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ANTIMICROBIAL EFFECT OF SAGE (*SALVIA OFFICINALIS* L.) AND ROSEMARY (*ROSMARINUS OFFICINALIS* L.) ESSENTIAL OILS ON MICROBIOTA OF CHICKEN BREAST

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The aim of the study was to evaluate the antimicrobial effect of sage and rosemary essential oils (EO) on microbiota of fresh chicken breast. Sample treatments were stored without packaging, vacuum-packaged, vacuum-packaged with EDTA 1.5% v/w, sage and rosemary EO treatment 0.2% v/w. Assessment of food quality was done by anaerobic plate count (APC), and Enterobacteriaceae*, lactic acid bacteria (LAB) and* Pseudomonas *spp. counts a period of 16 days of storage at 4 ± 0.5 °C. Bacterial species were identified with a MALDI TOF MS Biotyper. Antimicrobial activity of isolates against both EO were tested. The APC varied from 2.97 log CFU/g to 6.81 log CFU/g, LAB from 2.35 log CFU/g to 3.36 log CFU/g and* Enterobacteriaceae *from 0.00 log CFU/g on day 0 to 4.77 log CFU/g with the highest counts on day 16 and in control unpackaged samples.* Pseudomonas *spp. was found only on days 0, 4, 8, and 12, with counts from 0.00 log CFU/g on day 16 to 2.89 log CFU/g on day 4 in control unpackaged samples. APC were represented by* Staphylococcus *and* Kocuria*, LAB with* Lactobacillus *and* Enterobacteriaceae *with* Buttiauxella*,* Escherichia*,* Hafnia*,* Serratia *and* Yersinia*. The* Pseudomonas *genus was represented by ten species. The best antimicrobial effect on APC,* Enterobacteriaceae*, LAB and* Pseudomonas *was achieved by application of EO. The results suggest the potential use of* Salvia officinalis *L. and* Rosmarinus officinalis *L. EOs as natural food preservatives and potential sources of antimicrobial ingredients in the food industry.*

Key words: *chicken breast, sage and rosemary essential oils, ethylenediaminetetraacetic acid, vacuum-packaging, bacteria.*

INTRODUCTION

Poultry processing industries and researchers are eager to develop new techniques to minimise microbial growth and improve the microbiological quality of meat, including fresh chicken meat. Nowadays, increasing consumers concerns on side effects of chemical preservatives have resulted in a demand for application of natural preservatives in food and hence the use of natural preservatives has become more popular (Chouliara *et al*., 2008; Economou *et al*., 2009; Bazargani-Gilani *et al*., 2015). Recently, research has intensified on production of essential oil (EO) as additions to food and edible coatings, which are intended to extend the shelf life of foods (Lu *et al*., 2010; Fernández-Pan *et al*., 2014; Bazargani-Gilani *et al*., 2015; Raeisi *et al*., 2015).

Different plants have been used for production of EO and extracts for application in food processing, the pharmaceutical industry and the perfumery sector. EO and extracts not only exhibit natural aromas and flavours, but can have medicinal effect as well (Friedman *et al*., 2002). The genus *Rosmarinus* or Rosemary of the *Lamiaceae* family is a plant used for EO production. The *Rosmarinus* genus comprises of three species, however, *Rosmarinus officinalis* L. is the most productive and has been widely cultivated since antiquity as a herb, garden plant, and a source of EO (Porte *et al*., 2000). EO of rosemary has been used in various industry sectors and has been commercialised because of its antibacterial, antioxidant, antifungal, and anti-inflammatory activity. The application of rosemary EO in pest control products has been reported (Koul *et al*., 2008; Derwich *et al*., 2011).

The antimicrobial activity of *Salvia officinalis* has been recognised for decades. The antimicrobial effect has been associated with the presence of 1,8-cineole, thujone and camphor (Longaray Delamare *et al*., 2007). Recently, antimicrobial effect of sage extract has been shown experimentally. Dry sage leaves have been used, and still are used, as a traditional remedy for many diseases (Beheshti-Rouy *et al*., 2015). Possible application of rosemary and salvia EO in the food industry for treatment of meat and improvement of microbiological quality of products is an area of interest.

