rose Bengal Agar after incubation at 25 °C for 48 h.

The results were expressed as the mean of 6 determinations performed on 2 different steaks for each biological replicate.

The growth of *Pseudomonas* spp. and *B. thermosphacta* were modeled using the DMFit v 2.0 structured dynamic model (Baranyi and Roberts 1994).

Color was determined on the surface of water buffalo steaks. Measurements were performed at 0, 4, 8, 12, 15, 18, and 21 d of storage using the CIE L*, a*, b* system (Commission Internationale de l'Eclairage 1978) with a reflectance spectrophotometer (Minolta CR300b, Suitashi, Osaka, Japan). The results were expressed as the mean of 18 determinations performed in triplicate on 2 steaks for each biological replicate.

Sensory analysis was performed on steaks from all batches at 0, 3, 7, 10, 14, and 21 d of storage. To define the sensory profile, a descriptive panel of 15 judges was employed. The judges were trained in 3 preliminary sessions to produce a list of attributes useful to define the sensory profile of water buffalo steaks. On the basis of the frequency of citation (>60%), 4 descriptors were selected: general appearance, color, flavor, and off-flavor. Random samples were evaluated by assigning a score between 1 (poor) and 10 (excellent) for positive descriptors (general appearance, color, and flavor), and between 10 (unacceptable) and 1 (excellent) for the negative one (off-flavor).

Statistical methods

Mean values, medians, standard deviations, and the occurrence of statistically significant differences were determined with the OriginPro 7.5 software (OriginLab Corporation, Northampton, Mass., U.S.A.).

Results and Discussion

Chemical composition of *M. punicifolia* extract

GC-MS analysis of *M. punicifolia* extract led to the identification of 9 components in the volatile fraction (Table 2). The main relative composition of the extract, expressed as the percentage area contribution of each compound, was represented by furanes. These compounds are generally related to fruit aroma, but several authors also reported their antimicrobial activity (Baveja and others 2004; Sung and others 2007). The profile of vitamins and

Table 1–Dilutions of *M. punicifolia* extract used in the agar dilution susceptibility test.

Concentration of extract (mg/L)	Volume used (mL)	Distilled water added (mL)	Concentration obtained (mg/L)	Final concentration of extract (mg/L)[a]	Final concentration of extract (% w/v)[a]
80000.00[b]	1.00	0.00	80000.00	4000.00	0.4000
80000.00	1.00	1.00	40000.00	2000.00	0.2000
80000.00	1.00	3.00	20000.00	1000.00	0.1000
20000.00	1.00	1.00	10000.00	500.00	0.0500
20000.00	1.00	3.00	5000.00	250.00	0.0250
20000.00	1.00	7.00	2500.00	125.00	0.0125
2500.00	1.00	1.00	1250.00	62.50	0.0063
2500.00	1.00	3.00	625.00	31.25	0.0031
2500.00	1.00	7.00	312.50	15.63	0.0016
312.50	1.00	1.00	156.25	7.81	0.0008
312.50	1.00	3.00	78.13	3.91	0.0004
312.50	1.00	7.00	39.06	1.95	0.0002

[a]In medium after the addition of 19 mL of Muller Hinton agar.
[b]8000 mg/L (stock solution) corresponding to 8% w/v of *M. punicifolia* extract.

Table 2–Volatile compounds in *M. punicifolia* extract detected by GC-MS.

RT (min)	Compound	Retention index (Theorical)	Retention index (Real)	% area
6.54	2-furancarboxaldehyde	830	867	4.31
7.58	2-furanmethanol	885	894	1.76
9.36	2-Propenoic acid, 2-methyl- ethenyl ester	860	950	2.59
10.52	2-Furancarboxilic acid	970	987	1.62
12.56	2-furanpropionic	1050	1053	1.43
16.06	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1130	1170	4.03
18.30	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	1200	1214	43.79
21.53	5-(hydroxymethyl)-2-Furancarboxaldehyde,	1250	1258	3.52
24.50	3-hydroxy-5-methyl-2(5h)-furanone	1480	1502	1.47

Table 3–Vitamins and phenolic compounds in *M. punicifolia* extract detected by HPLC.

Peak	Time (min)	Compound	mg/g
1	3.21	Ascorbic acid (vitamin C)	95.06
2	3.30	Thiamine (vitamin B1)	22.59
3	8.53	Phenolic acid	5.31
4	9.51	Pheno diol	2.21
5	10.02	Flavanone	1.01
6	10.43	Flavanone	0.51
7	13.37	Isoflavone	0.70
8	13.45	Isoflavone	0.26

phenolic compounds (Table 3) was obtained using a RP-HPLC-DAD method previously described by Mendiola and others (2008). This method allows, in a single run, to analyze vitamins (water and fat soluble), phenolic compounds, and pigments, providing a general description of bioactive components present in a natural extract. The analysis highlighted the absence of fat-soluble vitamins and the high content of some water soluble vitamins such as ascorbic acid and thiamine, which were observed at a concentration of 95.06 and 22.59 mg/g extract, respectively (see Table 3). Although phenolic compounds were not completely identified, it was possible, by resemblance of UV-vis spectra, to assign them to certain families of phenolic compounds and to quantify them using the right standard. The content of phenolic compounds was correlated to the value obtained in the Folin–Ciocalteu test (121.16 ± 1.24 mg gallic acid/g extract).

