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CO_2 packaging increases shelf life through reduction of off-odor production by CO_2 tolerant bacteria in addition to growth inhibition of the spoilage bacteriota

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ABSTRACT

Optimized packaging conditions to improve the shelf life of chicken fillets is important to prevent food spoilage and food waste. Anaerobic packaging with CO_2 or by vacuum packaging is commonly used to increase the shelf life of skinless chicken fillets, but the literature is inconsistent about the spoilage bacteriota. The aim of this work was to determine which bacterial genera that spoil raw chicken fillets packaged under two common packaging conditions and how the packaging gas itself affects the production of off odors for these genera.

The spoilage potential of *Pseudomonas*, *Carnobacterium*, *Hafnia*, *Serratia*, *Brochothrix* and *Shewanella* isolated from spoiled chicken fillets was evaluated. Fresh chicken fillets were inoculated with mono- and multi genera strain cocktails (4 log CFU/cm^2) and packaged with 100% N_2 or 60% CO_2/40% N_2, stored at 4 °C, and bacterial numbers, bacteriota, gas in headspace and sensory profiles assessed. Additionally, the effect of CO_2 on the production of off-odors from fillets inoculated with similar levels of *Shewanella* spp. or *Brochothrix* spp. was determined by both sensory profiling and measuring volatile organic components.

All bacterial cocktails grew relatively well in chicken meat packed with 100% N_2, while 60% CO_2/40% N_2 resulted in growth inhibition of all isolates compared to 100% N_2. All genera except *Serratia* and *Pseudomonas* gave rise to off-odors after 11 days of storage in 100% N_2. During storage in 60% CO_2/40% N_2, only fillets with *Carnobacterium* spp. and *Brochothrix* spp. showed significantly higher intensities of off-odors compared to the reference fillets. *Shewanella* spp. and *Brochothrix* spp. also exhibited significantly higher intensities of negative odor attributes during storage in 100% N_2 compared to 60% CO_2/40% N_2, at a similar total bacterial count. Thus, CO_2 improves shelf life not only by reduction of the growth of CO_2 tolerant and sensitive bacteria, but also through inhibition of the production of off-odors.

1. Introduction

It is estimated that prolonged time of shelf life prevents and leads to a reduction in food waste especially for products with short shelf life (Lee et al., 2015). In that regard, improved knowledge of the bacterial quality of perishable products like chicken fillets is a prerequisite to ensure prolonged shelf life and prevent food waste for such products.

Molecular techniques to identify and monitor the bacteriota developing in raw meat have expanded in use during the last few years. Nevertheless, it cannot change the knowledge of the spoilage-associated bacteriota beyond giving an ecological identification (Doulgeraki et al., 2012). Still, some studies suffer from using methodologies that fail to identify the dominating bacteriota (Balamatsia et al., 2006; Herbert et al., 2015; Rossaint et al., 2015; Rotabakk et al., 2006), and it is difficult to conclude about which organisms that caused the observed spoilage. Most studies of bacteriota on chicken fillets define the spoilage bacteria based on the dominant strains at the end of shelf life without any sensory evaluation involved (Höll et al., 2016; Saenz-Garcia et al., 2020).

Overall, several studies on chicken bacteriota have used culture-dependent methods with both non-selective agars and selective agars, and it cannot be ruled out that the true spoilage bacteriota is not detected (Rouger et al., 2017). Interestingly, Höll et al. (2019) found a completely different dominating bacteriota on chicken stored with MAP comparing culture-dependent and independent methods (metatranscriptomics). The scientific literature is inconsistent about main spoilage

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organisms in raw poultry stored under vacuum or with CO_2 and a wide spectrum of genera/species has been identified as the important spoilage organisms, often in combination, including *Aeromonas* spp., *Brochothrix thermophacta, Carnobacterium* spp., *Lactobacillus* spp., *Lactococcus* spp., *Pseudomonas* spp., *Shewanella* spp., *Serratia* spp., *and Yersinia* spp. (Bailey et al., 1979; Bjorkroth et al., 2005; Chouliara et al., 2008; Holck et al., 2014; Höll et al., 2016; Wang et al., 2017). Using the former approach led to results pointing at *Brochothrix* and *Carnobacterium* as dominating bacteriota. While using meta transcriptomic sequencing, four out of six samples were dominated by *Photobacterium* spp. and it was predicted that growth would lead to the production of compounds overlapping with those of known potent meat spoilers. Another obvious reason for divergent results between investigations is differences in the initial bacteriota, packaging technologies and storage temperatures used.

The aim of the present study was to determine which bacterial genera that spoil raw chicken packaged under two common packaging conditions and how the packaging gas itself affects the production of off odors for these genera. The growth and spoilage potential of selected bacteria from chicken breast fillets, inoculated on fillets and packaged with restricted oxygen gas mixtures (100% N_2 or 60% CO_2/40% N_2) and stored at 4 °C were investigated. Furthermore, it was investigated how different anaerobic packaging atmospheres affected the odor profile and development of volatile metabolites during the storage of selected bacterial strains at different stages during growth.

2. Materials and methods

2.1. Collection and isolation of strains

Skinless chicken breast fillets delivered from two different processing plants in Norway were packaged into anaerobe and aerobe packages on the day of slaughter (vacuum packaging and packaged in trays with needle holes in the top film, respectively). In addition, consumer packaged samples from the same processing plants were bought in retail stores (modified atmosphere packaging with CO_2/N_2 and skin packaged).

Samples for bacterial plate count (total viable count) were taken after storage at 4 °C for six days (samples packaged at Nofima) and at the use-by date (consumer packages), respectively. Samples of 3 × 3 cm and 1 cm depth of the skinless fillets were homogenized in a stomacher for 60 s, 10-fold dilutions were made and spread on Plate count agar plates (PCA, Merck, Darmstadt, Germany) and incubated at 15 °C for 5–6 days. Up to 10 colonies were picked from a randomly chosen sector of the plates for sequencing. A selection of strains shown in Table 1 was stored in a freezer.

2.1.1. Identification of bacterial isolates

To determine the bacterial composition at the genus level, partial amplification and sequencing of the 16S rRNA gene were performed using universal primers (Nadkarni et al., 2002). Deoxyribonucleic acid (DNA) was isolated from the colonies by resuspending single colonies in 50 μl Tris-EDTA (TE) buffer and lysing by incubation at 99 °C for 10 min. Bacterial debris was removed by centrifugation, and 30 μl of the supernatant containing the DNA was transferred to a new tube, of which 1 μl was used as template in the polymerase chain reaction (PCR). Amplification and sequencing were performed as previously described (Hansen et al., 2021). The taxonomy was identified using the RDP (Ribosomal Database Project v 11) SeqMatch http://rdp.cme.msu.edu/, accessed on June 13th and November 4th of 2016. The thresholds used in the RDP search were as follows: both type and non-type strains, both uncultured and isolates, only good sequences >1200 bases and KNN = 1 (only the best match is displayed per sequence). The phylogenetic relationship between isolates was used to select different isolates per genera to get the best possible representation within a genus.

