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Research Paper

Effect of Plant Antimicrobial Agents Containing Marinades on Storage Stability and Microbiological Quality of Broiler Chicken Cuts Packed with Modified Atmosphere Packaging

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ABSTRACT

The food industry, including the meat industry, is currently looking for natural preservatives to prevent the growth of harmful microbes in foods. The potential of plant-derived antimicrobial extracts to increase the shelf life and to delay the microbiological spoilage of marinated broiler chicken cuts in modified atmosphere packages during cold storage was investigated in this study. We evaluated the impact of aqueous ethanolic extracts of Finnish sea buckthorn berries and lingonberries and supercritical CO_2-extracted herbal extracts from an antimicrobial blend and oregano leaves on the shelf life of broiler meat. The commercial antimicrobial blend extract and the oregano extract inhibited the growth of lactic acid bacteria (LAB) and *Brochothrix thermosphacta* in the marinated samples. The antimicrobial blend extract also reduced the growth of psychrotrophic aerobic bacteria, whereas the sea buckthorn and lingonberry extracts did not. Only minor antimicrobial activity against *Enterobacteriaceae* by all the extracts was observed. Plate count analysis, denaturing gradient gel electrophoresis, and quantitative real-time PCR indicated that LAB, which are the major spoilage group in marinated modified atmosphere–packaged poultry products, were not significantly affected by the berry extracts studied. During this shelf-life study, LAB isolates of *Lactobacillus* and *Leuconostoc* were identified in the marinated samples. Antimicrobial blends and oregano leaf extracts can act as antimicrobial agents in marinade blends, although tailoring of the dose is needed because of their strong taste. Further studies for exploiting synergistic effects of plant extracts could contribute to the development of potential and more effective antimicrobial blends. Studies are needed in meat matrices and in product applications to demonstrate the efficacy of these compounds.

Key words: Microbiological spoilage; Plant extract; Poultry; Shelf life

Food spoilage is a complex process, during which combinations of chemical and biological activities may render the product unacceptable for human consumption *(23)*. Huge amounts of food are lost due to microbiological spoilage even with modern preservation techniques *(6, 8)*. Consumer preferences for minimally treated ("natural") foods containing natural antimicrobial agents and fewer substances with E-numbers (codes for substances permitted by the European Food Safety Authority to be used as food additives within the European Union) has enhanced the research related to natural antimicrobial agents for food applications *(3, 32, 33)*. The antimicrobial activity of essential oils, herbs, spices, berries, berry-derived phenolic compounds, and other plant-derived antimicrobial compounds has been examined and reviewed *(2, 3, 9, 18, 20, 29, 33)*. Phytochemicals containing essential oils at 0.05 to 0.1% have had inhibitory activity against pathogens such as *Salmonella enterica,* pathogenic *Escherichia coli, Listeria monocytogenes, Bacillus cereus,* and *Staphylococcus aureus* in food systems *(32)*. However, proteins, lipids, complex carbohydrates, simple sugars, and cations can reduce this antimicrobial activity in food matrices *(5)*.

Modified atmosphere (MA) packaging is routinely applied in the poultry industry *(24, 31)*. Marination with salt, sugar, and acetic acid is a process commonly used to tenderize meat, improve sensory quality, and improve yield in the processing plant *(16)*. The spoilage microbes prevailing in the MA-packaged broiler products include psychrotrophic lactic acid bacteria (LAB), *Brochothrix thermosphacta,* and *Enterobacteriaceae (15, 16, 31)*. LAB frequently identified from spoiled meat products belong to the genera *Carnobacterium, Lactobacillus, Leuconostoc,* and *Weissella (17, 19)*. The sensory quality of fresh meat products deteriorates mainly because of the metabolites (e.g., acetoin, ethanol, and sulfur compounds) produced by the spoilage organisms *(4, 22, 35)*. LAB, especially *Leuconostoc gasicomitatum,* have been identified as the main spoilage bacteria in marinated broiler products and cause extensive bulging of the packages *(1, 15, 16)*. *Lactobacillus oligofermentans* has also been associated with spoilage of MA-packaged poultry products *(10)*.

The aim of this study was to examine the potential of plant-derived supercritical CO_2-extracted herbal extracts and

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Finnish berry extracts to increase the shelf life and delay microbiological spoilage of marinated broiler chicken cuts during cold storage in MA packages. The efficacy of two commercial antimicrobial herbal extracts (an antimicrobial blend and oregano leaf extract) and two novel Finnish berry extracts (sea buckthorn and lingonberry) for inhibiting spoilage bacteria was examined using both culture and DNA-based techniques.

MATERIALS AND METHODS

Antimicrobial extracts used in the shelf-life study. Two commercial supercritical CO_2 extracts, an antimicrobial blend (product code 238.001), and oregano leaf CO_2-se extract (product no. 103.002) were manufactured and provided by Flavex Naturextrakte GmbH (Rehlingen, Germany). According to information provided by the manufacturer, the concentrations of essential oils in the antimicrobial blend and oregano leaf extract were 27.7 and 81.9%, respectively. The oregano extract contained 50.1% carvacrol, 9.3% thymol, 8.2% *p*-cymene, and 5.1% thymoquinone. The antimicrobial blend was composed of seven supercritical extracts: 30% sage leaf (*Salvia fruticosa*), 20% hops (*Humulus lupulus*), 15% licorice root (*Glycyrrhiza uralensis*), 15% curcuma (*Curcuma xanthorrhiza*), 10% clove bud (*Syzygium aromaticum*), 5% oregano leaf, and 5% ajowain seed (*Trachyspermum ammi*) *(34)*. Two berry extracts with antimicrobial activity were selected for the shelf-life study. Extracts of sea buckthorn (*Hippopoaë rhamnoides* L. subsp. *rhamnoides* cultivar Tytti) and wild lingonberry (*Vaccinium vitis-idaea*) were prepared by extracting the fresh berries with 70% aqueous ethanol containing 1% acetic acid. The total phenolic concentration (gallic acid equivalent) was 245 and 8,666 μg/g in sea buckthorn extract and lingonberry extract, respectively, as determined by a Folin-Ciocalteau protocol.