Antimicrobial activity expressed *in vitro* by *M. Punicifolia*

The agar well diffusion showed that *M. punicifolia* extract, used at 8% concentration (stock solution), exerted a very strong inhibitory action (IH ratio ≤1) against *B. thermosphacta* DSMZ 20171^T (Table 4). A strong inhibition was appreciated against *P. fluorescens* DSMZ 50009^T (IH ratio, 1.07) and against *P. fragi* DSMZ 3456^T (IH ratio, 1.11), while a moderate inhibition was detected against *P. putida* DSMZ 291^T (IH ratio, 1.23). On the other hand, the hydroalcoholic solution without extract, used as control, produced no inhibition, as highlighted by the presence of microbial growth around the wells. Previous results pointed out that *M. punicifolia* extract has an important inhibitory action that could be associated to its composition. In fact, several compounds found in the extract, such as phenolic acids, flavonones, and furans (Table 2 and 3), were already described for their antimicrobial activity (Tiwari and others 2009; Ozen and others 2011; Ramalakshmi and Muthuchelian 2011; Chauhan and others 2012; Hyldgaard and others 2012; Nowak and others 2012a).

The results of the agar dilution test revealed interesting MIC values (Table 5). In fact, a concentration up to 0.025% completely inhibited the bacterial growth of all the assayed microorganisms, and the highest activity of the extract was detected against *B. thermosphacta* (MIC of 0.0063%). An important antimicrobial activity was also appreciated against *Pseudomonas* species, which showed MIC values of 0.0125% for both *P. fragi* and *P. fluorescens* and of 0.025% for *P. putida*. The highest antimicrobial effect measured against *B. thermosphacta* confirmed that Gram-positive bacteria are more sensitive than Gram-negative bacteria, in agreement with results on the inhibitory action of natural extracts obtained by other authors (Burt 2004; Kim and others 2013). MIC values also indicated that the extract of *M. punicifolia* has an antimicrobial effect against *Pseudomonas* and *B. thermosphacta* similar or higher than that exerted by other natural extracts (Gutierrez and others 2009; Tiwari and others 2009; Nowak and others 2012a).

Table 4–Antimicrobial activity exerted by *M. punicifolia* extract against *B. thermosphacta* and *Pseudomonas* spp.as detected by the agar well diffusion assay.

Strains	*M. punicifolia* extract (8% w/v)	Hydro alcoholic solution[b]	Streptomycin[c]	Tetracycline[c]	Ratio[d]
B. thermosphacta DSMZ 20171^T	24.02 (±0.05)[a]	Absent	–	23.5 (±0.04)	0.98
P. fluorescens DSMZ50009^T	19.53 (±0.04)	Absent	21.01 (±0.05)	–	1.08
P. fragi DSMZ3456 ^{T}	17.49 (±0.06)	Absent	19.51 (±0.03)	–	1.11
P. putida DSMZ291^T	15.01 (±0.03)	Absent	18.49 (±0.04)	–	1.23

[a]Inhibition halos (IH, mm).
[b]Hydroalcoholic solution was used as control.
[c]Streptomycin and tetracycline were used as reference controls for *Pseudomonas* spp. and *B. thermosphacta*.
[d]Ratio between IH (mm) mean value obtained with the reference control and IH (mm) mean value obtained with *M. punicifolia* extract.

Table 5–MIC values determined for *B. thermosphacta* and *Pseudomonas* spp. in presence of different concentrations of *M. punicifolia* extract (–, absence of growth; +, presence of growth).

Concentration of *M. punicifolia* extract	*B. thermosphacta* DSMZ20171^T	*P. fluorescens* DSMZ50009^T	*P. fragi* DSMZ3456^T	*P. putida* DSMZ291^T
0.4000	–	–	–	–
0.2000	–	–	–	–
0.1000	–	–	–	–
0.0500	–	–	–	–
0.0250	–	–	–	–
0.0125	–	–	–	+
0.0063	–	+	+	+
0.0031	+	+	+	+
0.0016	+	+	+	+
0.0008	+	+	+	+
0.0004	+	+	+	+
0.0002	+	+	+	+

Antimicrobial activity of *M. punicifolia* extract in water buffalo steaks

In situ experiments were carried out to investigate the efficacy of the extract when used in steaks stored under refrigerated conditions. The first test was performed on water buffalo steaks inoculated with a 4-strain cocktail of *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T. Steaks were then divided into 5 batches and were dipped with *M. punicifolia* extract at increasing concentrations (M1 to M4) or with the control solution (C). Immediately after dipping, microbiological analyses were performed on steaks from each batch to confirm the inoculum size at time zero (about