The aim of our study was to evaluate the antimicrobial effect of sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) essential oil on microbiota of fresh chicken breast, in comparison to the effect of application of other meat treatment methods.

MATERIALS AND METHODS

Essential oil preparation and analysis. The medicinal plants for EO production were donated by established growers. EOs were distilled in a large-scale distillation apparatus specifically designed for aromatic and medicinal plants. The apparatus consisted of the main distillatory apparatus, a steam condenser, steam boiler and apparatus for purifying the used water. There were two types of equipment — HV-3000 with height of 5250 mm, width of 2180 mm and container for 200 to 250 kg of dried plant material and HV-300 with height of 3400 mm, width 1300 mm and container for 40 to 50 kg of dried plant material. Chemical analysis of the essential oils was carried out with a Hewlett-Packard 5890/5970 GC-MSD system.

Sampling and preparation of samples. Fresh chicken breast meat (ca. 300 g, skinless and boneless fillet) was purchased from a local poultry processing plant within 1 h after slaughter and transferred to the laboratory of Slovak University of Agriculture, Nitra, Slovakia, in insulated polystyrene boxes on ice. The chicken meat was kept at 4 °C until the testing was initiated. For treatment of samples, EDTA (Invitrogen, USA), sage and rosemary EOs (Calendula, Nova Lubovna, Slovakia) were transferred onto meat with a micropipette to cover completely the surface of meat. Concentration of 1.5% v/w of EDTA ($(C_{10}H_{14}N_2O_8.Na_2.2H_2O)$, 99.5% purity, analytical grade) and 0.2% v/w of rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) EOs were used. Each sample was packed immediately after treatment and kept at 4 ± 0.5 °C for 16 days. A vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used for packaging.

Microbiological analysis. An amount of 10 g of chicken breast was transferred into a sterile stomacher bag containing 90 ml of 0.1% buffered peptone water (BPW, pH 7.0, Oxoid code CM0509, Basingstoke, UK) and homogenised for 60 s in a stomacher at room temperature. Sampling was carried out on 0, 4, 8, 12 and 16 days after initiation of the experiment. Replication was six samples (Table 1).

Table 1

CHICKEN MEAT TREATMENT METHODS APPLIED IN THE PRESENT STUDY

Treatment	Description
AC	Control samples: fresh chicken breast meat was packed into a polyethylene bag and stored aerobically at 4 °C
VPC	Control samples: fresh chicken breast meat was packed into a polyethylene bag and vacuum stored at 4 °C
VPEC	Control samples: fresh chicken breast meat was treated with EDTA solution of 1.50% w/w for 1 min, packed into a polyethylene bag and vacuum stored at 4 °C
VP+S	Treated samples: fresh chicken breast meat was treated with sage oil of 0.20% v/w concentration for 1 min, packed into a polyethylene bag and vacuum stored at 4 °C
VP+R	Treated samples: fresh chicken breast meat was treated with rosemary oil of 0.20% v/w concentration for 1 min, packed into polyethylene bag and vacuum stored at 4 °C

Serial decimal dilutions of the obtained 0.1% BPW suspensions were made. Amounts of 0.1 ml of serial dilution were spread on the surface of agar plates. For anaerobic plate counts (APC), plate count agar (PCA, Merck code 1.05463, Darmstadt, Germany) was inoculated and the plates were incubated at 30 °C for 72 h in anaerobic conditions. For detection of *Enterobacteriaceae*, the sample suspension was spread onto Violet Red Bile Glucose Agar (Oxoid code CM 485) and inoculated agars were incubated at 37 °C for 24 h. Lactic acid bacteria (LAB) were determined on Man Rogosa Sharpe agar (Oxoid code CM 361) after incubation at 25 °C for 120 h. *Pseudomonas* spp. were determined on Cetrimide Fucidin Cephaloridine agar (Oxoid code CM 559, supplemented with SR 103, Basingstoke, UK) after incubation at 25 °C for 48 h.

