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Comparative evaluation of spoilage-related bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging

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ABSTRACT

Microbial spoilage is a complex event to which different bacterial populations and metabolites can contribute depending on the storage conditions. This study explored the evolution of spoilage and related volatile organic compounds (VOCs) in chilled beef under air and vacuum packaging (VP). The results suggested that different storage conditions affected changes in bacterial communities and metabolites in beef and consequently affected the odor properties of the stored beef, thereby leading to spoilage. Bacterial species belonging to *Pseudomonadaceae* (*Pseudomonas* spp.) and lactic acid bacteria (*Lactobacillus* sp.) dominated the bacterial communities in beef stored under air and VP, respectively, with several VOCs associated with off-odors of the stored beef and most likely produced by both bacteria. Our results suggested several microbial VOCs that could be used as potential spoilage indicators, including acetic acid, butanoic acid, and 2-butanone in VP-stored beef and 3-methylbutan-1-ol, ethyl acetate, acetoin, 2-butanone, and diacetyl in air-stored beef. These findings might provide valuable information regarding the quality monitoring of beef during storage.

1. Introduction

Raw meat is a perishable product and can be considered unacceptable for human consumption when it spoils due to chemical and biological changes that alter its sensorial properties. It is well established that, in addition to lipid oxidation and autolytic enzymatic reactions, meat spoilage is mainly the result of decomposition and the formation of metabolites resulting from microbial growth and enzymatic activity (Casaburi et al., 2015). Therefore, preservation followed by adequate quality evaluation techniques is necessary to ensure meat quality and shelf-life.

Hurdle techniques of refrigeration and vacuum packaging (VP) have been widely applied for the preservation of primal cuts of red meats, such as beef (Chen et al., 2012). Temperature and packaging system represent the most important extrinsic factors that determine the development of spoilage-related microbial communities in stored meats. Spoilage usually occurs when specific spoilage organisms (SSO) grow to unacceptable levels, with the spoilage potential of SSOs depending on their ability to produce metabolites (Doulgeraki et al., 2012). SSOs represent a fraction of microorganisms that are favored by storage conditions (e.g., temperature and atmosphere) and can dominate other microorganisms, reach high populations, and produce several metabolites, thereby leading to the sensory rejection of the product (Wang et al., 2016). Understanding the dynamic changes in spoilage-related microbial communities and metabolites, as well as their impact on sensory quality, is a key factor for the development of a novel technique for meat-spoilage evaluation.

Various conventional evaluation techniques, including microbiological [e.g., total viable counts of aerobic microorganisms (TVC)], chemical [e.g., total volatile basic nitrogen (TVB-N)], pH, and sensorial (e.g., flavor/odor and color) assays have been broadly applied for meat-quality evaluation (Coombs et al., 2017; Wang et al., 2016). The recent development of next-generation sequencing technology in food studies has enabled researchers to study and understand microbial-community

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diversity and dynamics during storage, as well as identify core spoilage microorganisms (Cao et al., 2017). Recently, volatile organic compounds (VOCs) have attracted increasing attention because of their possible impact on meat quality (Casaburi et al., 2015). Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography mass spectrometry (GC-MS) has been employed to determine VOC levels to evaluate beef spoilage (Argyri et al., 2015; Jääskeläinen et al., 2016). A reliable compound for spoilage assessment should show good correlation with microbial growth, sensory score, and remaining shelf-life (Parlapani et al., 2015); therefore, establishing a new comprehensive study regarding relationships between VOC production, SSO growth dynamics, and sensorial and chemical changes is required in order to identify reliable VOCs for potential application as beef-spoilage indicators. Additionally, the use of high-resolution MS, such as time-of-flight MS (TOF/MS), has not previously been employed for VOC determination for the afore-mentioned purpose.

This study investigated the evolution of the spoilage of raw beef cuts stored under air and VP at 4 °C through determination of VOCs using HS-SPME and GC-TOF/MS along with its correlation with sensory (odor) changes, as well as correlations between VOCs and microbial community in order to obtain reliable microbial VOC metabolites potentially useful for evaluating beef spoilage. Conventional techniques, including TVC, TVB-N, and pH analyses, were also employed for quality monitoring. Our findings provide valuable information regarding the potential use of several microbial VOC metabolites to monitor the quality of raw beef cuts during storage.

2. Materials and methods

2.1. Sampling and storage of beef cuts

Fresh beef tenderloin cuts were purchased from a local slaughterhouse (Gyeonggi, Korea), and beef samples were transferred to the laboratory in an ice box within 2 h. In the laboratory, the beef samples used for experimental analyses were stored under air and VP at 4 °C for 11 and 21 days, respectively. For aerobic storage, samples were placed in permeable polyethylene bags (200 × 300 mm). For VP storage, samples were packed and sealed in polyethylene/polyamide bags (200 × 300 mm) with an O_2-transmission rate of 40 cm^3/m^2/day at 85% relative humidity and 23 °C. Each analysis, storage condition, and sampling time was performed using triplicate samples (25 g each).

2.2. Microbiological and chemical analysis

TVC in each 25 g beef sample were enumerated in plate count agar (PCA) according to a previously described method (Mansur et al., 2015), with a slight modification in that the PCA plates were incubated at 32 °C for 2 days. A sample containing a TVC level of ≥7 log CFU/g was considered to have unsatisfactory level of microbial contamination (HPA, 2009).

The pH of beef samples (10 g each) was measured using a digital pH meter (TOA DKK Corp., Tokyo, Japan) after calibration with commercial buffer solutions at pH 7 and 4. Each sample was homogenized with 40 mL distilled water, and the pH was recorded after signal stabilization.

A 10 g sample (from 25 g of beef) was used to determine the TVB-N content according to the Korean Food Code (MFDS, 2015a). A TVB-N value of 20 mg/100 g was considered a threshold to determine the freshness of beef samples (MFDS, 2015b).