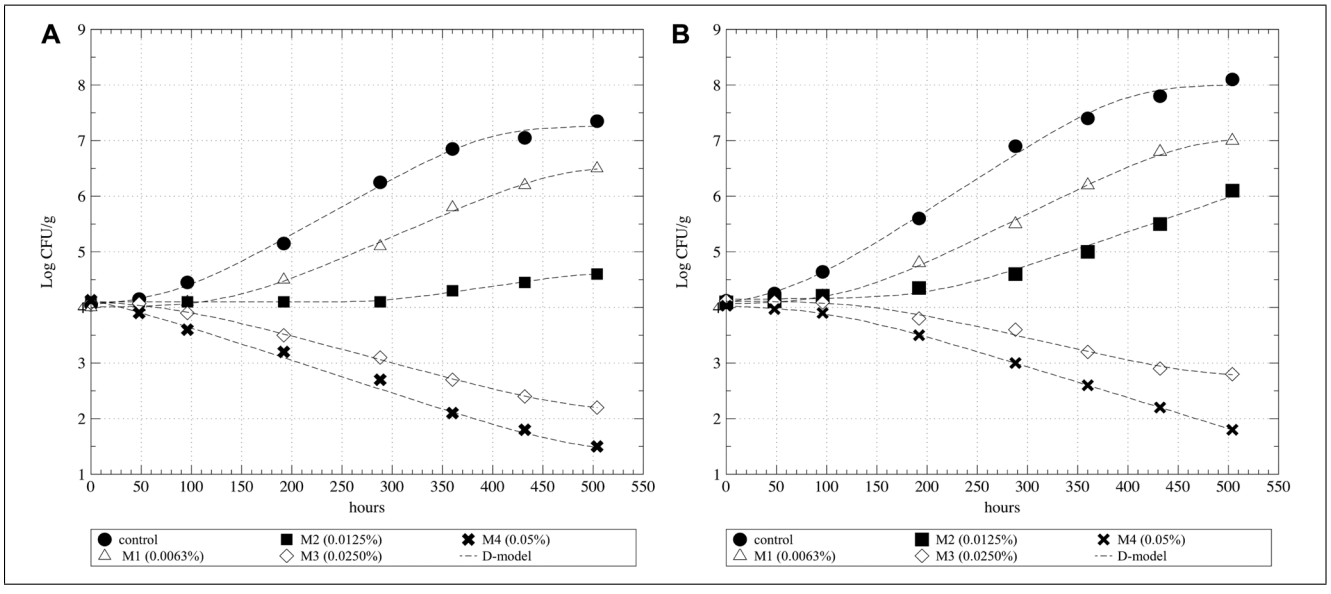

Figure 1–Kinetic data of *B. thermosphacta* (A) and *Pseudomonas* spp. (B) in water buffalo steaks stored at 4 °C for 21 d. Data were fitted with Baranyi's model (Baranyi and Roberts 1994). C, control steaks intentionally inoculated with *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T and without vegetal extract; M1, M2, M3, and M4, inoculated steaks treated with *M. punicifolia* extract at a concentration of 0.0063%, 0.0125%, 0.025%, and 0.05% (w/v), respectively.

Table 6–Kinetic parameters detected for *B. thermosphacta* and *Pseudomonas* spp. in water buffalo steaks intentionally inoculated with *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T. C, inoculated control steaks without vegetal extract; M1, M2, M3, and M4, inoculated steaks treated with *M. punicifolia* extract at a concentration of 0.0063%, 0.0125%, 0.025%, and 0.05% (w/v), respectively.

Microorganism	Batch	Initial values (log CFU/g)	Lag (h)	Shoulder (h)	Max rate (μmax/h)	Final values (log CFU/g)
B. thermosphacta	C	4.06	78.14	–	0.0102	7.26
	M1	4.01	138.58	–	0.0078	6.56
	M2	4.11	293.47	–	0.0027	4.62
	M3	4.10	–	76.32	−0.0049	2.11
	M4	4.13	–	18.36	−0.0059	1.33
Pseudomonas spp.	C	4.08	96.55	–	0.0120	8.21
	M1	4.01	108.42	–	0.0088	7.04
	M2	4.08	215.21	–	0.0067	6.03
	M3	3.99	–	180.23	−0.0043	2.71
	M4	4.01	–	116.31	−0.0061	1.78