Identification of microbiota with a MALDI TOF MS Biotyper. Qualitative analysis of microbial isolates was performed with a MALDI-TOF Mass Spectrometry (Bruker Daltonics, Germany). Isolates from the agar were transferred into 300 µl distilled water. Then, a quantity of 900 µl of ethanol was added and the tubes with bacterial suspension in water were centrifuged for 2 min at 14 000 npm. The supernatant was discarded and the pellet was centrifuged repeatedly. After the remaining ethanol was removed, the pellet was allowed to dry. An amount of 10 µl 70% formic acid was mixed with the pellet and a 10 µl acetonitrile was added. The tubes were centrifuged for 2 min at 14 000 npm and 1 µl supernatant was used for MALDI identification. Once dry, every spot was overlaid with 1 µl of HCCA matrix and left to dry at room temperature before analysis. Generated spectra were analysed on a MALDI-TOF Microflex LT (Bruker Daltonics) instrument using Flex Control 3.4 software and Biotyper Realtime Classification 3.1 with BC specific software. Criteria for reliable identification were a score of ≥2.0 at species level and ≥1.7 at genus level (DeMarco and Burnham, 2014).

Antimicrobial activity detection with disc diffusion method. The agar disc diffusion method was used for the determination of antimicrobial activity of EOs on chicken

meat microbiota. Briefly, a suspension of the tested microorganism in sterile water (0.1 ml of 10^5 cells per ml) was spread on Mueller Hinton Agar (MHA, Oxoid, code CM0337). Filter paper discs of 6 mm in diameter were impregnated with 15 µl EO and placed onto inoculated plates. Inoculated MHA plates were kept at 4 °C for 2 h and then incubated aerobically at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimetres. All tests were performed in triplicate.

Determination of minimum inhibitory concentration. Broth microdilution susceptibility assay was used to detect the minimum inhibitory concentration (MIC). All tests were performed in Mueller Hinton Broth (MHB, Oxoid, code CM0405) supplemented with Tween 80 detergent (0.5% v/w). Bacterial strains were cultured overnight at 37 °C on MHA. After cultivation, the tested strains were suspended in MHB to give a final density of 10^5 CFU/ml. The tested EO solution was made in dimethyl sulphoxide (DMSO, Penta, Prague, Czech Republic). EOs dilutions from 0.75 to 100 µg/ml were prepared in a 96-well microtitre plate, including one growth control (MHB + Tween 80), one sterility control (MHB + Tween 80 + test oil) and one negative control (pure DMSO) well. The plates were incubated aerobically at 37 °C for 24 h. In case of bacterial growth the presence of a white pellet on the well bottom was observed.

For detection of inhibition of microbial growth, the well absorbance at 450 nm was measured in an absorbance microplate reader Biotek EL808 with shaker (Biotek Instruments, USA). The difference between the absorbance of 96-microwell plates before and after each experiment was used as an estimate of growth. The measurement error for absorbance was 0.05. Eight replicates were used for determination of the MICs of the used EOs.

Statistical analysis. The mean values ± standard deviations were calculated. Analysis of variance was performed on the basis of mean values to test for significant differences ($p \leq 0.05$) between treatments. Statistical analysis was conducted using the SAS version 9.1.

RESULTS

Chemical composition of essential oils. Chemical composition of EO of *Rosmarinus officinalis* L. was: α-pinene (12.1%), camphene (7.8%), β-pinene (2.6%), limonene (12.2%), 1,8-cineole (33.9%), camphor (7.2%), borneol (4.3%), α-terpineol (3.4%) and boranyl acetate (4.7%). The chemical composition of *Salvia officinalis* L. EO was: α-pinene (9.4%), 1,8-cineole (11.8%), α-thujone (23.9%), β-thujone (5.4%), camphor (16.7%), β-caryophyllene (5.2%) and α-caryophyllene (2.6%).