Isolates selected for further study were also identified by MALDI-TOF MS (matrix-assisted laser desorption/ionization - time of flight mass spectrometry). The common direct transfer protocol was followed to obtain mass spectra. Briefly, ~0.1 mg of cell material was directly transferred from a bacterial colony to a target plate and overlaid with 1 μL of matrix solution (10 mg/mL a-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). MS analysis was performed on an Autoflex MALDITOF mass spectrometer (Bruker Daltonics, Germany) using MBT Compass 4.1 and FlexControl 3.4 software (Bruker Daltonics, Germany). Calibration was carried out with the Bacterial Test Standard (Bruker Daltonics, Germany). All MS spectra were measured automatically using Flex Control software according to the standard measurement method for microbial identification, MBT-autoX.axe autoExecute. The BioTyper 3.1 software (Bruker Daltonics, Germany) equipped with MBT 9607MSP Library (released November 2020) was used for classification. Score values: <1.7 was interpreted as an unreliable identification; 1.7–2.0 as a probable genus identification; 2.0–2.3 as a secure genus identification and probable species identification; and >2.3 was regarded as highly probable species identification.

Table 1
Bacterial strains (17) used in growth screening on chicken fillets packaged with either 100% N_2 or 60% CO_2, 40% N_2 (Challenge study, and Effect of packaging gas on sensory and volatile profiles of Brochothrix spp. and Shewanella spp.). The bacterial strains were selected from the fillets of Processing plant 1 and 2, or retail store samples delivered from the same processing plants. One strain was chosen from former industrial study at Plant 1(*) and two strains were chosen from Plant 1 samples in a pre-storage test (**). Bacterial taxonomy is assigned by partial 16S rRNA gene sequencing (16S) and RDP SeqMatch tool (KNN = 1), verified by use of MALDI-TOF MS (MALDI).

Bacterial mix	Bacterial taxonomy (16S)	Bacterial taxonomy (MALDI)	Source	Strain number
Carnobacterium	*Carnobacterium* sp.	*Carnobacterium maltaromaticum*	Skin, Plant 2	MF6482
	Carnobacterium divergens	*Carnobacterium divergens*	Skin, Plant 2	MF6483
	Carnobacterium sp.	*Carnobacterium maltaromaticum*	MAP, Plant 1	MF6484
Pseudomonas	*Pseudomonas psychrophila*	*Pseudomonas lundensis*	Vacuum, Plant 2	MF6485
	Pseudomonas syringae	*Pseudomonas lundensis*	Vacuum, Plant 2	MF6486
	Pseudomonas fluorescens	*Pseudomonas* sp.	Vacuum, Plant 1	MF6487
	Pseudomonas psychrophila	*Pseudomonas* sp.	Vacuum, Plant 1	MF6488
Serratia	*Serratia liquefaciens*	*Serratia liquefaciens*	Vacuum, Plant 2	MF6489
	Serratia quinivorans	*Serratia* sp.	Vacuum, Plant 2	
	Serratia fonticola	*Serratia fonticola*	From chicken industry, fillet Day 0*	MF6870
Brochothrix	*Brochothrix thermosphacta*	*Brochothrix thermosphacta*	Skin, Plant 2	MF6491
	Brochothrix thermosphacta	*Brochothrix* sp.	From chicken industry, equipment*	MF4817
	Brochothrix thermosphacta	*Brochothrix thermosphacta*	From chicken industry, fillet Day 0*	MF6860
Hafnia	*Hafnia* sp.	*Hafnia alvei*	Vacuum, Plant 2	MF6492
	Hafnia sp.	*Hafnia alvei*	Skin, Plant 2	MF6493
Shewanella	*Shewanella putrefaciens*	*Shewanella baltica*	Day 0, Plant 1**	MF6858
	Shewanella baltica	*Shewanella baltica*	Day 0, Plant 1 **	MF6859

2.2. Challenge study: Spoilage of chicken inoculated with potential spoilage bacteria and stored under different atmospheres

Seventeen isolates representing six genera collected from the Norwegian chicken were used in a challenge study (Table 1). Pieces of chicken breast meat were inoculated with strain-cocktails of either *Carnobacterium* spp., *Pseudomonas* spp., *Serratia* spp., *Brochothrix* spp., *Hafnia* sp., *Shewanella* spp. or a multi genera cocktail of all strains, packed in 100% N_2 or in 60% CO_2/40% N_2. The bacterial numbers, bacteriota, headspace gas measurement and descriptive sensory profile (odor) were determined after 11 days of 4 °C storage to assess the effect of different bacteria on the spoilage of chicken meat.

An initial screening study (data not shown) where all strain-cocktails were tested for growth under exposure to both packaging gases was performed similarly, with sampling after 7 and 9 days to determine experimental conditions in the main experiment. Based on this initial study, due to impaired growth *Serratia* spp. and *Pseudomonas* spp. were not included in 60% CO_2/40% N_2.

2.2.1. Raw materials

Chicken breast fillets of 220–250 g were purchased directly from a Norwegian commercial supplier of chicken meat on the day of slaughter and stored at Nofima overnight at 1 °C before inoculation. First, the surface of the fillets was briefly heat treated with a Bunsen burner (Flameboy) to reduce the surface contamination. The fillets were longitudinally cut in two with a sterile knife under strict hygienic conditions, resulting in two pieces of chicken meat of 5 × 10 cm^2 with a low bacterial number. The inner surface from the longitudinal cut was used as an inoculating surface. The pieces were wrapped in plastic sheets and stored at 1 °C overnight with ice before inoculation with bacteria. "Negative control" samples were added 500 µl sterile physiological water. Some fillets were not cut, and instead used as reference samples ("Reference").

2.2.2. Preparation and storage of samples

Cultures for application on chicken fillets were prepared by inoculating of one colony in Brain Heart Infusion (BHI, Oxoid) and incubating at 25 °C for 24 h. The cultures were reinoculated in fresh BHI and incubated at 25 °C for 24 h, followed by storage at 4 °C for 24 h to adapt bacteria to low temperatures. Each bacterial culture was diluted to approx. 10^6 CFU/ml in saline (0.9% NaCl). Strain cocktails of each bacterial genus were made by mixing these cultures in equal volumes. A multi genera cocktail of all strains was also prepared.

Volumes of 0.5 ml were applied to each piece of chicken meat (5 × 10 cm^2) on the cut surface and streaked out using a L-shaped spreader (VWR International, Oslo, Norway), resulting in an initial bacterial number of approx. 10^4 CFU/cm^2.