4.0 log CFU/g). Microbial counts revealed statistically significant differences ($P < 0.05$) between batches (data not shown), probably due to the washing out of some microbial cells during the dipping. So, the test was repeated in the same manner, except that prior to the treatment with the extract or with the control solution, inoculated steaks were incubated at 4 °C for 2 h to allow the bacterial adhesion to the meat surface (Rhoades and others 2013). This tool allowed the obtainment of constant initial microbial loads among batches, that we considered an important requisite not only to simulate the highest microbial contamination that can naturally occur in fresh meats (Ercolini and others 2010; Papadopoulou and others 2012), but also to compare the data of the antimicrobial efficacy of *M. punicifolia* extract used at different concentrations. The microbiological changes occurring in water buffalo steaks during 21 d of storage at 4 °C are shown in Figure 1 and 2. Results evidenced that *M. punicifolia* extract, when used at concentrations of 0.025% and of 0.05%, strongly affected the growth of *B. thermosphacta* (Figure 1A) and *Pseudomonas* spp. (Figure 1B) intentionally inoculated at 4.0 log CFU/g. The analysis of kinetic parameters for these bacteria (Table 6) underlined the effect of the extract used at different concentrations. In fact, *B. thermosphacta* increased in the control batch (C) after a lag phase of 78.14 h, reaching levels of 7.26 log CFU/g at the end of the storage with a maximum specific growth rate (μ_{max}/h) of 0.0102. In samples from batch M1 (0.0063% concentration of extract), *B. thermosphacta* reached 6.56 log CFU/g with a μ_{max} of 0.0078/h. Moreover, the lag phase of *B. thermosphacta* in the batch M1 was about twice as that detected in the control. A similar behavior was observed for *Pseudomonas* spp., but final counts were higher than those of *B. thermosphacta*. The *in situ* inhibitory activity of the extract appeared much more evident in the batch M2 (0.0125% concentration of the extract), where counts of *B. thermosphacta* and *Pseudomonas* spp. increased slightly during the entire storage period, and their lag phases were 3.7 and 2.2 times longer, respectively, than those observed in the control. A considerable inhibition of intentionally inoculated bacteria was found in batches M3 (0.025% concentration of the extract) and M4 (0.05% concentration of the extract). In fact, in the batch M3 counts of 2.11 for *B. thermosphacta* and

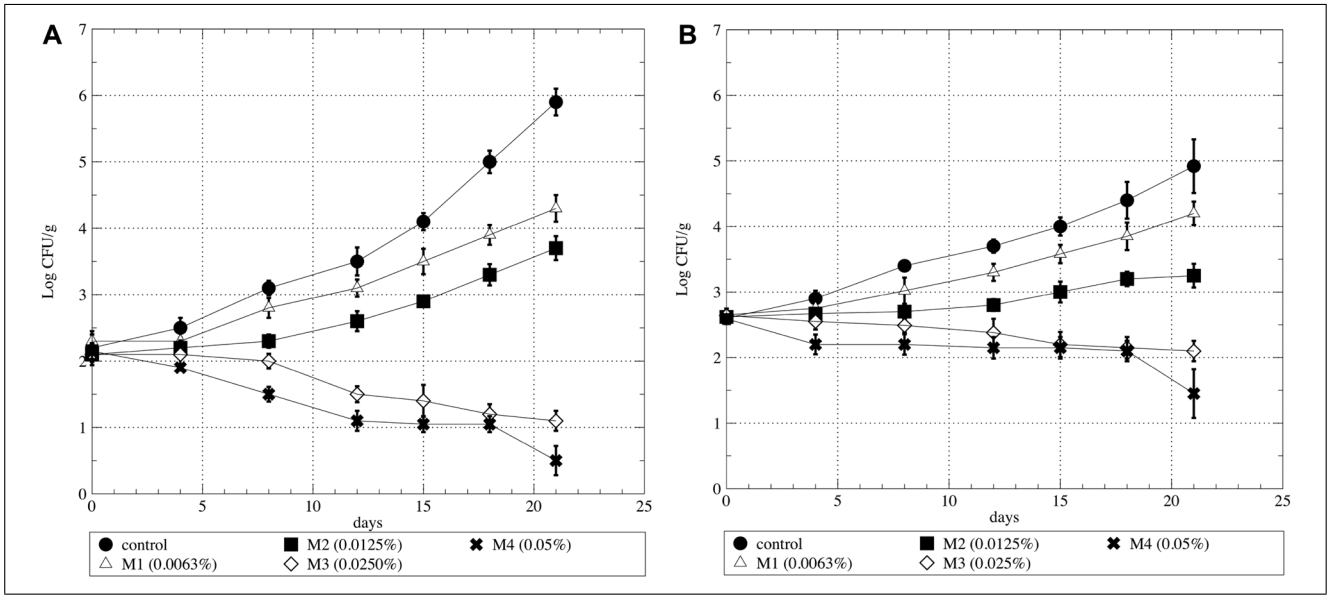

Figure 2–Behavior of total coliforms (A) and *Eumycetes* (B) in water buffalo steaks stored at 4 °C for 21 d. C, control steaks intentionally inoculated with *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T and without vegetal extract; M1, M2, M3, and M4, inoculated steaks treated with *M. punicifolia* extract at a concentration of 0.0063%, 0.0125%, 0.025%, and 0.05% (w/v), respectively.

Table 7–Lightness (L*), redness (a*), and yellowness (b*) values recorded after 21 d of refrigerated storage on the surface of water buffalo steaks intentionally inoculated with *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T. C, inoculated control steaks without vegetal extract; M1, M2, M3, and M4, inoculated steaks treated with *M. punicifolia* extract at a concentration of 0.0063%, 0.0125%, 0.025%, and 0.05% (w/v), respectively.