Microbiological quality of the chicken meat. The APC of chicken breast varied from 2.97 ± 0.04 log CFU/g on day 0 to 6.81 ± 0.02 log CFU/g on day 16 for AC (Table 2). *Enterobacteriaceae* counts ranged from 0.00 log CFU/g on day 0 to 4.77 log CFU/g on day 16 of storage in AC samples. The lowest *Enterobacteriaceae* counts (0.00 log CFU/g) for treated samples were observed for VPEC, VP+S and VP+R on day 16 of the experiment. Significant differences ($p \leq 0.05$) in *Enterobacteriaceae* counts were found between the AC, VPC, VPEC, VP+R and VR+S treatments, however, the differences between the VPEC, VP+S and VP+R treatments were not significant ($p \leq 0.05$) (Table 2).

The LAB counts were 2.35 log CFU/g on day 0 to 3.36 ± 0.14 log CFU/g on day 16 in AC samples. Among treatments, the lowest LAB count (2.45 ± 0.32 log CFU/g) was observed for VP+S on day 16 (Table 2). Significant differences in LAB counts ($p \leq 0.05$) were found between the AC and VPC and VP+R and VP+S treatments.

Table 2

MICROBIOLOGICAL QUALITY OF CHICKEN MEAT AFTER APPLICATION OF FIVE MEAT TREATMENT METHODS

Day	APC					*Enterobacteriaceae*				
	Bacterial count (log CFU/g)					Bacterial count (log CFU/g)				
	0	4	8	12	16	0	4	8	12	16
AC	2.97 ± 0.04	3.84 ± 0.07	4.35 ± 0.35	5.79 ± 0.06	6.81 ± 0.02	0	0	4.11 ± 0.15	4.72 ± 0.10	4.77 ± 0.10
VPC	2.97 ± 0.04	3.58 ± 0.22	4.59 ± 0.26	5.70 ± 0.04	6.75 ± 0.04	0	0	3.68 ± 0.84	3.67 ± 0.89	3.62 ± 0.29
VPEC	2.97 ± 0.04	3.21 ± 0.11	3.94 ± 0.11	4.70 ± 0.05	5.72 ± 0.06	0	0	0	0.38 ± 0.94	0
VP+S	2.97 ± 0.04	3.43 ± 0.10	3.49 ± 0.47	4.26 ± 0.06	5.62 ± 0.13	0	0	0	0	0
VP+R	2.97 ± 0.04	3.53 ± 0.17	3.86 ± 0.48	4.54 ± 0.28	5.70 ± 0.08	0	0	0	0	0
Day	Lactic acid bacteria (LAB)					*Pseudomonas* spp.				
	Bacterial count (log CFU/g)					Bacterial count (log CFU/g)				
	0	4	8	12	16	0	4	8	12	16
AC	2.35 ± 0.30	2.70 ± 0.46	3.28 ± 0.60	2.85 ± 1.02	3.36 ± 0.14	2.36 ± 0.25	2.89 ± 0.05	2.72 ± 0.53	2.83 ± 0.55	2.94 ± 0.48
VPC	2.35 ± 0.30	2.46 ± 0.41	3.75 ± 0.15	3.28 ± 0.90	3.26 ± 0.13	2.36 ± 0.25	2.86 ± 0.09	2.33 ± 0.20	2.53 ± 0.18	2.72 ± 0.17
VPEC	2.35 ± 0.30	2.28 ± 0.34	2.39 ± 0.26	2.61 ± 0.22	2.61 ± 0.51	2.36 ± 0.25	0	0	0	0
VP+S	2.35 ± 0.30	2.20 ± 0.24	2.17 ± 0.24	2.29 ± 0.06	2.45 ± 0.32	2.36 ± 0.25	0	0	0	0
VP+R	2.35 ± 0.30	2.08 ± 0.56	2.12 ± 0.10	2.17 ± 0.13	2.57 ± 0.52	2.36 ± 0.25	0	0	0	0