Two fillet pieces of both the inoculated fillets, Negative control and Reference, were packaged per HDPE (high density polyethylene, preformed) tray (1100 ml, RPC Promens, Kristiansand, Norway) with a Biaxer top web (Wipack, Finland), and stored at 4 °C. The oxygen transmission rate (OTR) of empty, sealed trays were measured to be 1.95 cm^3/package/d at 4 °C and 100% humidity, based on AOIR-method (Ambient oxygen ingress rate) described by (Larsen et al., 2000). The gas volume to product volume ratio (g/p ratio) was 5/1. The packaging gas used was a pre-mix of 60% CO_2 and 40% N_2 ("60% CO_2") and 100% N_2 ("100% N_2") (Linde Gas, Oslo, Norway).

2.2.3. Gas analysis

The CO_2 and O_2 in the headspace of the packages were analyzed at each sampling time by a CheckMate 9900 O_2/CO_2 analyser (PBI Dansensor, Ringsted, Denmark). The accuracy of the instrument is 0.01% O_2/CO_2.

2.2.4. Bacterial counts

A piece of 3 × 3 cm^2 and 1 cm depth was cut out with a sterile knife and diluted in approximately 90 mL peptone water until 1/10 dilution was attained. The samples were stomached for 60 s, serially diluted in peptone water. Samples were streaked out on PCA, and the plates were incubated at 25 °C. Numbers of total viable count (TVC) were calculated as log CFU/cm^2.

2.2.5. DNA extraction

Frozen stomacher solution was thawed, and 2 ml of the stomacher solutions were centrifuged at 13000×*g* for 5 min. DNA was extracted from the pellets using the Fast DNA-96 Soil Microbe kit (MP Biomedical) following the manufacturer's MP-96 Inhibitor Removal Plate protocol.

2.2.6. Bacteriota (16S rRNA gene) sequencing and data processing

16S rRNA gene PCR (V4 region) and paired end sequencing (2 × 150 bp) using the MiSeq Reagent Kit v3 on a MiSeq instrument (Illumina) were performed using the protocol presented by (Caporaso et al., 2012) as previously described.

The sequences were processed in QIIME2 (qiime2-2017.12 and qiime2-2019.1) as described in Moretro et al. (2021). The taxonomy- and feature table were exported to text files and further processed in Excel. The feature table was converted to relative values and taxa below an average of 0.1% was represented as "Other". For taxa above the genus level (e.g *Enterobacteriaceae* and *Lactobacilliales*) the representative sequences were compared to the sequences of the inoculated stains and submitted to BLAST nucleotide search (https://blast.ncbi.nlm.nih.gov) to get more information about possible genera. The Greengenes database still refers to *Serratia* and *Hafnia* as *Enterobacteriaceae* although *Serratia* now is under the family *Yersiniaceae* and *Hafnia* under the family *Hafniaceae* (Adeolu et al., 2016; Morales-Lopez et al., 2019). The plots are based on the Greengenes results, but with comments.

2.2.7. Sensory analysis

Samples for sensory analysis were frozen on the day of sampling, Day 11, and thawed overnight at 4 °C at the time of analysis.

To describe the sensory objective perception of the various samples, a highly trained panel of eight assessors (8 women; aged 37–64 years) at Nofima (Ås, Norway) performed a sensory descriptive analysis (DA) according to the "Generic Descriptive Analysis" as described by Lawless and Heymann (2010) and the ISO 13229 (2016). The assessors are regularly tested and trained according to ISO 8586 (2012), and the sensory laboratory follows the practice of ISO 8589 (2007).

For sensory evaluation, ten (eleven) sensory attributes for odor were evaluated: pungent, sweetish, metallic, cloying, fermented/sour, yeast, alcohol, sulfur, ammonia, sourness, and total intensity (only the last part of the study) (Table S1). In a pre-test session, the assessors were calibrated on samples that were considered the most different on the selected attributes typical for stored chicken.

In the main session, each assessor was served one piece of meat (width 2–3 cm, length 3–4 cm, thickness 1–2 cm) per sample. The slices were served at room temperature (19 ± 1 °C) in white plastic beakers covered with a metal lid. Samples were evaluated with the non-inoculated side of the fillet towards the bottom of the beaker.

All attributes were evaluated on an unstructured 15 cm line scale with labelled end points going from "no intensity" (1) to "high intensity" (9). Each assessor evaluated all samples at individual speed on a computer system for direct recording of data (EyeQuestion, Software Logic8 BV, Utrecht, the Netherlands). All samples were served to the panel coded with a three-digit number in duplicates following a randomized block design.

2.3. Effect of packaging gas on sensory and volatile profiles of Brochothrix spp. and Shewanella spp.

A new experiment testing the effect of the packaging gas on sensory and volatile profiles was performed with *Brochothrix* and *Shewanella*. The experiment was performed in the same way as the challenge study

(Chapter 2.2) with a few exceptions. One more sensory attribute was used: total odor intensity (Table S1). Also, an analysis of volatiles was performed. Selected samples (n = 14) of the two strain mixes from two storage timepoints per bacteria mix pr packaging gas and three Negative control samples pr packaging gases were included in the sensory analysis and analysis of volatile components: at the beginning and late of stationary phase; "Br-early", "Br-late", "Sh-early", "Sh-late" (shown in Fig. 3). The cocktails of *Brochothrix* spp. and *Shewanella* spp. were analyzed separately by the sensory panel on two consecutive days, in triplicate, samples following a randomized block design.

Analyses of volatile organic compounds (VOCs) were performed on the same samples as for the sensory analysis using headspace gas chromatography mass spectrometry (HS-C/MS). Samples (3 × 3 cm) were cut from the same fillet as performed for the bacterial analyses (and from the same package as for the sensory analysis) and homogenized. Five grams of homogenized chicken meat was weighed into Erlenmeyer bottles and ethyl heptanoate in methanol was added as an internal standard.

The content of volatiles was analyzed by dynamic headspace/GC-MS as described by Olsen et al. (2005) and Hansen et al. (2007) with small modifications to the method. The peaks were integrated, and compounds were tentatively identified with MSD Chemstation software (E.02.02.1431) and NIST/EPA/NIH Mass Spectral Library (version2.0 g, built Dec 4, 2012). Concentrations of the individual volatiles were calculated as μg/g sample based on an internal standard.

2.4. Calculations

All bacterial numbers were log transformed before calculating mean values and standard error of the mean. The statistical significance of the effect of packaging gases on the growth from inoculation to 11 days of storage was tested using the general linear model in Minitab (Minitab Statistical Software, Version 21.1).

For the sensory performance of study 2.2.7 paired 2 sample t-tests were used to determine if the means between the tested bacterial cocktails and Reference fillets per packaging gas mixtures (60% CO_2 and 40% N_2, and 100% N_2) were significantly different. Also, for study 2.3.4, paired 2 sample t-tests were used to determine if the means between the tested strain cocktails of either *Brochothrix* spp. or *Shewanella* spp, at early or late stationary phase and packaging gas mixtures (60% CO_2 and 40% N_2, and 100% N_2) were significantly different.