Preparation of the meat samples. Fresh brined skinless breast broiler chicken cuts were obtained directly from the production plant and processed at VTT (Espoo, Finland) 1 day after the slaughtering of the birds. After arrival at the laboratory, the samples were divided into six portions. The portions were mixed with either a commercial marinade base (control) or the marinade base spiked with one of the antimicrobial agents (13% of marinade). A commercial marinade base without preservatives, acids (citric acid, sodium benzoate, and potassium sorbate), antioxidants, and herbs was used as a reference (R1) and as a marinade base into which the antimicrobial compounds were mixed. To facilitate blending with the marinade base, the antimicrobial agent was diluted with rapeseed oil (1:10, w/w) and then mixed with the marinade base. The final concentration of the antimicrobial agents in the storage trial was 2 g/kg of product (0.2%). Commercial marinade base (R2) and unmarinated broiler chicken cuts (fresh brine, R3) were also included as control samples.

MA packaging and storage conditions. The marinated chicken cut samples were portioned into 120 ± 5 g in 210-mL tray packages (high-density polyethylene tray 564, Polimoon AS, Kristiansand, Norway). The packages were flushed with 65% CO_2 and 35% N_2 and sealed using the Dyno 462 VGA machine (lid material OPA 15/MDPF70, Schmid Maschinenbau, Sonnenbühl, Germany). The samples were stored in the dark at 6 ± 0.5°C for 13 days. Gas composition in the headspace of the packages was measured with a gas analyzer (CheckMate 9900, PBI-DanSensor A/S, Ringsted, Denmark). For each time point, three replicate packages were prepared for microbiological examination. During the shelf-life study, the quality of the broiler chicken cuts was evaluated as a function of time.

Small-scale sensory analysis. After 2 days of storage, the packed samples were opened, the odor of the samples was evaluated, and the samples were pan fried (5 min for the first side and 10 min for the other side). After cooking, the samples were cooled, and portions were served to a trained five-person panel. Product characteristics were assessed, and verbal descriptions were given for taste and odor, with an emphasis on possible off-flavors.

Culture-based microbiological analyses of potential spoilage bacteria. Microbiological analyses were performed after 2, 5, 9, and 13 days of storage at 6 ± 0.5°C. Three replicate samples were analyzed at each time point. Ten-gram subsamples were weighted and diluted with 90 mL of peptone saline and homogenized with a stomacher blender for 60 s (Lab Blender 400, Seward, Worthington, UK). Appropriate dilutions were plated on agar plates. Microbial groups for analysis with plate count techniques were chosen based on their role in broiler meat spoilage. LAB were plated on de Man Rogosa Sharpe agar (MRS; Oxoid, Basingstoke, UK) with sorbic acid at pH 5.8 and 25°C for 5 days. Aerobic psychrotrophic bacteria were plated on plate count agar (Difco, BD, Bordeaux, France) at 10°C for 7 days. *Enterobacteriaceae* were plated on violet red bile glucose agar (VRBGA; Lab M, Bury, UK) at 37°C for 1 day. *B. thermosphacta* (a gram-positive, facultatively anaerobic psychrotrophic bacterium) was plated on streptomycin sulfate thallium acetate actidione agar (STAA; Oxoid, Basingstoke, UK) at 25°C for 2 days. Anaerobic conditions (85% N_2, 5% CO_2, and 10% H_2) for LAB were generated by the evacuation replacement method (Anoxomat, Hart, Lichtenvoorde, The Netherlands). The pH of the samples was measured at each time point from the homogenized samples with a pH meter (UB-10x, Denver Instrument, Bohemia, NY). *B. thermosphacta* was analyzed only on days 9 and 13. Bacterial colonies were isolated from samples stored for 13 days. Dominant species were isolated from MRS, VRBGA, and STAA (five morphologically different isolates from each medium per marinade treatment if possible).

Quantification and characterization of bacterial populations with molecular techniques. For molecular biological analyses, two 50-g portions of the homogenized sample were centrifuged (200 × *g*, 5 min, 4°C). Supernatant was harvested (15 mL from both tubes) and further centrifuged (10,000 × *g*, 10 min, 4°C). Supernatant from the second centrifugation was discarded, and the pellets were collected and frozen (–70°C) for molecular biology analysis to be performed later. DNA extraction was performed with 0.2 g of sample (frozen pellet) as previously described *(12)*. Quantitative real-time PCR (qPCR) assays were performed as previously described *(30)* to quantify the levels of bacteria. Standard curves were obtained from genomic DNA templates isolated from pure cultures as previously described *(30)*, and the extracted DNA was quantified by using a spectrophotometer (NanoDrop 2000c, Thermo Scientific, Wilmington, DE). For each bacterium of interest, the number of cells present in the volume loaded to the qPCR was calculated on the basis of the genome size and the respective 16S rRNA copy number per cell, as identified through the National Center for Biotechnology Information genome database (http://www.ncbi.nlm.nih.gov/genome/).