Pseudomonas spp. counts varied from 2.36 log CFU/g on day 0 to 2.94 log CFU/g on day 16 in the AC treatment. The lowest value (0.00 log CFU/g) of LAB in treated samples on day 16 occurred in the VPEC, VP+S and VP+R treatments (Table 2). There were no significant differences between *Pseudomonas* spp. counts in VPEC and VP+R ($p \leq 0.05$) treatments. Significant differences ($p \leq 0.05$) were found between AC and VPEC, AC and VP+S, VPEC and VPC, VPC and VP+S, and VP+R and VP+S treatment pairs.

Identification of microbiota with a MALDI TOF MS Biotyper. Altogether, two genera (*Staphylococcus* and *Kocuria*) were identified from APC plates. Within the *Staphylococcus* genus, *S. warneri* was the most frequently isolated species, while *S. pasteuri* (11.76%) was isolated less frequently. There were no significant differences in abundance of *Kocuria kristinae* (11.76%) and *Kocuria rhizophila* (11.76%) (Table 3). Among LAB, only *Lactobacillus* spp. were isolated; *L. reuteri* (50.00%) was more abundant than *L. salivarius* (25.00%) and *L. acidophilus* (25.00%). The genus *Pseudomonas* was represented by ten species, of which *P. fluorescens* (14.29%), *P. synxantha* (14.29%) and *P. gessardii* (14.29%) were the most abundant. *Enterobacteriaceae* included *Buttiauxella*, *Escherichia*, *Hafnia*, *Serratia* and *Yersinia,* and *Serratia fonticola* (21.43%) and *Hafnia alvei* (21.43%) were the most abundant (Table 3).

Antimicrobial activity. The results on antibacterial activity of EOs assessed by disc diffusion method against the bacterial isolates from meat revealed that EOs were the most effective against *L. reuteri* and *Y. enterocolitica,* with inhibition zones of 10.00 and 12.33 mm for *S. officinalis*, and 12.33 and 12.67 mm for *R. officinalis*, respectively (Table 4). Determination of minimal inhibitory concentration of EOs showed that the best antimicrobial activity of *S. officinalis* was against *L. salivarius* and *B. izardii* with MIC50 = 3.125 and MIC90 = 6.25 μg/ml, respectively. *L. salivarius* and *B. izardii* were the most sensitive to *R. officinalis* EO activity, with MIC50 = 6.25 and MIC90 = 12.50 μg/ml (Table 4).

Table 3

IDENTIFICATION OF BACTERIA ISOLATED FROM CHICKEN MEAT AFTER TREATMENT

Species	No. of bacterial colonies/ No. of samples	Percentage %
Lactic acid bacteria (LAB)		
Lactobacillus salivarius	1/9	25.00
Lactobacillus reuteri	2/9	50.00
Lactobacillus johnsonii	1/9	25.00
Pseudomonas spp.		
Pseudomonas fluorescens	2/9	14.29
Pseudomonas synxantha	2/9	14.29
Pseudomonas chlororaphis	1/9	7.14
Pseudomonas gessardii	2/9	14.29
Pseudomonas libanensis	2/9	14.29
Pseudomonas orientalis	1/9	7.14
Pseudomonas veronii	1/9	7.14
Pseudomonas extremorientalis	1/9	7.14
Pseudomonas agarici	1/9	7.14
Pseudomonas lundensis	1/9	7.14
Anaerobic plate count (APC)		
Staphylococcus warneri	6/9	35.29
Staphylococcus epidermidis	5/9	29.41
Staphylococcus pasteuri	2/9	11.76
Kocuria kristinae	2/9	11.76
Kocuria rhizophila	2/9	11.76
Enterobacteriaceae family		
Serratia fonticola	3/9	21.43
Hafnia alvei	3/9	21.43
Serratia liquefaciens	1/9	7.14
Buttiauxella gaviniae	2/9	14.29
Buttiauxella izardii	1/9	7.14
Yersinia enterocolitica	1/9	7.14
Buttiauxella noackiae	2/9	14.29
Escherichia coli	1/9	7.14