2.3. Sensory (odor) analysis

Odor analysis was performed by at least 10 trained panelists. The beef samples (25 g each) were first equilibrated at room temperature for 30 min before analysis. Fresh beef samples within the same lot stored in the freezer (−20 °C) were used as references. The odor of the samples was determined using a five-point scale [1 = severe off-odor (spoiled), 2 = strong off-odor (spoiled), 3 = moderate off-odor (satisfactory), 4 = slight off-odor, and 5 = no off-odor)]. The odor score of fresh samples before storage was determined at 5, and the stored sample was considered spoiled when the median of the scale given was < 3.

2.4. Volatile organic compounds (VOC) analysis

A sample (2 g from 25 g of beef) was weighted and placed into a 20 mL glass vial and sealed with a polytetrafluoroethylene/silicone septum (Supelco; Sigma-Aldrich, St. Louis, MO, USA). The sealed vial containing the sample was heated at 40 °C for 15 min (equilibrium time) and extracted at headspace for 15 min using a carboxen/polydimethylsiloxane (Supelco; Sigma-Aldrich). The fiber was then thermally desorbed into the GS-injection system equipped with a TOF/MS (LECO Corp., St. Joseph, MO, USA). GC-TOF/MS conditions were the same as those described in our previous study (Mansur et al., 2017).

The raw chromatograms were processed using ChromaTOF software (v.4.22; LECO Corp.) and extracted with a signal-to-noise ratio of 20. The peaks were identified tentatively by comparing the obtained mass spectra with those available in the Wiley and NIST databases and using a minimum similarity match of 900. Peak area was calculated using the unique mass of each detected volatile compound. All of the peak information from different chromatograms was aligned using the Statistical Compare (SC) option in the ChromaTOF software in order to obtain an accurate comparison between the volatile profiles present in the beef samples. Results of the SC process were exported as .csv files and used in statistical analyses. The relative amounts of detected VOCs were expressed in arbitrary units using 3,3-dimethyl-2-butanol as the internal standard.

2.5. Microbial-community analysis

DNA in sample (2 g from 25 g of beef) was extracted using a QIAamp DNA stool mini kit (QIAGEN, Hilden, Germany), and concentration and purity were determined using a NanoDrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). The V1-V2 region of 16S rRNA genes was amplified by polymerase chain reaction using a range of universal primers (8F and 338R) with barcode sequences for multiplexing reads of each sample and a recombinant Taq polymerase (Takara, Shiga, Japan). A DNA library was constructed using the Ion Xpress Plus fragment library kit (Thermo Fisher Scientific, Waltham, MA, USA), and sequencing of the libraries was performed on a 318D sequencing chip using the Ion Sequencing 400 kit and Ion Torrent PGM system (Thermo Fisher Scientific). Quantitative insights into the microbial-ecology pipeline were used to analyze the reads (Caporaso et al., 2010). Operational taxonomic units (OTUs) were identified with 97% identity and a minimum cluster size of three using the Greengenes 13_5 reference database (McDonald et al., 2012). Beta diversity was calculated with Bray-Curtis distance and plotted using non-metric multidimensional scaling in R software (v.3.4.3; https://www.r-project.org/). The sequences obtained in this study were uploaded and made available through the DDBJ database under accession number DRA006545.

2.6. Statistical analysis

Spearman's correlation was used to calculate correlation coefficients between VOC data and odor changes, as well as VOC data and amplicon-sequencing data. Given that microbial communities at the beginning of storage were highly diverse, samples from day 0 were excluded from correlation analysis. Statistical tests were conducted using IBM SPSS software (v.20; SPSS Inc., Armonk, NY, USA).

Table 1
Total viable counts of aerobic microorganisms (TVC), pH, total volatile basic nitrogen (TVB-N), and odor changes in beef stored at 4 °C under air and vacuum packaging (VP).

Storage time (days)	TVC (log CFU/g)		pH		TVB-N (mg/100 g)		Odor	
	Air	VP	Air	VP	Air	VP	Air	VP
0	3.74 ± 0.17	3.74 ± 0.17	5.45 ± 0.04	5.45 ± 0.04	5.43 ± 0.22	5.43 ± 0.22	5.0 ± 0.0	5.0 ± 0.0
2	5.20 ± 0.25	3.13 ± 0.25	5.60 ± 0.02	5.56 ± 0.01	14.87 ± 2.61	11.96 ± 0.73	4.2 ± 0.6	4.0 ± 0.9
4	5.96 ± 0.17	3.16 ± 0.13	5.40 ± 0.01	5.51 ± 0.03	17.73 ± 0.73	10.69 ± 0.78	4.4 ± 0.5	4.3 ± 0.6
7	**8.81 ± 0.09**	4.13 ± 0.21	5.50 ± 0.02	5.45 ± 0.01	18.81 ± 3.81	11.55 ± 0.84	3.7 ± 0.5	4.4 ± 0.5
9	8.84 ± 0.04	4.97 ± 0.07	5.62 ± 0.01	5.42 ± 0.01	16.91 ± 1.94	13.50 ± 1.11	3.1 ± 0.8	3.9 ± 0.9
11	9.33 ± 0.26	5.24 ± 0.11	5.73 ± 0.02	5.22 ± 0.01	**30.12 ± 4.94**	13.73 ± 0.57	**2.7 ± 0.7**	3.2 ± 0.4
21	–	5.47 ± 0.19	–	4.94 ± 0.02	–	19.05 ± 0.34	–	**2.3 ± 0.5**

3. Results

3.1. Microbial, chemical, and odor changes

Microbial, pH, TVB-N, and sensory (odor) changes in beef samples stored under air and VP at 4 °C are shown in Table 1. The initial TVC in beef cuts before storage was 3.74 ± 0.17 log CFU/g, with TVC populations gradually increasing to 9.33 ± 0.26 log CFU/g and 5.47 ± 0.19 log CFU/g at the end of storage for beef samples stored under air and VP, respectively. The pH value of fresh beef cuts was 5.45 ± 0.04, with this increasing by 0.3 units to a final pH of 5.73 ± 0.02 under aerobic storage. By contrast, a decrease in pH by ~0.5 units was observed in beef stored under VP, reaching a final value of 4.94 ± 0.02. The initial content of TVB-N in beef samples before storage was 5.43 ± 0.22 mg/100 g, whereas at the end of storage, TVB-N in beef samples stored under air and VP was 30.12 ± 4.94 mg/100 g and 19.05 ± 0.34 mg/100 g, respectively. The TVB-N content in beef samples was considered unacceptable (≥20 mg/100 g) after 9 days of storage under aerobic conditions, whereas those stored in VP were considered acceptable up to 21 days of storage. The sensory (odor) scores of beef cuts stored under air and VP conditions both decreased gradually along with storage time, reaching unacceptable levels (< 3) after 9 and 11 days of storage for beef samples stored under air and VP conditions, respectively.