Bacterial community analyses were performed using two bacterial denaturing gradient gel electrophoresis (DGGE) methods validated at VTT. Predominant bacterial PCR-DGGE (Univ-DGGE and a *Lactobacillus* group DGGE for *Lactobacillus,*

TABLE 1. *Viable counts of lactic acid bacteria (LAB) and* Enterobacteriaceae *in chicken sample treatments during 13 days of storage at 6.0 ± 0.5°C*[a]

Treatment[b]	Mean ± SD LAB counts (log CFU g^{-1}) at:				Mean ± SD *Enterobacteriaceae* counts (log CFU g^{-1}) at:			
	2 days	5 days	9 days	13 days	2 days	5 days	9 days	13 days
Antimicrobial blend 238.001	3.4 ± 0.1***	5.3 ± 0.1***	7.4 ± 0.1***	8.1 ± 0.1***	2.6 ± 0.2**	4.2 ± 0.2	6.9 ± 0.0*	7.4 ± 0.1***
Oregano leaf	4.1 ± 0.1**	6.4 ± 0.3*	8.3 ± 0.0**	8.7 ± 0.0**	2.3 ± 0.2*	4.5 ± 0.8	6.3 ± 0.3*	7.0 ± 0.2
Sea buckthorn	5.0 ± 0.1	7.3 ± 0.1	8.8 ± 0.1	8.8 ± 0.2	2.9 ± 0.1	4.8 ± 0.1	6.8 ± 0.6	6.6 ± 0.4
Lingonberry	5.1 ± 0.1	7.3 ± 0.1	8.7 ± 0.1	8.9 ± 0.1	3.2 ± 0.6	5.0 ± 0.1	6.3 ± 0.0	6.4 ± 0.0
R1	4.9 ± 0.0	7.0 ± 0.1	8.8 ± 0.1	8.9 ± 0.1	2.8 ± 0.0	4.4 ± 0.1	7.1 ± 0.0	6.2 ± 0.1
R2	4.7 ± 0.1	7.0 ± 0.1	8.5 ± 0.1	8.9 ± 0.1	2.8 ± 0.1	5.0 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
R3	4.8 ± 0.1	7.6 ± 0.1	7.4 ± 0.1	8.4 ± 0.0	2.6 ± 0.1	5.4 ± 0.2	6.5 ± 0.1	6.8 ± 0.0

[a] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[b] R1, commercial marinade without preservatives, acids, antioxidants, or herbs, which was used as a base for other treatments; R2, commercial marinade with preservatives; R3, natural brine.

Leuconostoc, Pediococcus, and *Weissella*) were performed as previously described *(13)*. Profiles were compared by calculating the number of bands and a similarity percentage with BioNumerics software version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium). Clustering was performed with the Pearson correlation test and the unweighted pair group method. Amplicons with a total surface area of at least 1% were included in the similarity analysis *(11)*.

Identification of the bacterial isolates. Selected potential spoilage bacteria (57 isolates of *Enterobacteriaceae, B. thermosphacta,* and LAB) were identified with partial sequencing of the 16S rRNA gene and ribotyping. Automated ribotyping was performed with *Eco*RI according to the standard protocol (RiboPrinter Characterization System, DuPont Qualicon, Wilmington, DE) *(25)*. The similarity score was obtained by comparing the ribopatterns to other patterns in the VTT and DuPont identification databases. The cutoff value for reliable identification was a similarity score of 0.85. Sequencing of the partial 16S rRNA gene of the isolates was performed as previously described *(14)* using primers 7F and 1510R according to Satokari et al. *(26)*. Sequences were edited with the Sequence Scanner software v1.0 (Applied Biosystems, Foster City, CA) and identified using the SeqMatch tool of the Ribosomal Database Project II *(36)*.

Statistical analysis. Results from the culture-based microbiological analysis and characterization of bacterial populations with molecular techniques were analyzed using two-tailed unpaired Student's *t* test to determine differences; differences were considered significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

Effect of plant antimicrobial agents on pH. In the unmarinated broiler chicken cuts, the pH remained stable (5.9 to 6.0) throughout the storage. In the reference sample treated with commercial marinade (R2) and the samples marinated with berry extracts, clear decreases in pH occurred during storage; the pH was 5.4 to 5.3 after 9 days and 5.1 after 13 days. In samples with the antimicrobial blend and oregano leaf extract only minor changes in the pH occurred during storage; pH was 5.7 after 13 days. The decrease in pH was correlated with the increase in the viable numbers of LAB ($R^2 = 0.92$ at 9 days).

Effect of plant antimicrobial agents on the headspace CO_2 concentrations. The amount of residual oxygen in all the samples was below 1%. Headspace CO_2 concentrations after MA packaging were 65% ± 1%. After 5 days of storage, headspace CO_2 concentrations were at the lowest (36 to 38%) in all samples, except for the samples treated with the antimicrobial blend. The headspace CO_2 concentrations in unmarinated (natural brine) samples (R3) and in meat samples with antimicrobial blend marinade decreased from 65% to a low of 38 to 47% during storage. In the samples treated with commercial marinade, oregano leaf extract, sea buckthorn extract, and lingonberry extract, the headspace CO_2 concentration was 56 to 61% at the end of the trial.

Effect of plant antimicrobial agents on levels of viable potential spoilage bacteria. The antimicrobial blend and the oregano leaf extract significantly inhibited the growth of LAB and *B. thermosphacta* in the samples ($P < 0.05$, Tables 1 and 2). The efficacy of the antimicrobial blend and the oregano leaf extract for inhibiting the growth of LAB was greatest in the samples stored for 2 and 5 days. LAB levels in the sample with the antimicrobial blend were lower than those in the samples treated with R1, R2, and R3 by 1.4 to 1.7 log CFU/g (Table 1, $P < 0.05$). After 9 and 13 days of storage, *B. thermosphacta* levels in the sample with the antimicrobial blend were 2.8- and 3.8-log lower, respectively, than those in the samples treated with the R2 (Table 2, $P < 0.05$). The antimicrobial blend also prevented the growth of aerobic psychrotrophic bacteria, with a 0.8- to 1.1-log reduction during 9 days of storage (Table 2). None of the extracts had a strong effect on the growth of *Enterobacteriaceae* (Table 2). Sea buckthorn and lingonberry extracts did not have significant antimicrobial effects ($P > 0.05$) against any of the bacterial groups at the concentrations studied.