	Time (d)	C	M1	M2	M3	M4
L*	0	45.02 ± 0.89^a	45.56 ± 0.54^a	45.93 ± 0.63^a	45.74 ± 0.54^a	45.89 ± 0.71^a
	4	44.99 ± 0.73^a	45.07 ± 0.69^a	45.07 ± 0.47^a	46.02 ± 0.42^a	45.94 ± 0.63^a
	8	42.52 ± 0.66^b	44.92 ± 0.73^a	45.35 ± 0.42^a	46.21 ± 0.71^a	46.09 ± 0.38^a
	12	41.14 ± 1.22^{b,c}	45.66 ± 0.27^a	45.09 ± 0.67^a	46.11 ± 0.47^a	46.01 ± 0.53^a
	15	40.52 ± 0.71^c	45.66 ± 0.56^a	45.12 ± 0.88^a	46.01 ± 0.52^a	45.92 ± 0.66^a
	18	38.55 ± 0.53^d	44.83 ± 0.91^a	45.15 ± 0.92^a	45.95 ± 0.74^a	45.95 ± 0.54^a
	21	36.05 ± 0.54^e	42.73 ± 0.56^b	45.77 ± 0.61^a	46.11 ± 0.55^a	46.29 ± 0.48^a
a*	0	13.85 ± 0.45^a	13.75 ± 0.53^a	13.70 ± 0.23^a	13.95 ± 0.23^a	13.88 ± 0.24^a
	4	10.54 ± 0.47^b	11.42 ± 0.58^b	11.96 ± 0.42^b	13.72 ± 0.41^a	13.68 ± 0.42^a
	8	8.62 ± 0.71^c	10.43 ± 0.61^b	11.15 ± 0.51^b	13.32 ± 0.45^a	13.45 ± 0.38^a
	12	7.53 ± 0.71^{c,d}	10.01 ± 0.73^{b,c}	10.75 ± 0.45^b	13.15 ± 0.51^a	13.32 ± 0.27^a
	15	7.15 ± 0.49^d	9.10 ± 0.72^c	10.68 ± 0.57^b	12.85 ± 0.42^a	13.28 ± 0.31^a
	18	6.75 ± 0.56^d	8.82 ± 0.47^c	9.35 ± 0.22^c	12.95 ± 0.36^a	13.33 ± 0.29^a
	21	5.34 ± 0.52^e	7.61 ± 0.58^d	8.75 ± 0.29^c	12.45 ± 0.43^{a,b}	12.98 ± 0.30^a
b*	0	10.20 ± 0.63^a	10.85 ± 0.37^a	10.44 ± 0.41^a	10.75 ± 0.51^a	10.88 ± 0.31^a
	4	10.83 ± 0.67^a	11.03 ± 0.48^a	10.43 ± 0.57^a	10.83 ± 0.63^a	11.08 ± 0.42^a
	8	11.22 ± 0.48^a	11.42 ± 0.57^a	10.72 ± 0.51^a	11.32 ± 0.42^a	10.97 ± 0.33^a
	12	13.16 ± 0.59^b	12.33 ± 0.32^b	12.53 ± 0.47^b	10.70 ± 0.63^a	11.38 ± 0.41^a
	15	14.85 ± 0.57^c	13.22 ± 0.68^b	13.75 ± 0.51^b	10.96 ± 0.49^a	10.89 ± 0.65^a
	18	15.14 ± 0.44^c	14.95 ± 0.47^c	14.99 ± 0.42^c	11.40 ± 0.71^a	11.29 ± 0.46^a
	21	16.85 ± 0.38^d	15.12 ± 0.52^c	15.17 ± 0.61^c	11.10 ± 0.56^a	11.42 ± 0.52^a

Same letters in rows and columns indicate the absence of significant differences ($P > 0.05$).

2.70 log CFU/g for *Pseudomonas* spp. were appreciated after 21 d under refrigerated storage. Counts lower than 1.8 log CFU/g were registered in the batch M4.

In the light of previous results, it is possible to affirm that *M. punicifolia* extract, used *in situ* at concentrations of 0.025% and 0.05% (batches M3 and M4, respectively), was able not only to inhibit, but also to inactivate the assayed bacteria. Kinetic data (Table 6) showed that *B. thermosphacta* was more sensitive to the extract than *Pseudomonas* spp. In fact, the shoulders recorded in the survival curve of *B. thermosphacta* were 76.32 and 18.36 h in batches M3 and M4, respectively, followed by a linear decay. For *Pseudomonas* spp., higher lengths of the shoulders were evidenced (180 and 116 h in batches M3 and M4, respectively).

The inhibitory activity of *M. punicifolia* extract was also observed against undesired microorganisms other than those intentionally inoculated. In fact, a strong decrease in total coliforms was recorded in batches M3 and M4 during the storage period, in contrast with results characterizing the other batches (Figure 2A). The effect of the extract used at 0.025% concentration (batch M3) was lower against *Eumycetes* than that against total coliforms, whereas the concentration of 0.05% (batch M4) caused an important decrease of *Eumycetes* during time, similar to that characterizing total coliforms (Figure 2B).