3.2. VOC changes

The dynamic changes in VOCs produced by beef samples during storage at 4 °C under air or VP and their correlation with sensory (odor) changes (Table 1) are shown in Table 2. The VOCs in air-stored beef samples were generally more diverse as compared with those in VP-stored samples. The amount of VOCs in beef samples gradually increased during aerobic storage, with the highest amounts mostly produced between 9 and 11 days of storage and depending on VOC type. On the other hand, the amount of VOCs in VP-stored beef samples generally remained stable, with the largest changes occurring between 11 and 21 days of storage. Under aerobic storage, 2,3-butanediol, 3-methylbutan-1-ol, 3-methylbutanal, ethyl acetate, ethylidene diacetate, acetoin, 2-butanone, 3,3-dimethylbutan-2-one, 3-octanone, 2-heptanone, 3-hydroxypentan-2-one, diacetyl, and methyl thioacetate were found to be significantly ($p < 0.05$, $p < 0.01$) correlated with sensory (odor) changes. Additionally, acetic acid, butanoic acid, 2,3-butanediol, 2-butanone, 3,3-dimethylbutan-2-one, and methyl thioacetate were found to be significantly ($p < 0.05$, $p < 0.01$) correlated with odor changes in samples stored in VP. All of the significant correlations were negative, except that between methyl thioacetate and sensory (odor) changes in VP-stored samples.

3.3. Overall structural changes in beef bacterial communities

After quality control processing, we obtained a total 30,268 reads, with 27,912 reads assigned to OTUs, and 2356 reads clustered new reference OTUs. The relative abundance (%) of each bacterium at the family level is described in Fig. 1. Under aerobic storage, *Leuconostocaceae* (50.76 ± 4.56%), *Lactobacillaceae* (23.27 ± 4.66%), and *Pseudomonadaceae* (18.10 ± 9.40%) were predominant up to 4 days of storage. As the storage time increased, *Pseudomonadaceae* dominated the bacterial communities, accounting for > 74% of the total reads. After *Pseudomonadaceae*, *Flavobacteriaceae* was found to be predominant at the end of aerobic storage, accounting for ~20% of the total reads. In VP storage, *Lactobacillaceae* (59.11 ± 19.15%) and *Pseudomonadaceae* (25.49 ± 14.01%) were predominant bacteria up to 4 days of storage, with *Lactobacillaceae* dominating the bacterial communities as the storage time increased and accounting for > 80% of the total reads.

3.4. The diversity of bacterial communities in stored beef samples

Hierarchical clustering of the stored beef samples based on the relative abundance of the 10 most abundant OTUs is described in Fig. 2a. The result showed that air-stored beef samples were clustered into two groups, where beef samples stored for 2 and 4 days clustered separately from those sampled after 7–11 days of storage and starting to deteriorate. Similar to air-stored beef samples, the VP-stored beef samples also clustered into two groups, where the spoiled samples (day 21) were clearly separated from the rest of the samples. The bacterial communities at OTU level (Fig. 2b) in air- and VP-stored beef were compared in order to identify dominant bacterial species in each storage condition. The early bacterial communities in beef on days 4 of air storage were dominated by *Leuconostoc* sp. (OTU315506) and *Lactobacillus* sp. (OTU333726), representing > 70% of the total population. In the later aerobic storage phase (days 7–11), *Pseudomonas taetrolens* (OTU338200 and OTU339132) and *Pseudomonas fragi* (OTU295031, OTU930834 and OTU750018) were the dominant bacterial species, accounting for > 65% of the total population. Unlike air-stored beef samples, the bacterial communities in VP-stored beef samples were dominated by *Lactobacillus* sp. (OTU333726) from the early stage of storage (days 2 and 4), accounting for > 25% of the total population. The population of *Lactobacillus* sp. (OTU333726) in VP-stored beef samples increased along with storage time, reaching > 75% of the total population on days 7 through 21 of storage. Substantial increases in the populations of *Lactococcus* sp., *Carnobacterium* sp., and *Enterobacteriaceae* sp. (OTU558003, OTU686789, and OTU580295, respectively) in VP-stored beef were observed after 21 days of storage.

4. Discussion

Dynamic changes in microbial, chemical, and odor properties in beef cuts were greatly affected by the type of storage (Table 1). The growth of aerobic microorganisms (expressed as TVC) in beef stored under aerobic conditions was more accelerated as compared to that in beef stored under VP conditions. This might be due to the pH of the air-

Table 2
Volatile organic compounds (VOCs, a.u.) changes in beef cuts during storage and their correlation with sensory (odor) changes.