Identification of potential spoilage bacteria. LAB isolates identified from marinated samples included *Lactobacillus fuchuensis, L. oligofermentans, Lactobacillus sakei,* and *Leuconostoc gelidum* (Table 3). Similar LAB ribotypes

TABLE 2. *Viable counts of psychrotrophic bacteria and* Brochothrix thermosphacta *in chicken sample treatments during 13 days of storage at 6.0 ± 0.5°C*[a]

Treatment[b]	Mean ± SD psychrotrophic bacteria counts (log CFU g^{-1}) at:				Mean ± SD *Brochothrix thermosphacta* counts (log CFU g^{-1}) at:			
	2 days	5 days	9 days	13 days	2 days	5 days	9 days	13 days
Antimicrobial blend 238.001	3.8 ± 0.2**	6.4 ± 0.2**	7.6 ± 0.1***	8.4 ± 0.1*	ND	ND	1.5 ± 0.5**	3.5 ± 0***
Oregano leaf	4.1 ± 0.2**	6.8 ± 0.7	8.2 ± 0.4	8.4 ± 0.0*	ND	ND	3.8 ± 0.3	3.8 ± 0.3*
Sea buckthorn	4.8 ± 0.4	7.2 ± 0.1	8.6 ± 0.1	8.6 ± 0.3	ND	ND	4.5 ± 0.4	4.5 ± 0.1
Lingonberry	5.1 ± 0.3	7.4 ± 0.2	8.5 ± 0.1	8.5 ± 0.1	ND	ND	4.5 ± 0.4	4.5 ± 0.5
R1	4.9 ± 0.1	7.2 ± 0.1	8.7 ± 0.0	8.8 ± 0.2	ND	ND	4.0 ± 0.4	4.7 ± 0.3
R2	4.9 ± 0.1	7.2 ± 0.1	8.7 ± 0.0	8.6 ± 0.2	ND	ND	4.3 ± 0.1	4.3 ± 0.4
R3	4.9 ± 0.1	7.8 ± 0.2	7.6 ± 0.1	8.5 ± 0.2	ND	ND	4.6 ± 0.5	4.2 ± 0.3

[a] * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ND, not determined.
[b] R1, commercial marinade without preservatives, acids, antioxidants, or herbs, which was used as a base for other treatments; R2, commercial marinade with preservatives; R3, natural brine.

were isolated from each marinated sample type, except *L. sakei* and *Leuconostoc* were not isolated from the sample containing the antimicrobial blend. *L. fuchuensis, L. oligofermentans,* and *L. sakei* were also isolated from the unmarinated samples, whereas *Leuconostoc* was not. The isolated *L. fuchuensis* strains and the *L. fuchuensis* type strain belonged to the same ribogroup, indicating that these strains are closely related. The isolated *L. oligofermentans* strains had a riboprint pattern different from that of the *L. oligofermentans* type strain, indicating heterogeneity of these strains. *B. thermosphacta* could not be reliably identified by ribotyping comparison with the current identification databases of VTT or DuPont.

Hafnia alvei, which belongs to the family *Enterobacteriaceae,* was the main species isolated on VRBGA from both marinated and unmarinated samples. Isolates with the same fingerprint (belonging to similar ribotypes) were isolated both from marinated and unmarinated samples.

Quantification and characterization of bacterial populations. Predominant bacterial diversity as detected with the predominant bacterial DGGE decreased as a function of time from 5 to 13 days in most of the marinated samples (Fig. 1). However, the difference in predominant bacterial diversity differed greatly on day 5 between the marinade treatments. The highest diversity was in the samples with antimicrobial blend no. 238.001 (36 ± 4.4 bands) and the lowest in samples with sea buckthorn (24.5 ± 0.7 bands) (Fig. 1). The predominant bacteria detected with the qPCR assay were similar regardless of the sample type (Fig. 2), except that the levels were significantly lower ($P < 0.05$) in the antimicrobial blend samples than in the other marinated samples at 5 days. *Lactobacillus* group diversity (comprising *Lactobacillus, Leuconostoc, Pediococcus,* and *Weissella*), as detected with the group-specific DGGE, increased as a function of time from 5 to 9 days in all marinade treatments and stayed at a similar level from day 9 to day 13 (Fig. 3). After 9 days, the diversity was lowest in the antimicrobial blend samples. The levels of bacteria within the *Lactobacillus* group, as detected with the qPCR assay, increased as a function of time from 2 to 9 days in all sample types and stayed at a similar level from day 9 to day 13 (Fig. 4). The levels of *Lactobacillus* group bacteria were significantly lower ($P < 0.05$) in the antimicrobial blend samples than in the other samples at day 2 ($P < 0.05$; Fig. 4) and significantly lower than those in most of the other samples on days 5 and 9. After 13 days, the levels of *Lactobacillus* group bacteria were similar in all samples. Results based on molecular biology were similar to the culture-based results.

DISCUSSION

Because of the consumer trend of preference for "natural" products, the need to reduce the use of chemical preservatives in foods has recently emerged. Because consumers also want their food products to have a long shelf life, plant-based antimicrobial agents have been considered one option for replacing some chemical preservatives. In this study, we investigated the effects of two Finnish berry extracts (sea buckthorn and lingonberry) and two commercial CO_2 extracts on the shelf life of broiler meat. The commercial antimicrobial blend extract and the oregano extract inhibited the growth of LAB and *B. thermosphacta*. LAB and *B. thermosphacta* are important spoilage bacteria of MA- and vacuum-packaged meat products *(7)*; therefore, efficient methods for prevention of their growth are needed.