Previous results revealed that higher concentrations of *M. punicifolia* extract were required *in situ* than those required *in vitro*, and this fact was particularly evident against *B. thermosphacta*. In fact, the MIC value for *B. thermosphacta* was 0.0063%, but a concentration at least of 0.025% was required to assure the inhibitory effect in the steaks. These data are in agreement with other studies, which highlighted that the application *in situ* of natural extracts may be affected by the food composition (Tiwari and others 2009; Hyldgaard and others 2012). In this context, results obtained in our study are actually promising, considering that the difference between inhibitory concentrations of *M. punicifolia* extract *in vitro* and *in situ* was considerably lower than that reported in other studies on natural extracts (Cava-Roda and others 2012; Kyung 2012). For instance, Gill and others (2002) observed that cilantro oil had a significant antimicrobial activity *in vitro* at 0.018% concentration, while no antimicrobial activity was recorded in a food model using a concentration of 6% (over 300 times higher). In our study, the antimicrobial activity against *B. thermosphacta* was obtained by using *M. punicifolia* extract at concentrations of 0.0063% *in vitro* and 0.025% *in situ*, that is, only 4 times higher. This datum is particularly interesting, taking into account that the use of natural preservatives can alter the taste of food and exceed the flavor threshold acceptable to consumers (Lv and others 2011).

Among other factors able to influence the consumer choice, the color represents one of the most important visual clues for red fresh meat (Tremonte and others 2005). In our study, the color analysis performed on water buffalo steaks treated with *M. punicifolia* extract showed that the red color was preserved in samples from batches M3 and M4 ($a^* > 12.0$) until the end of the storage (Table 7), whereas a marked decrease in this value was observed in the samples from the other batches. The loss of redness in meat is generally related to the accumulation of met-myoglobin, caused to primary lipid oxidation products such as hydroperoxides and other free radicals, which oxidize the ferrous ion (Fe^{2+}) from oxymyoglobin into the ferric form (Fe^{3+}) present in metmyoglobin. Moreover, the accumulation of metmyoglobin in meat products is accelerated by secondary lipid oxidation products, such as unsaturated aldehydes (Faustman and others 2010). Phenolic acids, allowing the direct interaction with myoglobin, may retard myoglobin oxidation and discoloration (Kroll and Rawel 2001), as also reported by other authors with regard to the use of vegetable extracts in meat products (Gil-Chavez and others 2013; Kim and others 2013).

The positive effect expressed by *M. punicifolia* extract on the color of water buffalo steaks was also supported by the lightness (L*), and the yellowness (b*) measurements. In fact, these parameters revealed slight changes in batches M3 and M4, whereas a

Table 8–Mean values of sensory attributes evaluated after 21 d of refrigerated storage on water buffalo steaks inoculated with *P. putida* DSMZ 291^T, *P. fluorescens* DSMZ 50009^T, *P. fragi* DSMZ 3456^T, and *B. thermosphacta* DSMZ 20171^T. C, inoculated control steaks without vegetal extract; M1, M2, M3, and M4, inoculated steaks treated with *M. punicifolia* extract at a concentration of 0.0063%, 0.0125%, 0.025%, and 0.05% (w/v), respectively.

Batch	Flavor	Off-flavor	Color	General appearance
C	1.11 ± 0.35^a	9.80 ± 0.41^a	2.14 ± 0.63^a	1.32 ± 0.59^a
M1	1.63 ± 0.51^a	8.70 ± 0.45^a	4.12 ± 0.51^b	2.45 ± 0.63^a
M2	3.92 ± 0.25^b	5.83 ± 0.41^b	5.44 ± 0.50^b	4.91 ± 0.59^b
M3	8.12 ± 0.63^c	2.23 ± 0.56^c	8.56 ± 0.51^c	8.66 ± 0.50^c
M4	9.21 ± 0.51^c	1.31 ± 0.43^c	9.15 ± 0.35^c	9.42 ± 0.51^c

Same letters indicate the absence of significant differences ($P > 0.05$).

marked change was found in batches C and M1, where L* decreased and b* increased during the time of storage. Intermediate values were recorded in steaks from the batch M2.

The sensory analysis performed on water buffalo steaks (Table 8) confirmed previous results. In fact, after 21 d under refrigerated storage, steaks from batches M3 and M4 (0.025% and 0.05% concentration of the extract, respectively) were highly appreciated by the panelists, in terms of maintenance of red color, flavor, and general appearance, and low presence of off-flavors.

As expected, control steaks showed unacceptable sensory parameters at the end of the storage period, and similar values were attributed to steaks from the batch M1 ($P > 0.05$). Steaks from the batch M2 presented intermediate values between those appreciated in batches C/M1 and M3/M4. Generally, significant differences were not appreciated by panelists between batches M3 and M4 ($P > 0.05$).