VOCs	Unique mass	Air (days)						Correlation with odor change[a]	Vacuum packaging (days)							Correlation with odor change[a]
		0	2	4	7	9	11		0	2	4	7	9	11	21	
Acids																
Acetic acid	60	0.85	0.92	0.81	2.34	3.21	1.89	−.771	0.98	0.92	1.10	1.12	2.16	4.17	16.23	−.760*
Butanoic acid	60	0.14	0.36	0.35	0.54	0.37	0.08	−.086	0.42	0.55	0.66	0.84	1.02	2.74	3.80	−.857*
Alcohols																
2,3-Butanediol	45	nd[b]	nd	nd	2.24	6.62	2.29	−.880*	nd	nd	nd	nd	nd	0.11	0.72	−.802*
2-Butanol	45	nd	0.30	0.38	0.22	0.10	0.59	−.429	0.19	nd	0.22	0.18	nd	0.32	nd	.259
Ethanol	46	5.19	0.96	1.15	6.36	2.42	1.68	−.029	2.14	0.37	0.69	0.65	0.76	1.33	5.66	−.357
3-Methylbutan-1-ol	55	nd	nd	nd	1.66	7.72	13.59	−.941**	nd	nd	nd	nd	nd	nd	0.18	−.612
1-Hexanol	56	0.19	0.04	0.04	3.55	1.70	2.18	−.638	nd	nd	nd	nd	nd	nd	0.16	−.612
7-Octen-4-ol	57	0.04	0.03	0.08	0.87	0.72	1.37	−.771	nd	nd	nd	nd	nd	0.21	nd	—[c]
Aldehydes																
3-Methylbutanal	39	nd	nd	nd	nd	0.53	0.61	−.845*	nd	nd	nd	nd	nd	nd	nd	–
Esters																
Ethyl acetate	43	nd	nd	nd	16.18	75.91	46.50	−.880*	0.35	nd	nd	1.01	nd	0.10	0.49	.111
Ethylidene diacetate	43	nd	nd	nd	1.68	4.65	2.35	−.880*	nd	nd	nd	nd	nd	nd	nd	–
Ketones																
Acetoin	43	9.45	18.36	16.71	116.66	355.19	92.60	−.829*	23.67	32.19	37.99	30.89	32.85	25.80	1.96	.214
2-Butanone	43	2.34	3.23	2.57	4.00	11.29	27.50	−1.000**	2.37	2.58	2.61	2.79	3.51	3.29	6.73	−.821*
3,3-Dimethylbutan-2-one	57	0.23	0.48	0.13	0.69	1.03	1.84	−.943**	0.17	0.22	0.26	0.22	0.25	0.49	0.67	−.865*
3-Octanone	57	nd	nd	nd	0.07	0.02	0.38	−.880*	nd	nd	nd	nd	nd	nd	nd	–
2-Decanone	58	nd	nd	nd	nd	nd	1.38	−.655	nd	nd	nd	nd	nd	nd	0.42	−.612
2-Heptanone	58	nd	nd	nd	0.60	0.61	3.19	−.941**	nd	nd	nd	nd	nd	nd	nd	–
3-Hydroxypentan-2-one	59	nd	nd	nd	0.55	1.47	0.70	−.880*	nd	nd	nd	nd	nd	nd	nd	–
Diacetyl	86	0.15	0.29	0.25	3.45	13.22	0.86	−.829*	0.39	0.37	0.48	0.36	0.31	0.38	1.00	−.179
Sulfur compounds																
Methyl thioacetate	90	nd	nd	nd	nd	0.62	4.09	−.845*	0.05	0.02	0.04	0.03	nd	nd	nd	.927**
Dimethyl disulfide	94	nd	nd	nd	nd	nd	1.84	−.655	nd	nd	nd	nd	nd	nd	nd	–

[a] The values shown are Spearman's correlation coefficients with the significant level of 0.05 (*) and 0.01 (**).
[b] nd, not detected.
[c] —, not determined.

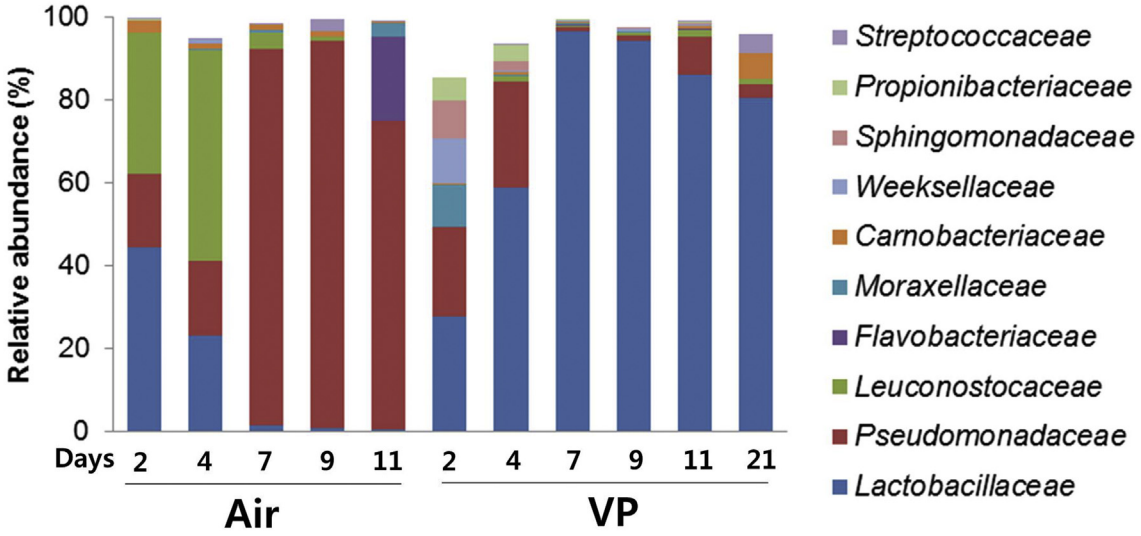

Fig. 1. Overall bacterial-community structure according to a stacked bar chart at the family level in beef stored at 4 °C under air and vacuum packaging (VP). The stacked bar chart shows the top eight bacterial families with an average relative abundance > 1%.

stored beef being higher than that of VP-stored beef. Wang et al. (2017) reported that a high pH in meat favors bacterial growth. As a result of the higher TVCs, the TVB-N content in air-stored beef was likely to be higher than that in VP-stored beef throughout the storage period. TVB-N content includes several microbial metabolites (e.g., amino acids and nucleotide catabolites) that result from degradation of protein and non-protein nitrogenous compounds (Qiao et al., 2017). Moreover, the sensory (odor) quality of air-stored beef was also likely affected by higher populations of TVC. Here, the unsatisfactory values for TVC (≥7 log CFU/g), TVB-N (≥20 mg/100 g) and odor quality (< 3) in air-stored beef were achieved earlier than those in VP-stored beef.