DISCUSSION

GC–MSD analysis of sage and rosemary EOs identified seven and nine components representing 75.00% and 88.20% of the total contents of the EOs, respectively. Previous studies (Raal *et al*., 2007; Jiang *et al*., 2011; Verma *et al*., 2015) showed similar phytochemical properties of rosemary EOs to those observed in our study. The compounds 1,8-cineole, thujone, camphor, and viridiflorol were reported in rosemary EOs originating from Ukraine and India (Raal *et al*., 2007, Verma *et al*., 2015). The main components of rosemary EO in another study (Jiang *et al*., 2011) were 1,8-cineole, α-pinene, camphor, camphene and β-pinene.

Microbiological testing results showed that the quality of chicken meat used in the present study was good. However, changes in bacterial counts were observed up to an APC of 6.81 ± 0.02 log CFU/g on day 16 of experiment in the AC treatment. Our findings indicated that untreated chicken meat stored in an anaerobic environment was the most subjected to spoilage. The absence of *Enterobacteriaceae* (0.00 log CFU/g on day 0) supported the assessment of quality of chicken meat as satisfactory. *Enterobacteriaceae* on raw beef, lamb, pork, poultry products and offal indicate the efficiency of hygiene during processing (Zeitoun *et al*., 1994; Pokorny *et al.* 2001; Khanjari *et al*., 2013). *Pseudomonas* spp. and LAB counts of 2.35 ± 0.30 and 2.36 ± 0.25 also showed that the quality of meat was acceptable. *Pseudomonas* spp. and LAB are associated with meat spoilage, and the presence of these bacteria can accelerate deterioration of quality of meat (Oussalah *et al*. 1996; Frantianni *et al*., 2010).

Table 4

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AGAINST BACTERIAL ISOLATES ORIGINATED FROM CHICKEN MEAT AFTER TREATMENT

	Diameter of inhibition zone in mm		Minimal inhibition concentration in μg/ml			
EOs	S. *officinalis*	*R. officinalis*	S. *officinalis*		*R. officinalis*	
Microorganisms			MIC 50	MIC 90	MIC 50	MIC 90
L. salivarius	3.00 ± 1.00	6.00 ± 1.00	3.125	6.25	6.25	12.50
L. reuteri	10.00 ± 1.00	12.33 ± 2.52	6.25	12.50	12.50	25.00
L. johnsonii	5.00 ± 0.00	4.67 ± 0.58	25.00	50.00	25.00	50.00
P. fluorescens	4.33 ± 0.58	7.00 ± 2.00	25.00	50.00	12.50	25.00
P. synxantha	4.66 ± 0.58	4.00 ± 0.57	25.00	50.00	12.50	25.00
P. chlororaphis	4.33 ± 0.58	2.33 ± 0.57	12.50	25.00	25.00	50.00
P. gessardii	2.33 ± 0.58	7.66 ± 1.53	6.25	12.50	12.50	25.00
P. libanensis	4.33 ± 0.58	9.00 ± 1.00	25.00	50.00	25.00	50.00
P. orientalis	2.33 ± 0.58	8.67 ± 0.58	50.00	100.00	50.00	100.00
P. veronii	4.67 ± 0.58	10.00 ± 1.00	50.00	100.00	25.00	50.00
P. extremorientalis	4.33 ± 0.58	3.00 ± 1.00	6.25	12.50	25.00	50.00
P. agarici	7.33 ± 0.58	3.00 ± 1.00	25.00	50.00	25.00	50.00
P. lundensis	4.33 ± 0.58	3.00 ± 1.00	25.00	50.00	25.00	50.00
S. warneri	2.00 ± 1.00	5.33 ± 0.58	50.00	100.00	25.00	50.00
S. epidermidis	4.66 ± 0.58	5.00 ± 1.00	50.00	100.00	25.00	50.00
S. pasteuri	6.67 ± 1.53	5.33 ± 0.57	6.25	12.50	12.50	25.00
K. kristinae	9.67 ± 1.53	5.67 ± 1.53	50.00	100.00	50.00	100.00
K. rhizophila	4.67 ± 0.58	5.00 ± 1.00	50.00	100.00	50.00	100.00
S. fonticola	4.33 ± 0.58	7.33 ± 0.58	25.00	50.00	25.00	50.00
H. alvei	6.00 ± 1.00	4.33 ± 0.58	25.00	50.00	12.50	25.00
S. liquefaciens	4.33 ± 0.58	4.33 ± 0.58	25.00	50.00	12.50	25.00
B. gaviniae	5.33 ± 0.58	2.67 ± 0.58	25.00	50.00	25.00	50.00
B. izardii	2.67 ± 1.15	3.33 ± 0.58	3.125	6.25	6.25	12.50
Y. enterocolitica	12.33 ± 1.53	12.67 ± 1.15	6.25	12.50	12.50	25.00
B. noackiae	4.67 ± 0.58	3.33 ± 0.58	25.00	50.00	25.00	50.00
E. coli	8.67 ± 0.58	4.33 ± 0.58	25.00	50.00	12.50	25.00