The antimicrobial blend extract also had inhibitory effects on psychrotrophic aerobic bacteria in the marinated chicken cut samples, but sea buckthorn and lingonberry extracts did not. Only minor antimicrobial activity against *Enterobacteriaceae* was observed with the antimicrobial blend. Thus, although buckthorn and lingonberry had in vitro antimicrobial activity, in the food matrix studied no effect against gram-negative bacteria was seen. In complex food matrices, factors such as fat content, protein content, water activity, pH, and enzymes can decrease the activity of plant-derived antimicrobial compounds *(3)*. The chemical composition of the matrix in which the antimicrobial agent should be active, e.g., the amount of divalent cations or lipids in food, is an additional factor, because many

TABLE 3. *Identification of bacterial isolates from marinated and unmarinated chicken samples*[a]

VTT code	RDP II nearest group[b]	Similarity score	Length of unique oligos	GenBank identification	RiboPrint database identification	Similarity scores for VTT/DuPont databases	Ribogroup
Marinated samples							
E-153431	*Hafnia paralvei* (T); type strain: ATCC 29927	0.999	758	S001156681	*Hafnia alvei*	0.91/0.90	638-S-8
E-153436	*H. paralvei* (T); type strain: ATCC 29927	0.999	747	S001156681	*H. alvei*	0.90/0.92	1881-S-2
E-153440	*Brochothrix thermosphacta* (T); DSMZ 20171	0.994	738	S000369470	Closest to *B. thermosphacta*	0.67/0.76	1879-S-1
E-153441	*B. thermosphacta* (T); DSMZ 20171; AY543023	0.996	771	S000369470	Closest to *B. thermosphacta*	0.75/0.70	1879-S-2
E-153447	*B. thermosphacta* (T); DSMZ 20171; AY543023	0.997	810	S000369470	Closest to *B. thermosphacta* or *Klebsiella pneumoniae*	0.72/0.72	1879-S-5
E-153419	*Lactobacillus oligofermentans* (T); AMKR18; AY733084	0.997	756	S002967794	Closest to *Lactobacillus sakei*	0.60/0.59	1878-S-1
E-153420	*L. fuchuensis* (T); B5M10875 (=JCM 11249, =DSM 14340); AB370	0.996	757	S000978963	*L. fuchuensis*	0.99/–	1877-S-4
E-153421	*Leuconostoc gelidum* (T); DSM5578; AF175402	0.997	728	S000388588	*L. gelidium*	0.97/–	189-S-8
E-153423	*L. sakei* (T); CCUG 34545; AY204889	0.997	744	S000417782	*L. sakei*	0.83/0.80	1877-S-7
E-153426	*L. oligofermentans* (T); AMKR18; AY733084	0.997	722	S002967794	*L. oligofermentans* or *Lactobacillus* sp.	0.71/0.62	1878-S-3
E-153429	*L. sakei* (T); CCUG 34545; AY204889	0.996	736	S000417782	*L. sakei*	0.76/0.75	1878-S-6
Unmarinated samples							
E-153439	*H. paralvei* (T); type strain: ATCC 29927; FM179943	0.999	764	S001156681	*H. alvei*	0.89/0.88	638-S-8
E-153449	*B. thermosphacta* (T); DSMZ 20171; AY543023	0.982	757	S000369470	*B. thermosphacta* or *Staphylococcus hyicus*	0.74/0.75	1879-S-3
E-153430	*L. fuchuensis* (T); B5M10 (=JCM 11249, =DSM 14340); AB370875	0.997	776	S000978963	*L. fuchuensis* or *L. sakei*	0.98/0.70	1877-S-4
E-153451^T	*L. fuchuensis* (T); DSM 14340						1877-S-4
E-153452^T	*L. oligofermentans* (T); DSM 15707						1880-S-8

[a] Results from isolates belonging to different ribogroups are presented.
[b] RDP, Ribosomal Database Project.

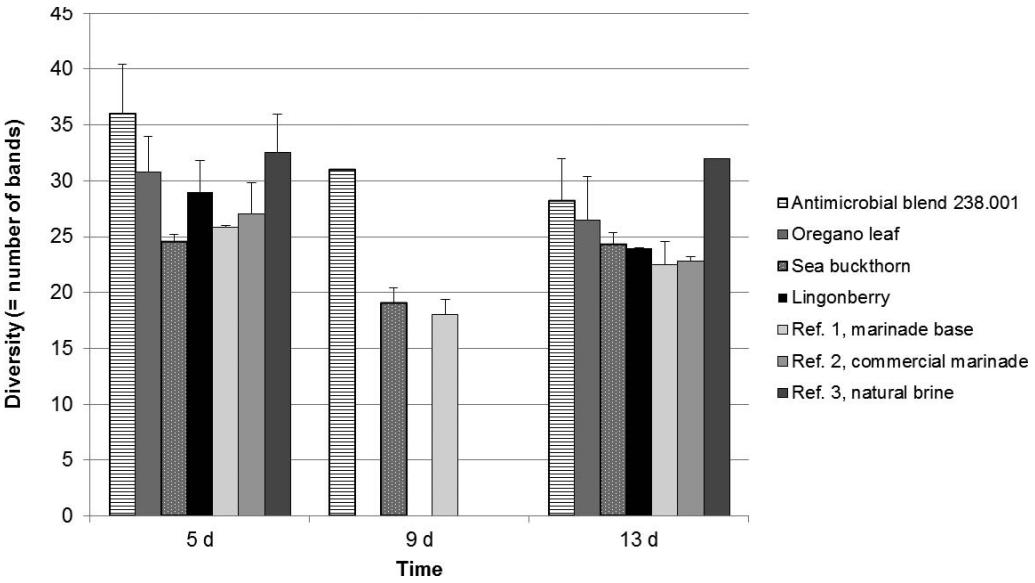

FIGURE 1. *Predominant bacterial diversity as detected with the predominant bacterial DGGE.*

antimicrobial compounds can react with food chemicals and the activities of several antimicrobial agents may be significantly decreased *(28)*. This type of interaction might explain why sea buckthorn and lingonberry did not have antimicrobial effects in the present experiments. Meat and poultry products are regarded as the most difficult food matrices for application of natural antimicrobial agents because of the high concentrations of nonhomogenous substrates, lipids, and proteins and a neutral pH *(5)*. Schirmer and Langsrud *(27)* observed that thymol, cinnamaldehyde, allyl thiocyanate, citric acid, ascorbic acid, a rosemary extract, and a grapefruit seed extract at concentrations up to 10 times the MICs obtained in vitro had no effect on total bacterial levels in vacuum-packed pork meat.