Dynamic changes in bacterial communities (Figs. 1 and 2a, and b) were also greatly affected by the type of storage. The chilling process selects psychrotrophic bacteria in meat, whereas further selection is mostly dependent upon packaging type (Doulgeraki et al., 2012). Two lactic acid bacteria (LAB) belonging to *Leuconostocaceae* (*Leuconostoc gelidum*) and *Lactobacillaceae* (*Lactobacillus algidus*) dominated the bacterial communities until 4 days of aerobic storage (Figs. 1 and 2b);

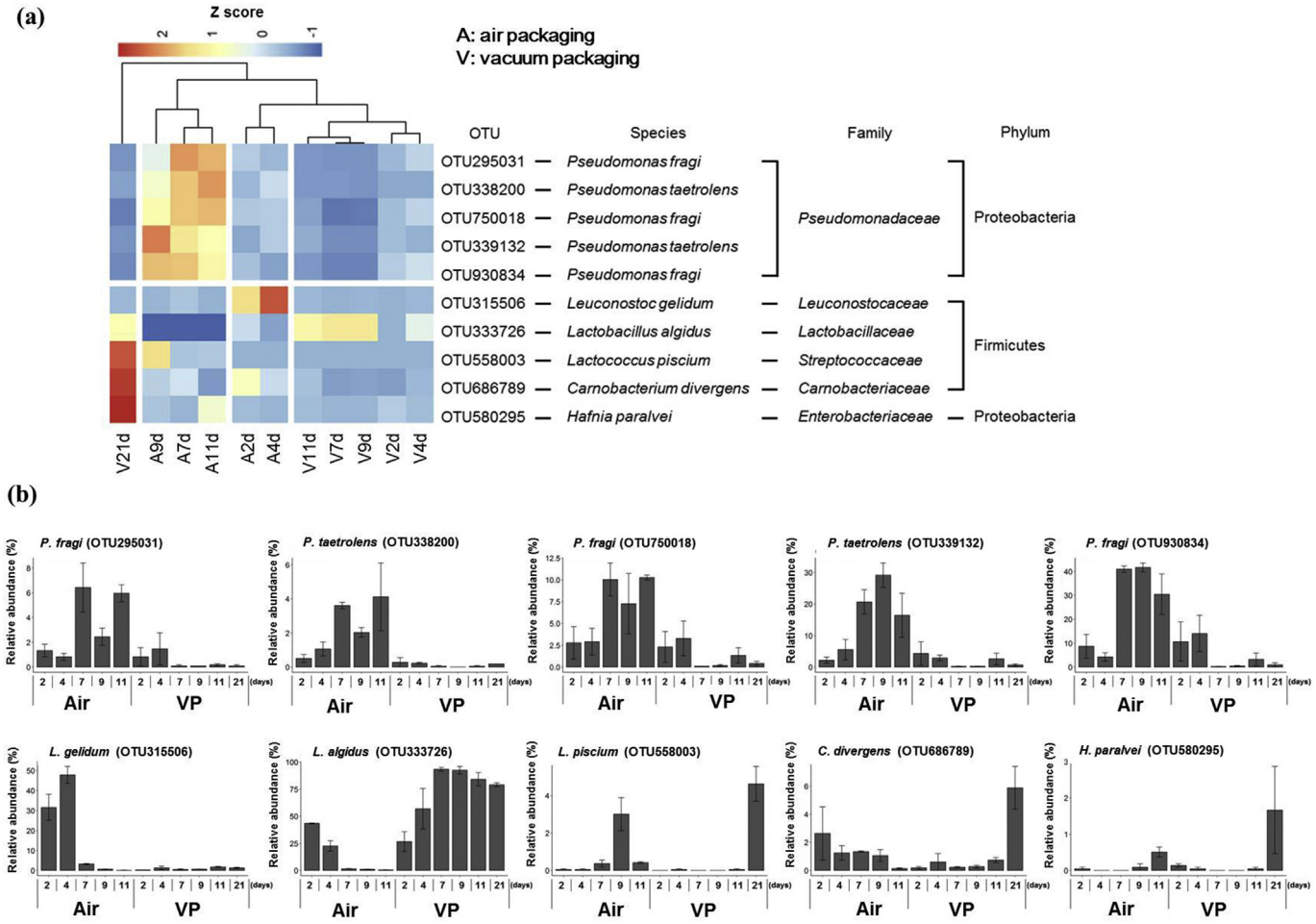

Fig. 2. OTU distribution in beef under air and vacuum packaging (VP) by heatmap according to Z-score (a) and changes in the relative abundance of each OTU according to storage condition (b). The top 10 OTUs with an average relative abundance > 1% are shown. Z-score is calculated as follows: $z = (x - \mu)/\sigma$. z: Z-score; x: individual data point; μ: mean value of each row; σ: standard deviation of each row.

however, these populations decreased over storage time, indicating that both might be involved only in the early stages of beef spoilage. This confirmed the results of a previous study reporting that psychrotrophic *Leuconostoc* spp. and *Lactobacillus* spp. are involved in beef spoilage stored under high oxygen atmosphere (Jääskeläinen et al., 2016). Unlike both bacterial species, the population of *Pseudomonadaceae* (*Pseudomonas taetrolens* and *P. fragi*) increased over storage time and reached over 65% of the total population, particularly after 7–11 days of air storage (Figs. 1 and 2b), during which the off-odor in beef samples started to develop (Table 1). This finding confirmed previous reports, indicating that *Pseudomonas* species dominate the bacterial communities in beef after 7 days of aerobic storage (Ercolini et al., 2011) and play a key role in the spoilage of chilled meat under aerobic storage (Remenant et al., 2015). Additionally, *Pseudomonas* species produce proteolytic and lipolytic enzymes involved in food spoilage (Stanborough et al., 2018). This proteolytic activity causes undesirable changes in meat, such as breaking down protein and producing a variety of odor defects, when *Pseudomonas* spp. reaches high populations (Gram et al., 2002). Three bacterial species belonging to *Pseudomonadaceae,* including *P. taetrolens* (OTU338200) and *P. fragi* (OTU295031 and OTU750018) (Fig. 3), also likely contributed to the higher content of TVB-N in chilled beef under aerobic storage (Table 1). At the end of aerobic storage (day 11), we observed a substantial increase in the population of *Flavobacteriaceae* (Fig. 1), which mainly consisted of a bacterial species named *Myroides odoratimimus* (data not shown). This bacterial species is known as a pathogen causing human infections (Endicott-Yazdani et al., 2015), thereby implying a potential pathogenic risk in long-term chilled beef stored under aerobic condition. *Brochothrix thermospacta* is a dominant spoilage organism that plays a role in shortening the shelf-life of aerobically stored meat (Borch et al., 1996; Russo et al., 2006). In the present study, *B. thermospacta* was not included in the top 10 OTUs, but was observed under aerobic conditions and in the early stage of VP-storage (Supplementary Fig. 1).