MIC, minimum inhibitory concentration

The results showed significant differences in bacterial contamination between the samples treated with different methods and especially with the EO treatments. Inhibition of APC and *Enterobacteriaceae* in the VP+S and V+R treatments, in comparison with AC and VPC treatments, was likely due to the antibacterial effects of the vacuum packaging and treatment with sage and rosemary EOs. Rosemary EOs, which contain ursolic acid, phenolic acids, flavones, and other compounds, can inhibit the growth of *E. coli*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis*, *S. aureus,* and *K. pneumonia* (Prabuseenivasan *et al*., 2006). Miladinović and Miladinović (2000) reported that EO from sage leaves exhibits antimicrobial activity against *B. subtilis, St. aureus* ATCC 6538, *E. coli*, *Salmonella enterica* serovar Enteritidis and *Aspergillus niger*. However, Ntzimani *et al*. (2010) described the presence of *Enterobacteriaceae* on semi-cooked chicken meat samples after treatment with EDTA and rosemary EO, indicating the possible survival of the microorganisms.

Significantly lower counts ($p \leq 0.05$) of LAB in VP+R and VP+S treatments than in AC and VPEC treatments indicated that treatment with EOs had significant effect on growth of LAB in chicken meat. The effect of rosemary EO observed in the present report was in agreement with Zaika *et al*. (1983) who showed a reduction of 4 log CFU/g in LAB populations after the addition of 4 g/l (0.4%) of oregano EO. Differences in the activity of EOs may be attributed to the type of food and composition of EO and this should be taken into consideration when the EOs effect is evaluated (Zaika *et al*. 1983; Burt, 2004). The results of our study on a combined effect of EOs and VP are in agreement with Chouliara *et al*. (2007), who described the inhibitory effect of EO meat treatment on microbial growth in chicken meat.

Reduction of the *Pseudomonas* spp. population in VTEC meat had been described to occur after EDTA treatment, which increases sensitivity to antibacterial agents against

which *Pseudomonas* spp. are normally resistant (Ntzimani *et al*., 2011). Bioactive components of EOs possess relatively weak activity against *Pseudomonas* spp., due to differences in cell wall composition between Gram-negative and Gram-positive bacteria (Burt, 2004). Inhibitory effect of EOs on growth of *Pseudomonas* spp. associated with meat spoilage was observed in a study on the effect of 60 different EOs in concentrations from 0.003 to 0.8% (v/w) (Oussalah *et al*. (2006).