Predominant bacterial diversity as detected with the predominant bacterial DGGE decreased as a function of time in most of the marinated samples, indicating population changes in the microbiota regardless of whether plant extracts were added to the marinade. The levels of the predominant bacteria as detected with the qPCR assay (which cannot differentiate between live and dead cells) were similar regardless of the marinade type. *Lactobacillus* group diversity (*Lactobacillus, Leuconostoc, Pediococcus,* and *Weissella*) detected with group-specific DGGE and *Lactobacillus* group levels detected with the qPCR assay increased in the beginning of the study. Plate count analysis and the qPCR assay gave parallel results for LAB, but the actual LAB levels were higher with the qPCR assay. Higher levels are commonly obtained in qPCR studies mainly because culture-based methods measure only live bacterial cells able to multiply whereas the qPCR assay measures both live and dead cells *(17)*.

During the shelf-life study, *Lactobacillus* and *Leuconostoc* isolates were identified in the marinated samples. In previous studies, marination changed the composition of bacterial communities in broiler fillet strips and favored leuconostocs and lactobacilli over carnobacteria and lactococci *(16)*. Nieminen et al. *(16)* reported that in marinated

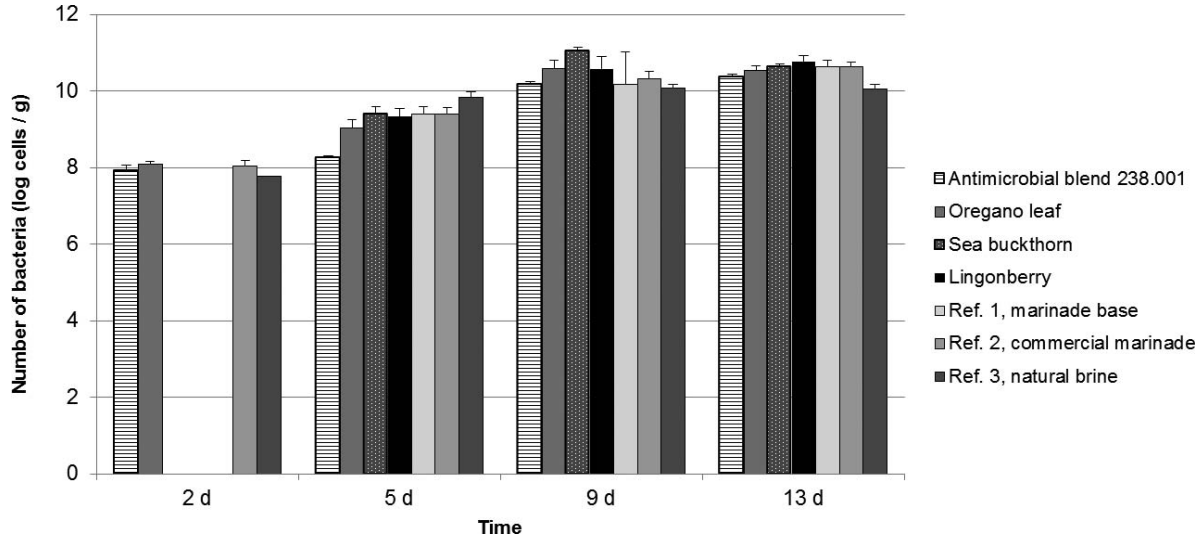

FIGURE 2. *Predominant bacterial levels (per gram of sample) as detected with the predominant bacterial qPCR assay.*

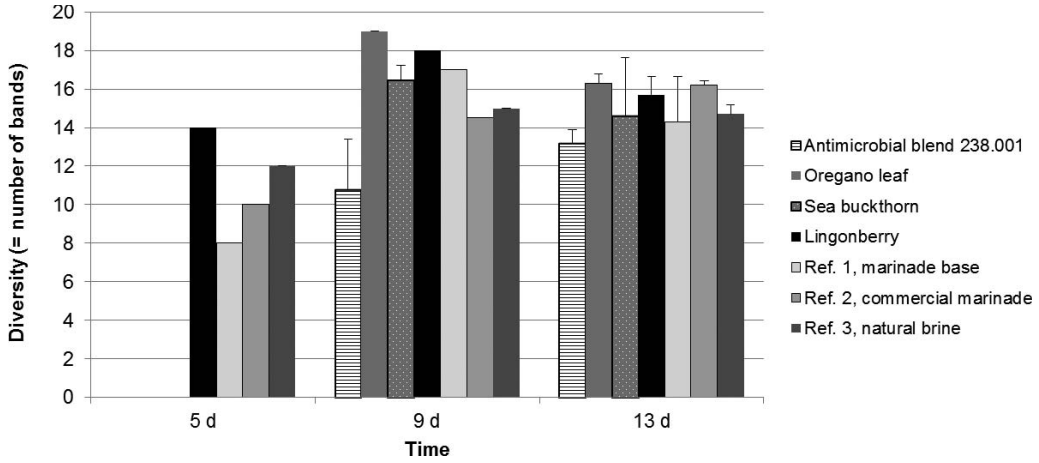

FIGURE 3. Lactobacillus *group (*Lactobacillus, Leuconostoc, Pediococcus, *and* Weissella*) diversity as detected with the* Lactobacillus *group–specific DGGE.*

poultry meat LAB metabolism during storage caused a decrease in pH, and higher headspace CO_2 concentrations were also linked to growth of LAB. In our experiments, higher headspace CO_2 concentrations were found in samples where more LAB growth was detected on day 9. The increase in LAB levels and decrease in pH were correlated ($R^2 = 0.92$, day 9).