Unlike in air-stored beef, *L. algidus* dominated the bacterial communities in VP-stored beef from the early stage of storage (Fig. 2a and b), and this population increased along with storage time, reaching > 75% of the total population from day 7–21 of storage, during which the sensory (odor) properties of the beef started to deteriorate (Table 1). Substantial increases in the population of other LAB genera (*Lactococcus piscium* and *Carnobacterium divergens*) in VP-stored beef were observed after 21 days of storage. Previous studies also reported LAB as dominant in VP-stored beef bacterial communities, mainly comprising *Lactobacillus* spp. (Hernandez-Macedo et al., 2012; Jääskeläinen et al., 2016), whereas *Lactococcus* and *Carnobacterium* are able to grow at low temperatures and frequently predominant in the microbial community of chilled beef (Sakala et al., 2002). LAB can inhibit the growth of spoilage and pathogenic microorganisms by producing antimicrobial substances, including organic acids and bacteriocins (Jalilsood et al., 2015). The lower population of TVC in VP-stored beef (Table 1) as compared with that in air-stored beef might be due to the inhibition of microbial growth by LAB, mainly *Lactobacillus*. We also observed a substantial increase in the population of *Enterobacteriaceae* species (*Hafnia paralvei*) in spoiled VP-stored beef at the end of storage. *Enterobacteriaceae* are

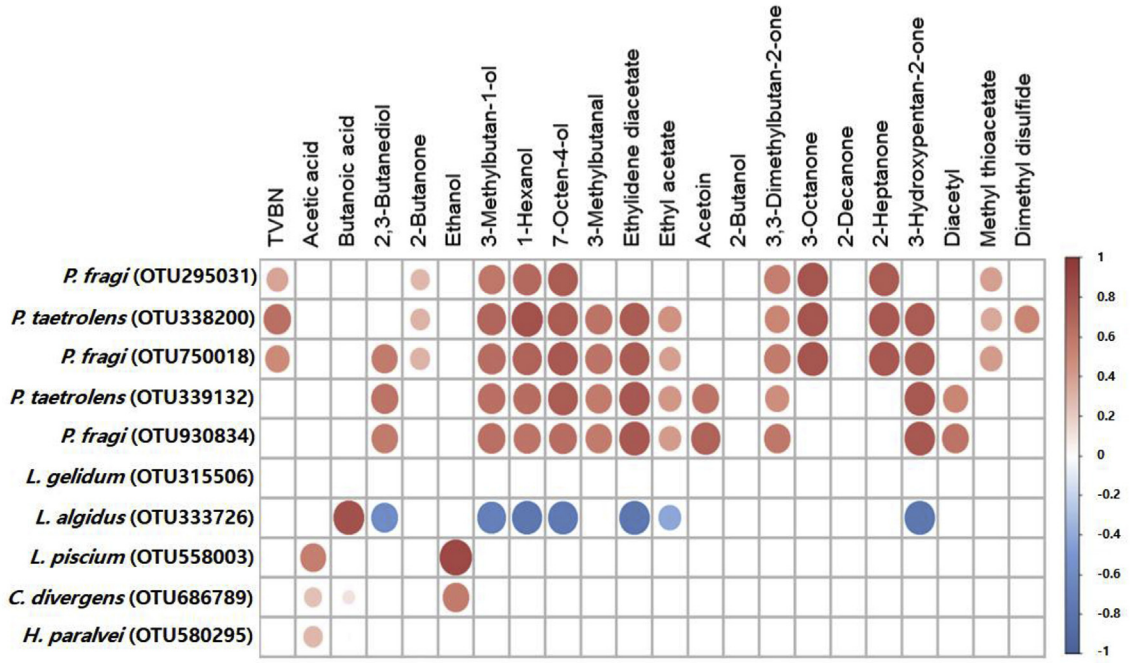

Fig. 3. Heatmap of Spearman metabolite-microbiota correlation coefficients in beef cuts. Colors represent the correlation coefficients, with red indicating a positive correlation and blue indicating a negative correlation. Statistically significant correlations ($p < 0.05$) are indicated with colors and a blank suggesting no significance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Gram-negative bacteria that encompass psychrotrophic species associated with chilled meat spoilage (Nychas et al., 2008). It is surprising that some other families, such as *Moraxellaceae*, *Weeksellaceae*, and *Sphingomonadaceae*, are uncommon in beef meat and these emerged after 2days of storage at 4 °C under VP as a replacement for *Leuconostocaceae* under air (Fig. 1). We speculate that there are likely few studies on microbiota associated with beef meat in Korea likely due to regional differences.