Differences in volatile organic compounds between the two packaging gases per group ("Br-early", "Br-late", "Sh-early", "Sh-late") were analyzed using One-way analysis of variance (ANOVA, was performed (Minitab Statistical Software, Version 21.1)) with significance defined at $p < 0.05$.

3. Results

3.1. Isolation of strains

A total of 111 strains were collected from chicken breast fillets delivered from two industrial processing plants and identified by partial 16S rRNA gene sequencing. From Plant 1 *Shewanella* spp. was detected among the initial contaminants, together with a diverse bacteriota consisting of *Pseudomonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Leucobacter* spp., *Chryseobacterium* spp. and *Carnobacterium* spp. (Table S2). After 6 days of aerobic storage (at 4 °C), *Pseudomonas* spp. dominated, but also *Acinetobacter* spp. were detected on fillets from both processing plants, in addition to *Microbacterium* spp. (Plant 1) and *Serratia* spp. (Plant 2).

The fillets packaged anaerobically with vacuum or skin were dominated by *Carnobacterium* spp. and *Pseudomonas* spp. (Plant 1) or by *Pseudomonas* spp., *Hafnia* spp., *Serratia*, spp. *Carnobacterium* spp. and *Brochothrix* (Plant 2). Fillets packaged by modified atmosphere (MAP) (only from Plant 1) were dominated by *Carnobacterium* spp., with a minor part consisting of *Providencia* spp. and *Yersinia* spp.

Initial total viable count (TVC) was 2.9 ± 0.8 log CFU/cm^2 and the TVC for the different stored samples ranged from 5 to 8 log CFU/cm^2, including retail store samples analyzed at the "use by" date (Table S2).

Based on these results and available literature, a selection of 17 strains (representing 6 different genera) was chosen (Table 1) to be used in a challenge study to assess the effect of different bacterial genera on the spoilage of chicken meat.

3.2. Challenge study: Chicken inoculated with potential spoilage bacteria and stored under different anaerobic atmospheres

3.2.1. Growth of different bacterial genera

Six different strain cocktails, each representing different genera (Table 1), were prepared and inoculated on chicken fillets. In addition, one multi-genera cocktail was used.

All inoculated bacterial cocktails grew relatively well in chicken meat packaged with 100% N_2, with 3.0–4.5 log increase in cell numbers from inoculation to 8 days of 4 °C storage (Fig. 1a). *Pseudomonas* spp. and *Shewanella* spp. grew significantly slower than the other bacterial genera ($p < 0.05$) during the first 8 days of storage. After 8 days maximum numbers of bacteria were reached for all fillets, except for the Negative control (inoculated with sterile physiological water) and fillets inoculated with *Shewanella* cocktail. Bacteriota analysis confirmed that the natural background bacteriota did not outcompete the inoculated bacterial genera (Supplementary file S1). Fillets (average of three parallels) inoculated with the multi genera cocktail were dominated by *Enterobacteriaceae* (77%: most likely Serra*tia* spp. (58%) and *Hafnia alvei* (19%)), followed by *Shewanella* spp. (10%) after storage in 100% N_2. (Fig. 2). The Negative control and the Reference (whole fillets) were dominated by *Pseudomonas* spp. and *Shewanella* spp. after 11 days of storage in 100% N_2. For details see Supplementary file S1.

In fillets packaged with 60% CO_2 the growth rates of bacteria were generally lower compared to fillets packaged with 100% N_2 ($p < 0.001$), with 1.0–3.5 log increase in cell numbers from inoculation to 8 days of storage (Fig. 1b). Among the cocktails inoculated, *Serratia* spp. and *Shewanella* spp. grew significantly slower than the other bacterial genera ($p < 0.05$) the 8 first days of storage. While *Serratia* spp. appeared to reach a stationary phase and a TVC of 6 log CFU/cm^2 after 8 days and *Brochothrix* spp. reached a TVC of 7 log CFU/cm^2 after 11 days, *Carnobacterium* spp. reached a TVC of 8 log CFU/cm^2 after 11 days similarly to N_2-packaged fillets. *Shewanella* spp. seemed to grow rapidly from day 8–11 reaching similar numbers as fillets stored under 100% N_2. Bacteriota analysis confirmed that the bacterial genera added to the chicken fillets also dominated after storage. Fillets (average of three parallels) inoculated with the multi genera cocktail were dominated by *Carnobacterium* spp. (28%), *Shewanella* spp. (24%), *Brochothrix* spp. (23%) and *Lactobacilliales* (18%: most likely *Carnobacterium*) after storage in CO_2-containing atmosphere (Fig. 2). The Negative controls (n = 2) were dominated by *Enterobacteriaceae* (59%: most likely *Hafnia* spp. and *Serratia* spp.), followed by *Lactobacilliales* (24%: most likely *Carnobacterium*). The Reference (n = 3) was dominated by *Lactobacilliales* (95%: most likely *Carnobacterium* spp.) (Fig. 2). For details see Supplementary file S1.

3.2.2. Odor description

The odor attributes of chicken meat inoculated with mono genus cocktails or a multi genera cocktail were compared to the Reference after 11 days of storage. The Reference fillets stored under 100% N_2 had a TVC of 6.7 log CFU/g and a mixed bacteriota (Fig. 2). Chicken fillet inoculated with *Hafnia alvei* (TVC 8.5 ± 0.1 log CFU/cm^2) had significantly higher intensities of the negative attributes pungent, cloying, fermented/sour, sulfur, and ammonia (Table 2a). Those inoculated with *Brochothrix* spp. (TVC 7.9 ± 0.1 log CFU/cm^2) showed significantly higher intensity of yeast odor compared to the Reference, and the attributes cloying, fermented/sour and ammonia. Fillets inoculated with

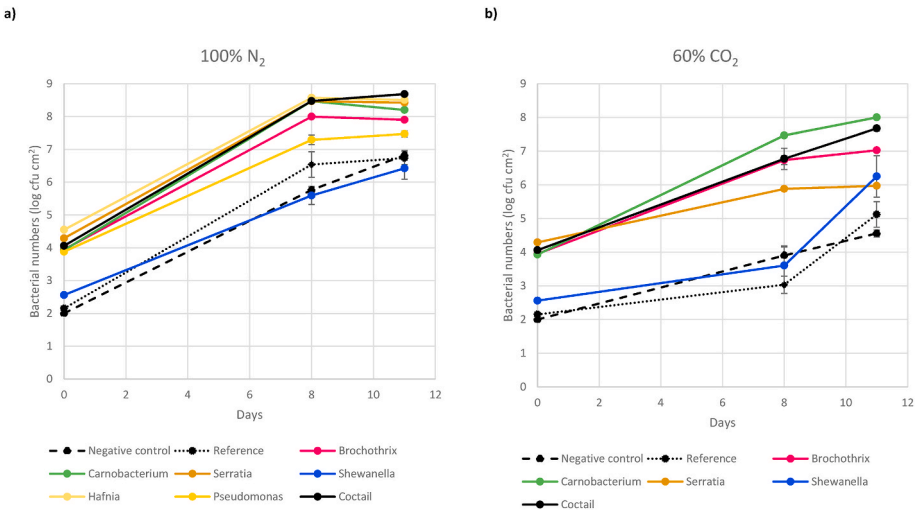

Fig. 1. Changes in total viable count (log CFU/cm^2) for the inoculated samples, the negative control fillets, and the reference fillets during 4 °C storage under a) 100% N_2 or b) 60% CO_2/40% N_2 gas mixtures. Values are mean ± SE (n = 3).