The application of EOs of *Salvia officinalis* 0.2% and *Rosemarinus officinalis* 0.2% had strong antimicrobial effect on growth of APC and *Enterobacteriaceae,* LAB and *Pseudomonas* spp. It is important to point out that not all EOs exhibit inhibitory activity. Anise, eugenol, coriander, clove, oregano, spearmint and thyme oils were found to be effective in inhibiting spoilage flora in meat products and to cause marked initial reduction in the number of recoverable cells (Tsigarida *et al.*, 2000; Skandamis and Nychas, 2001; Kačániová *et al*., 2016). Ntzimani *et al*. (2010) reported that vacuum-packaged coated chicken samples with the addition of rosemary EO showed a 7-day shelf-life extension. Borneol from sage and rosemary had an inhibitory effect against Gram-positive and Gram-negative bacteria at 2% while the concentrations of 0.3% and 0.5% were bacteriostatic and bactericidal for Gram-positive bacteria (Bajpai *et al*., 2008).

CONCLUSION

Our results show that the combined use of vacuum-packing and application of EOs treatment can extend the shelf-life of the product up to 16 days at +4 °C. The identified inhibitory effect of sage and rosemary oil on *Enterobacteriaceae* in vacuum packed chicken breast meat was potential use in provision of good microbiological quality of the product.

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SALVIJAS (*SALVIA OFFICINALIS* L.) UN ROZMARĪNA (*ROSMARINUS OFFICINALIS* L.) ĒTERISKO EĻĻU ANTIMIKROBIĀLĀ IEDARBĪBA UZ CĀĻA FILEJAS MIKROFLORU

Pētījuma mērķis bija noteikt salvijas un rozmarīna ēterisko eļļu antimikrobiālo ietekmi uz cāļa filejas mikrofloru. Eksperimentiem tika sagatavoti sekojoši cāļa filejas paraugi: 1) vakuuma iepakojumā; 2) vakuuma iepakojumā ar EDTA (1,5% v/m); 3) vakuuma iepakojumā pēc apstrādes ar salvijas un rozmarīna ēteriskajām eļļām (0,2% v/m). Savukārt cāļa filejas paraugs bez iepakojuma izmantots kā kontroles paraugs. Sagatavotos cāļu filejas paraugus uzglabāja 16 dienas 4 ± 0,5 °C. mikrobioloģiskās kvalitātes izmaiņas tika analizētas, nosakot anaerobo, *Enterobacteriaceae* dzimtas, pienskābes un *Pseudomonas spp.* baktēriju skaitu. Baktēriju sugas tika identificētas, lietojot MALDI TOF MS Biotyper. Eksperimentu gaitā kontroles paraugā tika konstatēta intensīvāka mikrooganismu attīstība. Eksperimentā kopumā anaerobais baktēriju skaits mainījās no 0. uzglabāšanas dienā no 2,97 log KVV g^{-1} līdz 6,81 KVV g^{-1} 16. uzglabāšanas dienā, bet pienskābes baktēriju skaits attiecīgi no 2,35 log KVV g^{-1} līdz 3,36 KVV g^{-1} un *Enterobacteriacea* dzimtas baktēriju skaits no 0,00 log KVV g^{-1} līdz 4,77 log KVV g^{-1} . *Pseudomonas* spp. baktērijas identificēja 0., 4., 8. un 12. eksperimenta dienā, un to skaits mainījās no 0,00 KVV g^{-1} 16. uzglabāšanas dienā līdz 2,89 KVV g^{-1} 4. uzglabāšanas dienā. Pētījumu gaitā identificēja tādas baktēriju ģintis kā *Staphylococcus*, *Kocuria*, *Lactobacillus, Buttiauxella, Escherichia, Hafnia, Serratia, Yersinia* un *Pseudomonas*. Eksperimentos izteiktāks antibaktēriālais efekts, ietekme uz mikroorganismu skaitu, konstatēta cāļa filejas paraugos, pēc apstrādes ar ēteriskajām eļļām. Pētījuma rezultāti norāda uz iespējamo salvijas (*Salvia officinalis* L.) un rozmarīna (*Rosmarinus officinalis* L.) ēterisko eļļu izmantošanu pārtikas produktu gatavošanā kā dabīgu konservantu un antimikrobiālo vielu avotu.