Recently, VOC composition has been extensively studied in order to understand the evolution of microbial spoilage of chilled beef; however, its potential impact on sensory quality has not been well explored (Casaburi et al., 2015; Wang et al., 2016). A suitable compound for microbial-spoilage assessment should represent a microbial metabolite produced by core spoilage microorganisms such as *Pseudomonas* and H_2S-producing bacteria, which are initially absent or at low levels, increase during storage, and show good correlations with sensory score and remaining shelf-life (Jay, 1986). Accordingly, several VOCs, including acids, alcohols, aldehydes, esters, ketones, and sulfur compounds, were identified and semi-quantitatively examined. The majority of identified VOCs (Table 2) were previously reported and associated with beef spoilage (Argyri et al., 2015; Casaburi et al., 2015; Jääskeläinen et al., 2016; Wang et al., 2016), except 7-octen-4-ol, ethylidene diacetate, 3,3-dimethylbutan-2-one, and 3-hydroxypentan-2-one. Levels of VOCs in beef samples gradually increased during storage, with the largest changes occurring between 9 and 11 days of aerobic storage and between 11 and 21 days of VP storage, at which points the sensory (odor) quality of the beef samples started to deteriorate (Table 1). Major VOCs represented by ketones (acetoin, 2-butanone, and diacetyl), alcohols (3-methylbutan-1-ol and 2,3-butanediol), and esters (ethyl acetate) were likely to contribute to the development of off-odor in beef samples under aerobic storage. Among the ketones, acetoin and diacetyl produce buttery, creamy, and cheesy odors, whereas 2-butanone produces an acetone-like ethereal, fruity odor. Among the alcohols, 3-methylbutan-1-ol produces a fermented, fusel, alcoholic, pungent, and ethereal odor, whereas 2,3-butanediol produces a creamy, buttery, and fruity odor. Ethyl acetate, as a major VOC from the ester group, produces an ethereal, fruity odor (Casaburi et al., 2015). Minor VOCs encompassing aldehyde (3-methylbutanal), ester (ethylidene diacetate), ketone (3,3-dimethylbutan-2-one, 3-octanone, 2-heptanone, and 3-hydroxypentan-2-one), and sulfur compounds (methyl thioacetate) might also contribute to negative correlations with sensory (odor) changes in air-stored beef (Table 2). The development of off-odors in VP-stored beef on days 21 (Table 1) might be due to the release of two acids (acetic and butanoic acid) and 2-butanone as major VOCs, followed by 2,3-butanediol and 3,3-dimethylbutan-2-one as minor VOCs. Acetic acid produces a pungent, acidic, cheesy, and vinegar odor (Hernandez-Macedo et al., 2012), whereas butanoic acid produces a sharp acidic, cheesy, buttery, and fruity odor (Ercolini et al., 2011).

Correlation analysis between the top 10 OTUs and VOCs (Fig. 3) showed multiple significant correlations between beef microbiota and VOC production. The dominant OTUs in air-stored beef and belonging to *Pseudomonadaceae* (Fig. 2a and b) were significantly ($p < 0.05$) positively correlated with all VOCs that might significantly contribute to the development of off-odors in air-stored beef (Table 2). *Pseudomonas* is the major spoilage bacteria that produce different types of VOCs in meat (Casaburi et al., 2015). For example, *P. fragi and P. taetrolens* are associated with the production of acetoin, 2-butanone, diacetyl, 3-methylbutan-1-ol, 2,3-butanediol, and ethyl acetate in chilled meat stored under aerobic conditions (Argyri et al., 2015; Ercolini et al., 2010; La Storia et al., 2012). In the present study, we found that LAB (*L. algidus*, *L. piscium*, and *C. divergens*) and *Enterobacteriaceae* sp. were significantly ($p < 0.05$) positively correlated with acetic and/or butanoic acid, with both acidic compounds likely to produce significant off-odors in VP-stored beef (Table 2). This agreed with previous studies reporting that LAB produce acetic and butanoic acid in VP-stored meat (Hernandez-Macedo et al., 2012; Jääskeläinen et al., 2016). Moreover, *Lactococcus* sp. (OTU558003), *Carnobacterium* sp. (OTU686789), and *Enterobacteriaceae* sp. (OTU580295) were likely involved in spoilage and VOC production such as acetic acid and ethanol in VP-stored beef after 21 days based on the significant increases in their population (Fig. 2a and b). Additionally, we observed a negative correlation between *L. algidus* (OTU333726) and VOC production, particularly alcohols and esters (Fig. 3) suggesting that in addition to its important role

in meat spoilage, *Lactobacillus* OTU333726 is considered as protective OTU and might inhibit spoilage-bacterial growth, thereby reducing the production of several spoilage-related VOCs. However, *L. gelidum* (OTU315506) was not associated with VOC production.

5. Conclusions

Here, we reported that storage conditions affected dynamic changes in bacterial communities and metabolites in beef. These changes consequently affected sensory (odor) properties, thereby promoting spoilage of the beef. Bacterial species belonging to *Pseudomonadaceae* dominated the bacterial communities in air-stored beef, whereas LAB, mainly *Lactobacillus* sp., dominated those in VP-stored beef. *P. fragi and P. taetrolens* are core spoilage microorganisms that might contribute to the production of the most promising compounds, which are significantly associated with odor changes, as spoilage indicators, including 3-methylbutan-1-ol, ethyl acetate, acetoin, 2-butanone and diacetyl in air-stored beef. In VP-stored beef, *L. algidus*, *L. piscium*, *C. divergens* and *H. paralvei* were significantly positively correlated with acetic and/or butanoic acid and both acidic compounds likely to produce significant off-odors. Our results indicated that several microbial VOC metabolites could be used as potential spoilage indicators. Quantitative analysis, however, is needed in order to determine the threshold values for each VOC used as a spoilage indicator. Moreover, extensive and large-scale experiments are also required to validate this approach and using beef from different breeds and contaminated with different spoilage strains.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fm.2018.09.006.

References

Argyri, A.A., Mallouchos, A., Panagou, E.Z., Nychas, G.J.E., 2015. The dynamics of the HS/SPME–GC/MS as a tool to assess the spoilage of minced beef stored under different packaging and temperature conditions. Int. J. Food Microbiol. 193 (16), 51–58.

Borch, E., Kant-Muermans, M.-L., Blixt, Y., 1996. Bacterial spoilage of meat and cured meat products. Int. J. Food Microbiol. 33, 103–120.

Cao, Y., Fanning, S., Proos, S., Jordan, K., Srikumar, S., 2017. A review on the applications of next generation sequencing technologies as applied to food-related microbiome studies. Front. Microbiol. 8, 1829.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336.

Casaburi, A., Piombino, P., Nychas, G.J., Villani, F., Ercolini, D., 2015. Bacterial populations and the volatilome associated to meat spoilage. Food Microbiol. 45, 83–102.

Chen, J.H., Ren, Y., Seow, J., Liu, T., Bang, W.S., Yuk, H.G., 2012. Intervention technologies for ensuring microbiological safety of meat: current and future trends. Compr. Rev. Food Sci. Food Saf. 11, 119–132.

Coombs, C.E., Holman, B.W., Friend, M.A., Hopkins, D.L., 2017. Long-term red meat preservation using chilled and frozen storage combinations: a review. Meat Sci. 125, 84–94.