The antimicrobial blend extract and the oregano leaf extract appeared useful as antimicrobial agents in the marinade blends used in this study, although tailoring of the concentrations is needed because of the strong taste of these extracts. In the sensory evaluation, the odor and taste of both antimicrobial blend and oregano leaf extracts were characterized as herbal and strong (data not shown). The taste and odor of sea buckthorn and lingonberry samples were reported as mild and acceptable (data not shown). However, sea buckhorn and lingonberry extracts added to the marinade were not effective against spoilage microbes at the concentrations studied. Some researchers have reported synergistic antimicrobial activity for essential oils and plant-derived antimicrobial extracts *(3, 9)*. Radha Krishnan et al. *(21)* reported that in raw chicken meat the highest antimicrobial activity, longest extension of shelf life, and highest degree of inhibition of lipid oxidation were obtained with a spice extract mixture of clove, cinnamon, oregano, and mustard. Tarvainen et al. *(34)* also noted the antioxidant activity of antimicrobial blend and oregano leaf extracts in food matrix trials.

Further studies for exploiting the synergistic effects of compounds or processing techniques could contribute to more effective antimicrobial treatments. Additional research should focus on the performance of plant extracts in food matrices and the interaction of plant extracts with food matrix components. Indigenous microbiota in raw materials, processing technologies, packaging types, and storage conditions also have an effect on the efficacy of antimicrobial treatments.

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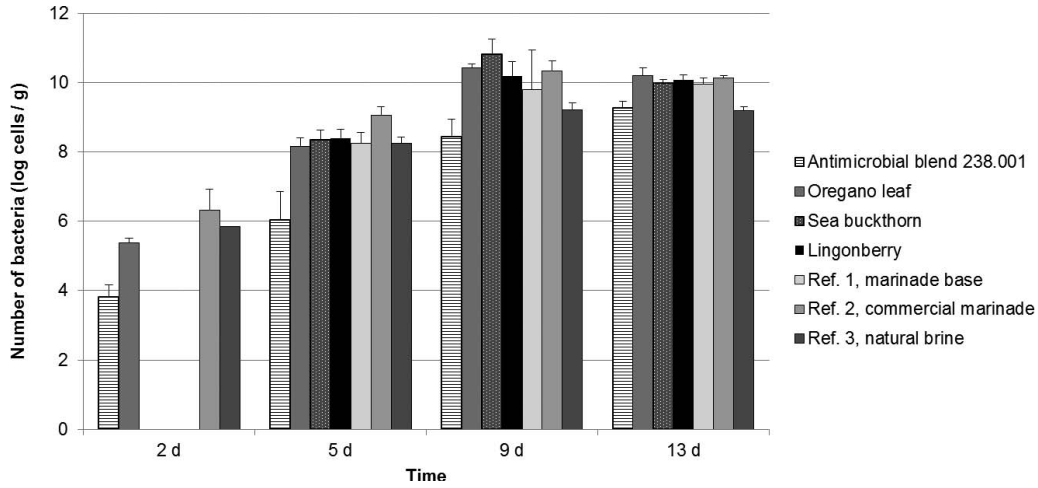

FIGURE 4. Lactobacillus *group (*Lactobacillus, Leuconostoc, Pediococcus, *and* Weissella*) levels as detected with the* Lactobacillus *group–specific qPCR assay.*