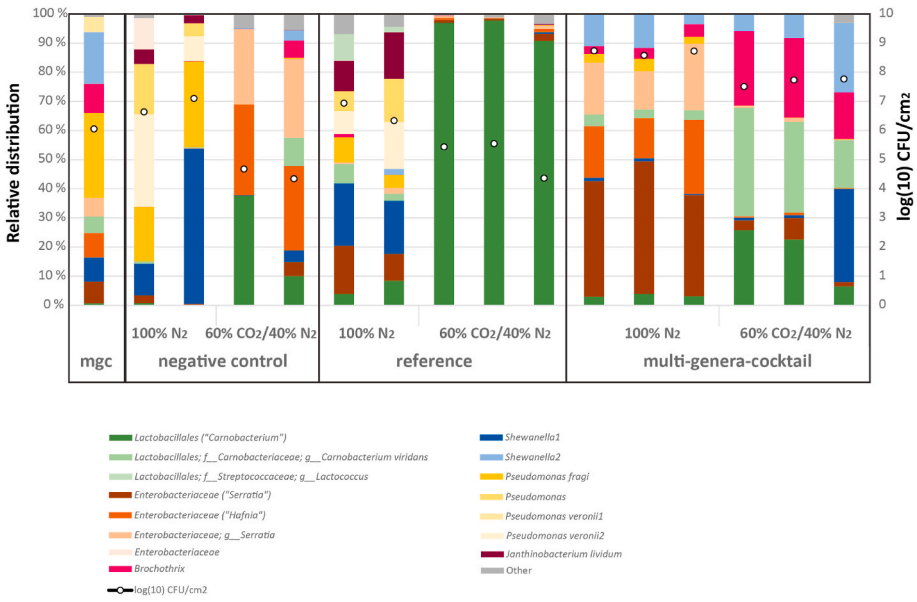

Fig. 2. Challenge study. The dominating taxa of the fillets inoculated by the multi genera cocktail prior storage ("mgc"), the negative control fillets (added sterile physiological water), the Reference (whole fillets) and the fillets added the multi-genera-cocktail, after 11 days of storage (4 °C) in either 100% N_2 or 60% CO_2/40% N_2 (n = 2 or 3). Taxa with average over all samples above 1% or max value above 5% is represented. The remaining taxa is represented as "Other". The taxa are colored according to order, family, or genus affiliation. For taxa above the genus level (e.g *Enterobacteriaceae* and *Lactobacilliales*) the representative sequences were compared to the sequences of the inoculated stains and submitted to BLAST nucleotide search (https://blast.ncbi.nlm.nih.gov) to get more information about possible genera. The Greengenes database still refer to Serratia and Hafnia as Enterobacteriaceae although Serratia now are under the family *Yersiniaceae* and Hafnia under the family *Hafniaceae* (Adeolu et al., 2016; Morales-Lopez et al., 2019). The total viable count (log CFU/cm^2) is represented by an open circle.

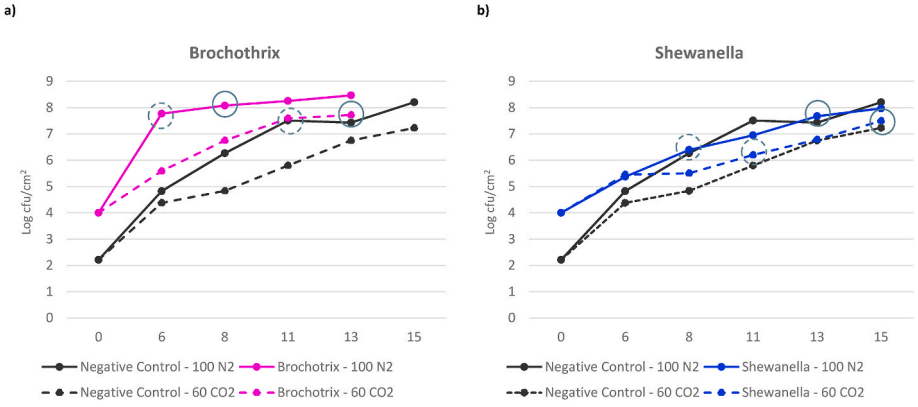

Fig. 3. Total viable count (log CFU/cm^2) of chicken fillets inoculated with *Brochothrix* (a) and *Shewanella* (b) and stored under different packaging atmospheres (4 °C): 100% N_2 and 60% CO_2/40% N_2. Fillets at early stage of stationary phase and late of stationary phase were chosen per bacterial strain for the sensory descriptive analyses for four different groups ("Br-early", "Br-late", "Sh-early", "Sh-late"). Values are mean ± standard deviation (n = 3).

Table 2
Sensory descriptive analysis (odor attributes) of inoculated chicken breast fillets after 11 days of storage (4 °C) for the two package atmospheres (a) 100% N_2, b) 60% CO_2 and 40% N_2). Values are means of two replicates times 8 assessors, compared to corresponding Reference samples (*p < 0.05, **p < 0.01, ***p < 0.005, bold numbers: strain < Reference). The order of the attributes in the table is the same as the order of evaluation by the assessors.

a)

100% N_2	Multicocktail	*Shewanella*	*Serratia*	*Carnobacterium*	*Brochothrix*	*Hafnia*	*Pseudomonas*	Reference
Log CFU/cm^2	8.7 ± 0.1	6.4 ± 0.6	8.4 ± 0.1	8.2 ± 0.1	7.9 ± 0.1	8.5 ± 0.1	7.5 ± 0.2	6.7 ± 0.3
Pungent	5.11***	3.88	3.27	3.63	4.57	4.96*	2.36	3.09
Sourness	1.18	1.24	1.27	1.05	1.12	1.12	1.68*	1.26
Sweetish	3.91	3.23	3.10	3.48	3.30	4.33	2.78	3.34
Metallic	3.84*	3.56	3.57	3.01	3.04	3.69	2.90	3.22
Cloying	6.17***	5.29	4.66	4.99	6.07*	6.72***	**3.12***	4.51
Fermented/sour	4.81*	3.84	3.94	4.83*	5.03*	5.12***	2.26	2.98
Yeast	3.26*	1.80	2.01	2.80	5.71***	2.16	1.79	1.90
Alcohol	1.46	1.31	1.36	1.66	2.44	1.29	1.62	1.83
Sulfur	5.24**	5.62***	3.19	2.60	2.59	6.11***	**1.86***	3.39
Ammonia	2.88**	2.01	2.18	2.35	2.94**	2.79*	1.45	1.53

b)