Conclusions

Results reported in this study highlight that *M. punicifolia* extract is able to produce a strong antagonistic effect against *Pseudomonas* species and *B. thermosphacta*, involved in the spoilage of fresh meat products. *In vitro* assays evidenced low MIC values against the assayed spoilage bacteria, and promising results were also obtained *in situ*. The strong antimicrobial activity of *M. punicifolia* extract could be due to the presence of several compounds belonging to different bioactive groups, which represent a series of hurdles (more or less strong) for the microbial growth. Moreover, the presence of bioactive compounds also allowed the preservation of sensorial properties in water buffalo steaks.

References

Argyri AA, Doulgeraki AI, Blana VA, Panagou EZ, Nychas G. 2011. Potential of a simple HPLC-based approach for the identification of the spoilage status of minced beef stored at various temperatures and packaging systems. Int J Food Microbiol 150:25–33.

Baranyi J, Roberts TA. 1994. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol 23:277–94.

Baveja J, Willcox M, Hume E, Kumar N, Odell R, Poole-Warren L. 2004. Furanones as potential anti-bacterial coatings on biomaterials. Biomaterials 25:5003–12.

Bekhit A, Geesink G, Ilian M, Morton J, Bickerstaffe R. 2003. The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. Food Chem 81:175–87.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94:223–53.

Casaburi A, Di Martino V, Ercolini D, Parente E, Villani F. 2014. Antimicrobial activity of *Myrtus communis* L. water-ethanol extract against meat spoilage strains of *Brochothrix thermosphacta* and *Pseudomonas fragi* in vitro and in meat. Ann Microbiol 65:841–50.

Cava-Roda RM, Taboada-Rodríguez A, Valverde-Franco MT, Marín-Iniesta F. 2012. Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling *Listeria monocytogenes* and *Escherichia coli* O157: H7 in milk. Food Bioproc Tech 5:2120–31.

Chauhan R, Siddiqi A, Dwivedi J. 2012. An approach to regioselective synthesis of pyrazole and isoxazole derivatives and study of their antimicrobial effect. Pharm Chem J 46:316–20.

Commission Internationale de l'Eclairage. 1978. International Commission on Illumination recommendations on uniform color spaces, color difference equations, psychometric color terms. Supplement 2 to C.I.E. publication 15 (E-1.3.1) 1971/(TC-1.3). Bureau Central de la Commission Internationale de l'clairage, Paris.

Doulgeraki AI, Nychas G. 2013. Monitoring the succession of the biota grown on a selective medium for pseudomonads during storage of minced beef with molecular-based methods. Food Microbiol 34:62–9.

Ercolini D, Russo F, Torrieri E, Masi P, Villani F. 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl Environ Microbiol 72:4663–71.

Ercolini D, Casaburi A, Nasi A, Ferrocino I, Di Monaco R, Ferranti P, Mauriello G, Villani F. 2010. Different molecular types of *Pseudomonas fragi* have the same overall behaviour as meat spoilers. Int J Food Microbiol 142:120–31.

European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). 2000. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin Microbiol Infect 6:509–15.

Faustman C, Sun Q, Mancini R, Suman SP. 2010. Myoglobin and lipid oxidation interactions: mechanistic bases and control. Meat Sci 86:86–94.

Gil-Chávez JG, Villa JA, Ayala Zavala JF, Heredia JB, Sepulveda D, Yahia EM, González Aguilar GA. 2013. Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Compr Rev Food Sci F 12:5–23.

Gill A, Delaquis P, Russo P, Holley R. 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int J Food Microbiol 73:83–92.

Gutierrez J, Barry-Ryan C, Bourke P. 2009. Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interactions with food components. Food Microbiol 26:142–50.

Herrero M, Mendiola JA, Cifuentes A, Ibáñez E. 2010. Supercritical fluid extraction: recent advances and applications. J Chromatogr A 1217:2495–511.

Holley RA, Patel D. 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol 22:273–92.

Hyldgaard M, Mygind T, Meyer RL. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbiol 3:1–24.

Iturriaga L, Olabarrieta I, de Marañón IM. 2012. Antimicrobial assays of natural extracts and their inhibitory effect against *Listeria innocua* and fish spoilage bacteria, after incorporation into biopolymer edible films. Int J Food Microbiol 158:58–64.

Kim SJ, Cho AR, Han J. 2013. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. Food Control 29:112–20.

Koşar M, Dorman H, Hiltunen R. 2005. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected *Lamiaceae* species. Food Chem 91:525–33.

Kroll J, Rawel H. 2001. Reactions of plant phenols with myoglobin: influence of chemical structure of the phenolic compounds. J Food Sci 66:48–58.

Kyung KH. 2012. Antimicrobial properties of allium species. Curr Opin Biotechnol 23:142–7.

Liao CH. 2006. *Pseudomonas* and related genera. In Blackburn CDW, editor. Food spoilage microorganisms. Cambridge: Woodhead Publishing Ltd. p 507–40.

Lv F, Liang H, Yuan Q, Li C. 2011. *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res Int 44:3057–64.