REFERENCES

1. Björkroth, J., R. Geisen, U. Schillinger, N. Weiss, P. De Vos, W. Holzapfel, H. Korkeala, and P. Vandamme. 2000. Characterization of *Leuconostoc gasicomitatum* sp. nov. associated with spoiled raw tomato-marinated broiler meat strips packaged under modified-atmosphere conditions. *Appl. Environ. Microbiol.* 66:3764–3772.
2. Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* 94:223–253.
3. Calo, J. R., P. G. Grandall, C. A. O'Bryan, and S. C. Ricke. 2015. Essential oils as antimicrobials in food systems—a review. *Food Control* 54:111–119.
4. Casaburi, A., P. Piombino, G.-J. Nychas, F. Villani, and D. Ercolin. 2015. Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiol.* 45:83–102.
5. Davidson, P. M., F. J. Critzer, and T. M. Taylor. 2013. Naturally occurring antimicrobials for minimally processed foods. *Annu. Rev. Food Sci. Technol.* 4:163–190.
6. Gram, L., L. Ravn, M. Rasch, J. B. Bruhn, A. B. Christensen, and M. Givskov. 2002. Food spoilage—interactions between food spoilage bacteria. *Int. J. Food Microbiol.* 78:79–97.
7. Gribble, A., and G. Brightwell. 2013. Spoilage characteristics of *Brochothrix thermosphacta* and *campestris* in chilled vacuum packaged lamb, and their detection and identification by real time PCR. *Meat Sci.* 94:361–368.
8. Hammond, S. T., J. H. Brown, J. R. Burger, T. P. Flanagan, T. S. Fristoe, N. Mercado-Silva, J. C. Nekola, and J. G. Okie. 2015. Food spoilage, storage, and transport: implications for a sustainable future. *BioScience* 65:758–768.
9. Hyldgaard, M., T. Mygind, and R. L. Meyer. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front. Microbiol.* 3:1–24.
10. Koort, J., A. Murros, T. Coenye, S. Eerola, P. Vandamme, A. Sukura, and J. Björkroth. 2005. *Lactobacillus oligofermentans* sp. nov. associated with spoilage of modified-atmosphere–packaged poultry products. *Appl. Environ. Microbiol.* 71:4400–4406.
11. Maukonen, J., K.-L. Kolho, M. Paasela, J. Honkanen, P. Klemetti, O. Vaarala, and M. Saarela. 2015. Altered fecal microbiota in paediatric inflammatory bowel disease. *J. Crohns Colitis* 9:1088–1095.
12. Maukonen, J., J. Mättö, R. Satokari, H. Söderlund, T. Mattila-Sandholm, and M. Saarela. 2006. PCR DGGE and RT-PCR DGGE show diversity and short term temporal stability in the *Clostridium coccoides–Eubacterium rectale* group in the human intestinal microbiota. *FEMS Microbiol. Ecol.* 58:517–528.
13. Maukonen, J., J. Mättö, M.-L. Suihko, and M. Saarela. 2008. Intraindividual diversity and similarity of salivary and faecal microbiota. *J. Med. Microbiol.* 57:1560–1568.
14. Maukonen, J., C. Simões, and M. Saarela. 2012. The currently used commercial DNA extraction methods give different results of clostridial and actinobacterial populations derived from human fecal samples. *FEMS Microbiol. Ecol.* 79:697–708.
15. Nieminen, T. T., K. Koskinen, P. Laine, J. Hultman, E. Säde, L. Paulin, A. Paloranta, P. Johansson, K. J. Björkroth, and P. Auvinen. 2012. Comparison of microbial communities in marinated and unmarinated broiler meat by metagenomics. *Int. J. Food Microbiol.* 157:142–149.
16. Nieminen, T. T., H. Välitalo, E. Säde, A. Paloranta, K. Koskinen, and K. J. Björkroth. 2012. The effect of marination on lactic acid bacteria communities in raw broiler fillet strips. *Front. Microbiol.* 3:1–8.
17. Nieminen, T. T., E. Vihavainen, A. Paloranta, J. Lehto, L. Paulin, P. Auvinen, M. Solismaa, and K. J. Björkroth. 2011. Characterization of psychrotrophic bacterial communities in modified atmosphere–packed meat with terminal restriction fragment length polymorphism. *Int. J. Food Microbiol.* 144:360–366.
18. Nohynek, L., H.-L. Alakomi, M. P. Kähkönen, M. Heinonen, I. M. Helander, K.-M. Oksman-Caldentey, and R. H. Puupponen-Pimiä. 2006. Berry phenolics—antimicrobial properties and mechanisms of action against severe human pathogens. *Nutr. Cancer* 54:18–32.
19. Nychas, G.-J. E., D. L. Marshall, and J. N. Sofos. 2007. Meat, poultry, and seafood, p. 105–140. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology: fundamentals and frontiers, 3rd ed. ASM Press, Washington, DC.
20. Puupponen-Pimiä, R., L. Nohynek, S. Hartmann-Schmidlin, M. Kähkönen, M. Heinonen, K. Määttä-Riihinen, and K. M. Oksman-Caldentey. 2005. Berry phenolics selectively inhibit growth of intestinal pathogens. *J. Appl. Microbiol.* 98:991–1000.
21. Radha Krishnan, K., S. Babuskin, P. Azhagu Saravana Babu, M. Sasikala, K. Sabina, G. Archana, M. Sivarajan, and M. Sukumar. 2014. Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *Int. J. Food Microbiol.* 171:32–40.
22. Rajamäki, T., H.-L. Alakomi, T. Ritvanen, E. Skyttä, M. Smolander, and R. Ahvenainen. 2006. Application of an electronic nose for quality assessment of modified atmosphere packaged poultry meat. *Food Control* 17:5–13.
23. Remenant, B., E. Jaffres, X. Dousset, M.-F. Pilet, and M. Zagorec. 2015. Bacterial spoilers of food: behavior, fitness and functional properties. *Food Microbiol.* 45(A):45–53.
24. Rossaint, S., S. Klausmann, and J. Kreyenschmidt. 2015. Effect of high-oxygen and oxygen-free modified atmosphere packaging on the spoilage of poultry breast fillets. *Poult. Sci.* 94:96–103.
25. Satokari, R., T. Mattila-Sandholm, and M.-L. Suihko. 2000. Identification of pediococci by ribotyping. *J. Appl. Microbiol.* 88:260–265.
26. Satokari, R. M., E. E. Vaughan, A. D. L. Akkermans, M. Saarela, and W. M. De Vos. 2001. Bifidobacterial diversity in human feces detected by genus-specific PCR and denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 67:504–513.
27. Schirmer, B. C., and S. Langsrud. 2010. Evaluation of natural antimicrobials on typical meat spoilage bacteria in vitro and in vacuum-packed pork meat. *J. Food Sci.* 75:M98–M102.
28. Scotter, M. J., and L. Castle. 2004. Chemical interactions between additives in foodstuffs: a review. *Food Addit. Contam.* 21:93–124.
29. Seow, Y. X., C. R. Yeo, H. L. Chung, and H. G. Yuk. 2014. Plant essential oils as active antimicrobial agents. *Crit. Rev. Food Sci. Nutr.* 54:625–644.
30. Simões, C. D., J. Maukonen, J. Kaprio, A. Rissanen, K. H. Pietiläinen, and M. Saarela. 2013. Habitual dietary intake is associated with the stool microbiota composition of Finnish monozygotic twins. *J. Nutr.* 143:417–423.
31. Smolander, M., H. L. Alakomi, T. Ritvanen, J. Vainionpää, and R. Ahvenainen. 2004. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. A. Time-temperature indicators as quality-indicating tools. *Food Control* 15:217–229.
32. Tajkarimi, M., and S. A. Ibrahim. 2012. Phytochemicals as antimicrobial food preservatives, p. 207–235. *In* A. K. Patra (ed.), Dietary phytochemicals and microbes. Springer, Dordrecht, The Netherlands.
33. Tajkarimi, M. M., S. A. Ibrahim, and D. O. Cliver. 2010. Antimicrobial herb and spice compounds in food. *Food Control* 21:1199–1218. doi:10.1016/j.foodcont.2010.02.003.
34. Tarvainen, M., K.-W. Quirin, H. Kallio, and B. Yang. 2016. CO_2 plant extracts reduce cholesterol oxidation in fish patties during cooking and storage. *J. Agric. Food Chem.* 64:9653–9662.
35. Vainionpää, J., M. Smolander, H.-L. Alakomi, T. Ritvanen, T. Rajamäki, M. Rokka, and R. Ahvenainen. 2004. Comparison of different analytical methods in the monitoring of the quality of modified atmosphere packaged broiler chicken cuts using principal component analysis. *J. Food Eng.* 65:273–280.
36. Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73:5261–5267.