60% CO_2, 40% N_2	Multicocktail	*Shewanella*	*Serratia*	*Carnobacterium*	*Brochothrix*	Reference
Log CFU/cm^2	7.7 ± 0.1	6.2 ± 1.0	6.0 ± 0.1	8.0 ± 0.1	7.0 ± 0.1	5.1 ± 0.6
Pungent	4.18***	1.51	1.60	3.79*	2.70	1.77
Sourness	**1.14****	1.92	1.62	**1.02*****	**1.24***	1.99
Sweetish	3.08	2.11	2.29	3.84*	3.10	2.52
Metallic	3.34	2.68	2.43	2.94	3.56	2.91
Cloying	5.51***	2.12	1.99	5.44***	4.2***	2.18
Fermented/sour	4.84***	1.62	1.61	4.66***	3.54**	1.73
Yeast	3.44***	1.09	1.47	2.77***	1.99	1.23
Alcohol	1.94*	1.11	1.56*	1.30	1.34	1.01
Sulfur	2.65	1.88	2.02	3.05*	3.61*	2.06
Ammonia	2.63*	1.01	1.05	1.57	1.31	1.46

Carnobacterium spp. (TVC 8.2 ± 0.1 log CFU/cm^2) showed significantly higher intensity of fermented/sour. Although the numbers of *Shewanella* spp. in this experiment only reached TVC of 6.4 ± 0.6 log CFU/cm^2 after 11 days of storage of the fillets (probably due to the low inoculation concentration), inoculated fillets showed significantly higher intensity of sulfur compared with the Reference fillets. Fillets added *Serratia* spp. on the other hand gained relative high numbers (TVC 8.4 ± 0.1 log CFU/cm^2)) but did not show any odor attributes that were significantly different from the Reference. The fillets inoculated with *Pseudomonas* spp. possessed quite high TVC (7.5 ± 0.2 log CFU/cm^2) but showed no difference in any of the negatively associated odor attributes compared to the Reference fillets. Fillets inoculated with a multi genera cocktail resulted in higher intensities of several odor attributes compared to the Reference fillets when stored under 100% N_2; pungent, metallic, cloying, fermented/sour, yeast, sulfur, and ammonia odor.

The Reference fillets stored under 60% CO_2 reached a TVC of 5.1 log CFU/cm^2 and were completely dominated by *Lactobacillales* (most probably *Carnobacterium* spp.) after 11 days of storage. Compared to the Reference fillets, both fillets inoculated with *Carnobacterium* spp. (8.0 ± 0.1 log CFU/cm^2) and *Brochothrix* spp. (7.0 ± 0.1 log CFU/cm^2) showed odor attributes with significantly higher intensity scores of attributes such as cloying, fermented/sour, and sulfur (Table 2b) and lower intensity of the sourness. Additionally, fillets with *Carnobacterium* spp. had higher intensity scores by the yeast and sweetish odor attributes compared to the Reference fillets. Fillets inoculated with *Serratia* spp. only reached a TVC of 6 log CFU/cm^2 and only one attribute scored higher than the Reference fillets, alcohol. The fillets inoculated with *Shewanella* spp. also had low TVC (6.2 ± 1.0 log CFU/cm^2) and showed no differences in odor attributes compared to the Reference fillets. The multi genera cocktail showed higher intensities for six of the negative associated attributes compared to the corresponding Reference fillets, similar as for storage under the 100% N_2 atmosphere (Table 2b). A main difference between the atmospheres for the fillets with the multi genera cocktail, were the higher intensity of sulfur odor for fillets stored under 100% N_2 (TVC of 8.7 ± 0.1 log CFU/cm^2) compared to storage under 60% CO_2 (TVC of 7.7 ± 0.1 log CFU/cm^2) ($p < 0.001$). There were also differences in the bacterial diversity with a dominance by *Enterobacteriaceae* (most likely *Serratia* spp., *Hafnia* alvei, *Shewanella* spp.) when stored under 100% N_2, compared to the dominance of *Carnobacterium* sp p., *Shewanella* spp. and *Brochothrix* spp. when stored under 60% CO_2.

3.2.3. Gas concentrations in package headspace

Fillets packaged by using 100% N_2 showed an initial O_2 level in headspace of 0.02% and below 0.08% during the entire storage period for all the inoculated fillets. The CO_2 levels were higher in the packages that contained fillets inoculated with *Hafnia*, *Serratia* and the multi genera cocktail (containing 77% *Enterobacteriaceae*) compared to the other inoculated fillets (5.0–5.6% vs. 1.8–3.0% CO_2) with an initial level of 0.05% CO_2.

The CO_2 level for fillets packaged with 60% CO_2/40% N_2 was 51.29 ± 0.25% after 11 days of storage (initial g/p ratio of 1/5). The residual oxygen level immediately after packaging for this gas mixture was 0.02 ± 0.01% O_2. Fillets inoculated with *Shewanella* spp. showed higher residual oxygen levels under 60% CO_2 atmosphere compared to 100% N_2 atmosphere (0.13% ± 0.04 vs 0.00% ± 0.00 O_2) after 11 days of storage. A similar difference was found for fillets inoculated with *Serratia* spp. (0.17% ± 0.01 vs 0.00% ± 0.00 O_2).

The fillets inoculated with *Brochothrix* or *Carnobacterium* did not show any differences in residual oxygen levels between atmospheres.

3.3. Effect of packaging gas on sensory and volatile profiles of *Brochothrix spp. and Shewanella spp.*

There was a tendency that fillets inoculated with either *Brochothrix* spp. or *Shewanella* spp. showed higher intensities of negative associated odor attributes when stored under N_2 than CO_2, given similar TVCs. New experiments were done to determine if the presence of CO_2 reduces the spoilage process of *Brochothrix* spp. and *Shewanella* spp. beyond inhibiting growth.

3.3.1. Odor development

Sensory descriptive analyses were performed for fillets sampled at an early and late stage of the stationary phase of bacterial growth, corresponding to TVC of about 7.7 and 7.9 log CFU/cm^2 for *Brochothrix* spp., and 6.4 and 7.5 log CFU/cm^2 for *Shewanella* spp. for the respectively packaged gases, shown in Fig. 3a and b.

In the early stage of stationary phase, the odor attributes total intensity, pungent, cloying, fermented/sour, yeast, sulfur, and ammonia showed significantly higher scores under N_2 atmosphere compared to CO_2 atmosphere (Table 3, $p < 0.05$) for fillets inoculated with *Shewanella* spp. In a later stage of stationary phase, no difference in sulfur and pungent odor was achieved. However, there were still higher scores for the total intensity, cloying, fermented/sour, yeast, and ammonia for fillets stored under the N_2 atmosphere compared to the CO_2 atmosphere.