Mendiola JA, Marin FR, Señoráns FJ, Reglero G, Martín PJ, Cifuentes A, Ibáñez E. 2008. Profiling of different bioactive compounds in functional drinks by high-performance liquid chromatography. J Chromatogr A 1188:234–41.

Muñoz M, Guevara L, Palop A, Tabera J, Fernandez P. 2009. Determination of the effect of plant essential oils obtained by supercritical fluid extraction on the growth and viability of *Listeria monocytogenes* in broth and food systems using flow cytometry. LWT-Food Sci Technol 42:220–7.

Naveena B, Muthukumar M, Sen A, Babji Y, Murthy T. 2006. Improvement of shelf-life of buffalo meat using lactic acid, clove oil and vitamin C during retail display. Meat Sci 74:409–15.

Nowak A, Kalemba D, Krala L, Piotrowska M, Czyzowska A. 2012a. The effects of thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) essential oils on *Brochothrix thermosphacta* and on the shelf life of beef packaged in high-oxygen modified atmosphere. Food Microbiol 32:212–6.

Nowak A, Rygala A, Oltuszak-Walczak E, Walczak P. 2012b. The prevalence and some metabolic traits of *Brochothrix thermosphacta* in meat and meat products packaged in different ways. J Sci Food Agric 92:1304–10.

Nychas G, Skandamis PN, Tassou CC, Koutsoumanis KP. 2008. Meat spoilage during distribution. Meat Sci 78:77–89.

Ozen T, Darcan C, Aktop O, Turkekul I. 2011. Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey. Comb Chem High T Scr 14:72–84.

Papadopoulou OS, Doulgeraki AI, Botta C, Cocolin L, Nychas G-JE. 2012. Genotypic characterization of *Brochothrix thermosphacta* isolated during storage of minced pork under aerobic or modified atmosphere packaging conditions. Meat Sci 92:735–8.

Ramalakshmi S, Muthuchelian K. 2011. Analysis of bioactive constituents from the ethanolic leaf extract of *Tabebuia rosea* (Bertol.) DC by gas chromatography-mass spectrometry. Int J Chem Tech Res 3:1054–9.

Rhoades J, Kargiotou C, Katsanidis E, Koutsoumanis KP. 2013. Use of marination for controlling *Salmonella enterica* and *Listeria monocytogenes* in raw beef. Food Microbiol 36:248–53.

Schelz Z, Hohmann J, Molnar J. 2010. Recent advances in research of antimicrobial effects of essential oils and plant derived compounds on bacteria. In: Chattopadhyay D, editor. Ethnomedicine: a source of complementary therapeutics. Kerala, India: Research Signpost. p 281–304.

Schreckinger ME, Lotton J, Lila MA, de Mejia EG. 2010. Berries from South America: a comprehensive review on chemistry, health potential, and commercialization. J Med Food 13:233–46.

Settanni L, Palazzolo E, Guarrasi V, Aleo A, Mammina C, Moschetti G, Germanà MA. 2012. Inhibition of foodborne pathogen bacteria by essential oils extracted from citrus fruits cultivated in Sicily. Food Control 26:326–30.

Soultos N, Tzikas Z, Abrahim A, Georgantelis D, Ambrosiadis I. 2008. Chitosan effects on quality properties of Greek style fresh pork sausages. Meat Sci 80:1150–6.

Sung WS, Jung HJ, Park K, Kim HS, Lee IS, Lee DG. 2007. 2, 5-dimethyl-4-hydroxy-3 (2H)-furanone (DMHF); antimicrobial compound with cell cycle arrest in nosocomial pathogens. Life Sci 80:586–91.

Tipaldi L, Tremonte P, Reale A, Succi M, Di Renzo T, Pannella G, Coppola R, Sorrentino E. 2011. Effects of natural compounds on food-related microorganisms. Proceedings in 6th International CIGR Technical Symposium. Towards a sustainable food chain: food process, bioprocessing and food quality management. Nantes, France, p 18–21.

Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen P. 2009. Application of natural antimicrobials for food preservation. J Agric Food Chem 57: 5987–6000.

Tremonte P, Sorrentino E, Succi M, Reale A, Maiorano G, Coppola R. 2005. Shelf life of fresh sausages stored under modified atmospheres. J Food Prot 68:2686–92.

Tremonte P, Succi M, Reale A, Di Renzo T, Sorrentino E, Coppola R. 2007. Interactions between strains of *Staphylococcus xylosus* and *Kocuria varians* isolated from fermented meats. J Appl Microbiol 103:743–51.

Tremonte P, Reale A, Di Renzo T, Tipaldi L, Di Luccia A, Coppola R, Sorrentino E, Succi M. 2010. Interactions between *Lactobacillus sakei* and CNC (*Staphylococcus xylosus* and *Kocuria varians*) and their influence on proteolytic activity. Lett Appl Microbiol 51:586–94.

Turgis M, Han J, Borsa J, Lacroix M. 2008. Combined effect of natural essential oils, modified atmosphere packaging, and gamma radiation on the microbial growth on ground beef. J Food Prot 71:1237–43.