Doulgeraki, A.I., Ercolini, D., Villani, F., Nychas, G.J.E., 2012. Spoilage microbiota associated to the storage of raw meat in different conditions. Int. J. Food Microbiol. 157, 130–141.

Endicott-Yazdani, T.R., Dhiman, N., Benavides, R., Spak, C.W., 2015. Myroides odoratimimus bacteremia in a diabetic patient. SAVE Proc. 28 (8), 342–343.

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 (1–2), 120–131.

Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., La Storia, A., Laghi, L., Mauriello, G., Guerzoni, M.E., Villani, F., 2011. Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. Appl. Environ. Microbiol. 77 (20), 7372–7381.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage—interactions between food spoilage bacteria. Int. J. Food Microbiol. 78 (1–2), 79–97.

Health Protection Agency (HPA), 2009. Guidelines for Assessing the Microbiological Safety of Ready-to-eat Foods. http://webarchive.nationalarchives.gov.uk/20110930033343/http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1259151921557, Accessed date: 23 July 2018.

Hernandez-Macedo, M.L., Contreras-Castillo, C.J., Tsai, S.M., Da Cruz, S.H., Sarantopoulos, C.I., Padula, M., Dias, C.T., 2012. Gases and volatile compounds associated with micro-organisms in blown pack spoilage of Brazilian vacuum-packed beef. Lett. Appl. Microbiol. 55 (6), 467–475.

Jääskeläinen, E., Hultman, J., Parshintsev, J., Riekkola, M.L., Björkroth, J., 2016. Development of spoilage bacterial community and volatile compounds in chilled beef under vacuum or high oxygen atmospheres. Int. J. Food Microbiol. 223, 25–32.

Jalilsood, T., Baradaran, A., Song, A.A., Foo, H.L., Mustafa, S., Saad, W.Z., Yusoff, K., Rahim, R.A., 2015. Inhibition of pathogenic and spoilage bacteria by a novel biofilm-forming Lactobacillus isolate: a potential host for the expression of heterologous proteins. Microb. Cell Factories 14, 96.

Jay, J.M., 1986. Microbial spoilage indicators and metabolites. In: Pierson, M.D., Sterm, N.J. (Eds.), Food-borne Microorganisms and Their Toxins: Developing Methodology. Marcel Dekker, Inc., New York, N.Y, pp. 219–240.

La Storia, A., Ferrocino, I., Torrieri, E., Di Monaco, R., Mauriello, G., Villani, F., Ercolini, D., 2012. A combination of modified atmosphere and antimicrobial packaging to extend the shelf-life of beefsteaks stored at chill temperature. Int. J. Food Microbiol. 158 (3), 186–194.

Mansur, A.R., Tango, C.N., Kim, G.H., Oh, D.H., 2015. Combined effects of slightly acidic electrolyzed water and fumaric acid on the reduction of foodborne pathogens and shelf life extension of fresh pork. Food Contr. 47, 277–284.

Mansur, A.R., Nam, T.G., Jang, H.W., Cho, Y.S., Yoo, M., Seo, D., Ha, J., 2017. Determination of 2-propenal using headspace solid-phase microextraction coupled to gas chromatography–time-of-flight mass spectrometry as a marker for authentication of unrefined sesame oil. J. Chem. 2017, 9106409.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618.

Ministry of Food and Drug Safety (MFDS), 2015a. Food Code (No.2015-4_20150203, Standards and Specifications for Each Food Product). http://www.mfds.go.kr/eng/brd/m_15/list.do?page=3&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_seq_2=0&multi_itm_seq=0&company_cd=&company_nm=, Accessed date: 25 July 2018.

Ministry of Food and Drug Safety (MFDS), 2015b. Processing Standards and Ingredient Specifications for Livestock Products (MFDS Notice No. 2015–94, 12/16/2015). http://www.mfds.go.kr/eng/brd/m_15/list.do?page=2&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_seq_2=0&multi_itm_seq=0&company_cd=&company_nm=, Accessed date: 25 July 2018.

Nychas, G.J.E., Skandamis, P.N., Tassou, C.C., Koutsoumanis, K.P., 2008. Meat spoilage during distribution. Meat Sci. 78 (1–2), 77–89.

Parlapani, F.F., Haroutounian, S.A., Nychas, G.J.E., Boziaris, I.S., 2015. Microbiological spoilage and volatiles production of gutted European sea bass stored under air and commercial modified atmosphere package at 2 °C. Food Microbiol. 50, 44–53.

Qiao, L., Tang, X., Dong, J., 2017. A feasibility quantification study of total volatile basic nitrogen (TVB-N) content in duck meat for freshness evaluation. Food Chem. 237, 1179–1185.

Remenant, B., Jaffrès, E., Dousset, X., Pilet, M.F., Zagorec, M., 2015. Bacterial spoilers of food: behavior, fitness and functional properties. Food Microbiol. 45, 45–53.

Russo, F., Ercolini, D., Mauriello, G., Villani, F., 2006. Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. Food Microbiol. 23, 797–802.

Sakala, R.M., Hayashidani, H., Kato, Y., Hirata, T., Makino, Y., Fukushima, A., Yamada, T., Kaneuchi, C., Ogawa, M., 2002. Change in the composition of the microflora on vacuum-packaged beef during chiller storage. Int. J. Food Microbiol. 74 (1–2), 87–99.

Stanborough, T., Fegan, N., Powell, S.M., Singh, T., Tamplin, M., Chandry, P.S., 2018. Genomic and metabolic characterization of spoilage-associated Pseudomonas species. Int. J. Food Microbiol. 268, 61–72.

Wang, Y., Li, Y., Yang, J., Ruan, J., Sun, C., 2016. Microbial volatile organic compounds and their application in microorganism identification in foodstuff. Trends Anal. Chem. 78, 1–16.

Wang, G.Y., Wang, H.H., Han, Y.W., Xing, T., Ye, K.P., Xu, X.L., Zhou, G.H., 2017. Evaluation of the spoilage potential of bacteria isolated from chilled chicken *in vitro* and *in situ*. Food Microbiol. 63, 139–146.