In the early stage of the stationary phase the attributes fermented/sour and sulfur were higher under the N_2 atmosphere compared to the CO_2 atmosphere (Table 3, $p < 0.05$) for fillets inoculated with *Brochothrix* spp. Sulfur odor was also higher in the late stage of the stationary phase, in addition to the attributes of yeast, pungent and total intensity, under the N_2 atmosphere compared to the CO_2 atmosphere.

3.3.2. Volatile organic compounds (metabolites)

The *Shewanella* and *Brochothrix* inoculated fillets showed typical microbial metabolites like ketones (2-butanone, 2,3-butanedione, 3-hydroxy-2-butanone, acetone), alcohols (ethanol, 2-propanol, 3-methyl-1-butanol, 1-ethoxy-2-propanol), esters (ethyl acetate), nitrogen compounds (trimethylamine), and fatty acids during storage. Also, aldehydes as heptanal, octanal and nonanal (typical oxidation products) were found. The only sulfur compound detected was dimethyl trisulfide, but this was only detected in one of the *Brochothrix* spp. inoculated fillets (Supplementary file S3).

There were few VOCs showing significantly different levels between N_2 and CO_2 stored fillets within the groups tested: Sh-Early, Br-Early, Sh-Late and Br-Late. Significantly higher levels were observed for fillets inoculated with *Shewanella* spp. at the late stationary phase stored under 100% N_2 compared to the 60% CO_2 for the 3-methyl-1-butanol (27.8 ± 2.5 μg/g and 11.8 ± 7.4 μg/g, $p = 0.012$) and for the 2-butanone (35.4 ± 14.0 μg/g and 11.5 ± 2.0 μg/g, $p = 0.04$). Fillets inoculated with *Brochothrix* spp. showed higher levels of TMA at early in stationary phase under 100% N_2 compared to fillets stored under 60% CO_2 (11.5 ± 3.3 vs. 4.3 ± 1.1 μg/g, $p = 0.023$).

3.3.3. Bacteriota

Although attempts were made to make the inoculated side of the fillets as sterile as possible and the fillets were inoculated with a relatively high load of the specific bacterial genera, bacteriota analysis showed that other bacteria were present to a varying degree during storage. The bacteriota of fillets inoculated with *Brochothrix* (100% N_2) also included small relative amounts of *Carnobacterium*, *Lactobacilliales* (*Carnobacterium*, *Enterococcus* or *Vagococcus*) and *Enterobacteriaceae* during storage. When packed with 60% CO_2 the bacteriota also included *Carnobacterium* and *Lactobacilliales* (*Carnobacterium*, *Enterococcus* or *Vagococcus*). The bacteriota of fillets inoculated with *Shewanella* and packed either with 100% N_2 or 60% CO_2/40% N_2 also included small relative amounts of *Lactobacilliales* (*Carnobacterium*, *Enterococcus* or *Vagococcus*) during storage. The detailed bacterial composition of each sample was essential for the later interpretation of the results (Supplementary file S2).

The bacteriota of the negative controls are shown in Fig. S1. For details see Supplementary file S2.

3.3.4. Gas in headspace of the packages

The initial O_2 level was 0.05 ± 0.04% O_2 and 0.01 ± 0.00% O_2 for the 100% N_2 and for the 60% CO_2/40% N_2, respectively, for both inoculates. During storage the O_2 levels were lower under the 100% N_2 atmosphere than under the 60% CO_2-containing atmosphere, most pronounced for *Shewanella* (Fig. 4 a and b).

In packages with 100% N_2, the CO_2 content in headspace during storage was measured to be 0.0% CO_2 immediately after packaging, 1.5 ± 0.2% CO_2 after 6 days of storage, and 2.5 ± 0.3% CO_2 after 13 days of storage (average for all the packaged fillets). All fillets stored under the 60% CO_2 had a CO_2 content from 60.0 ± 0.2% to 54.3 ± 0.3% CO_2 after 6 days of storage, and slightly reduced to 52.8 ± 0.4% CO_2 after 13 days of storage (no significant differences between inoculates).

4. Discussion

4.1. Effect of packaging atmospheres on spoilage

The use of CO_2 containing atmosphere did not only retard the bacterial growth rate, but also led to a lower intensity of off odors during storage of the chicken fillets compared to the atmosphere without CO_2, even at similar numbers of *Shewanella* spp. and *Brochothrix* spp. This was especially profound for the *Shewanella* spp. inoculated fillets and early in stationary phase, with a significant effect of the gas atmosphere on odor attributes both at bacterial limits lower and around what is often reported as the range where spoilage occurs (6.5–8 log CFU/cm^2) (Bailey et al., 1979; Balamatsia et al., 2006; Holck et al., 2014; Rossaint et al., 2015). The observed differences in intensity between the atmospheres were especially evident for the sulfur, cloying and total odor (about 2 score units in difference). Even at a bacterial level of 6.4 log CFU/cm^2, the intensity of the odor attributed could probably have been noticeable

Table 3
Sensory scores of odor attributes of chicken fillets stored under different anaerobic atmospheres (100% N_2 or 60% CO_2, 40% N_2), sampled early ("Sh-Early" and "Br-Early") in stationary phase, and late ("Sh-Late" and "Br-Late") in stationary phase of the bacterial growth. P-values refer to comparison between the different atmospheres. 1 = low intensity, 9 = high intensity (mean ± sd, three samples times 8 assessors). Log CFU/cm^2 gives the total bacterial count numbers (mean ± sd). The attributes sweetish, metallic and alcohol odor did not significantly differ between samples and thereby not shown.

Log CFU/cm^2	Sh-early-N_2	Sh-early-CO_2	p-value	Sh-late-N_2	Sh-late-CO_2	p-value	Br-early-N_2	Br-early-CO_2	p-value	Br-late-N_2	Br-late-CO_2	p-value
	6.4 ± 0.5	6.3 ± 0.1		7.6 ± 0.3	7.3 ± 0.4		7.9 ± 0.1	7.6 ± 0.1		8.1 ± 0.1	7.7 ± 0.2	
Total odor intensity	5.57	3.60	**<0.001***	5.67	4.66	**0.005***	4.99	4.80	0.583	6.04	5.47	**0.042***
Pungent	3.02	1.62	**<0.001***	3.45	2.60	**0.025***	2.86	2.67	0.499	3.98	3.14	**0.024***
Sourness	1.45	2.20	**0.008****	1.09	1.50	0.060	1.16	1.49	0.117	1.00	1.21	0.070
Cloying	5.13	2.63	**<0.001***	5.65	4.34	**0.008***	5.26	4.63	0.252	6.44	5.63	0.051
Fermented/sour	3.29	2.07	**0.009***	4.82	3.02	**<0.001***	4.29	3.31	**0.025***	4.93	4.53	0.297
Yeast	1.68	1.24	**0.037***	2.60	1.94	**0.044***	2.85	2.61	0.517	3.70	2.90	**0.040***
Sulfur	4.97	2.54	**<0.001***	3.40	2.97	0.196	3.33	2.80	**0.047***	3.46	2.84	**0.025***
Ammonia	2.01	1.22	**0.016***	2.44	1.47	**0.010***	1.89	1.60	0.247	2.13	1.87	0.471

*inoculated strain > Negative control. **inoculated strain < Negative control.

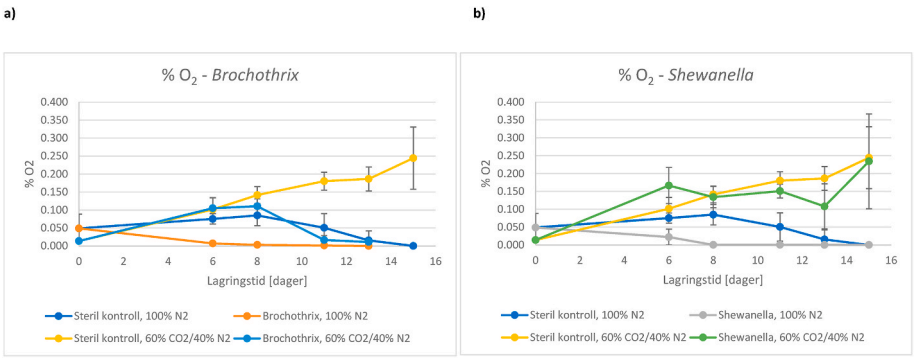

Fig. 4. Levels (%) of O_2 in headspace during storage (4 °C) of chicken fillets inoculated with Shewanella spp. (a) and Brochothrix spp. (b), packaged with two different packaging gases: 100% N_2 and 60%CO_2/40%N_2. Values are means ± st.dev (n = 3).

to consumers. The differences in off odors between the storage atmospheres were less evident for the *Brochothrix* inoculated fillets, still significantly higher intensity of off odors under 100% N_2 were detected. This might correspond to the consumption of the residual O_2 levels, as there were fewer differences between the atmospheres for the fillets inoculated with *Brochothrix* spp. as it was for the *Shewanella* spp. inoculated fillets. The consumption of residual O_2 in the headspace of packages during storage might be an indication of increased off odor development, but it has to our knowledge, not previously been studied. It is well known that CO_2, restrictive levels of O_2, and high/toxic levels of O_2 can inhibit bacterial growth and thereby improve preservation of the initial freshness and prolong shelf life (Farber, 1991). This study additionally showed that CO_2 inhibit the spoilage process of *Shewanella* spp. and *Brochothrix* spp. However, the O_2 consumption as shown here, and different metabolic pathways as performed by Höll et al. (2020), will be useful to study further.

4.2. Spoilage associated bacteria under 100% N_2 atmosphere

A wide spectrum of genera or species has previously been suggested to be important spoilage organisms, often in combination with others and at certain TVC, at a specific time of storage or by a total odor evaluation/acceptance or by a single chemical component (Morales et al., 2016; Rotabakk et al., 2006). Even with dominance during storage, it is important to verify whether they directly cause unpleasant changes (specific spoilage organisms; SSO) or not (spoilage associated organisms; SAO) (Gram et al., 2002; Saenz-Garcia et al., 2020).

Despite relatively high TVC, fillets with the cocktails of *Pseudomonas* spp. or of *Serratia* spp. did not show any development of negative associated odors. *Pseudomonas* spp. have previously been reported to spoil meat under aerobic conditions (Ercolini et al., 2010) and to vary in phenotypic and genotypic characterization between species and strains (Morales et al., 2016). Former studies refer to *Pseudomonas* spp. as spoilage bacteria due to dominance at time of spoilage (Doulgeraki et al., 2012). Furthermore, *Pseudomonadaceae* is described to include bacteria that can cause spoilage under aerobic storage conditions (Saenz-Garcia et al., 2020; Wang et al., 2017) and under anaerobic conditions (Casaburi et al., 2015; Kolbeck et al., 2021). An initial screening performed prior to the presented study included the same *Pseudomonas* spp. mix as presented here, stored under 60% CO_2/40% N_2, and it did not result in any growth from the inoculated (initial) level of 4.0 CFU/cm^2 and no development of off-odors (results not shown). We can therefore conclude that the *Pseudomonas* strains used in this study were not specific spoilage organisms of chicken fillets under any of the anaerobic conditions used in our experiment, or at least at the TVC level achieved under 100% N_2 (7.5 CFU/cm^2).

The *Serratia* cocktail contained at least two different species (*Serratia liquefaciens*, *Serratia fonticola*, Table 1). To our knowledge, evaluation of *Serratia* sp. under different packaging atmospheres has not previously been described as related to odor development. Despite relatively high TVC under 100% N_2 atmosphere, no difference was found compared to the Reference fillets. Both Wang et al. (2017) and Höll et al. (2016) report *Serratia* to be among the dominating bacteriota after CO_2/N_2 storage, and it is detected under vacuum packaging (Pennacchia et al., 2011), but without any sensory analyses performed. In general, *Serratia* spp. is commonly found in meat under different storage conditions (Doulgeraki et al., 2012).

Hafnia spp., which probably contained the species *H. alvei*, showed a high degree of spoilage activity due to the development of different off odors. *Hafnia* spp., together with *Shewanella* spp., were the two genera that developed high levels of sulfur odor, though with a higher TVC (8.5 log CFU/cm^2.) for the *Hafnia* spp. mix compared to the *Shewanella* spp. mix. This corresponds to odor development reported by Irlinger et al. (2012) and Russell et al. (1995).

4.3. Specific spoilage bacteria under CO_2-containing atmosphere

As expected, the initial mixture of 60% CO_2 and 40% N_2 with a relatively high gas to product volume ratio of 5/1, prolonged the lag phase of bacterial growth during the 4 °C storage compared to the 100% N_2 atmosphere. During storage under these packaging conditions, we found that only *Carnobacterium* spp. and *Brochothrix* spp. were able to spoil the fillets, especially with the development of cloying and fermented/sour odors compared to Reference fillets, but also higher scores of sulfur odor. *C. maltaromaticum* has previously been associated with spoilage of meat due to being part of the spoilage communities under CO_2/N_2 atmosphere (Höll et al., 2016) and development of volatile organic components under vacuum packaging (Ercolini et al., 2009). Studies also report a dominance of lactic acid bacteria at time of spoilage for chicken packaged with 60–65% CO_2 (Alakomi et al., 2017; Chouliara et al., 2008; Holck et al., 2014; Höll et al., 2016; Pettersen et al., 2021; Tsafrakidou et al., 2021). The uninoculated fillets were dominated by *Carnobacterium* spp., but with too low TVC to cause spoilage. Casaburi et al. (2011) found negligible contribution to meat spoilage by *Carnobacterium maltaromaticum* at TVC of 7 log CFU/g, though with a higher intensity of dairy and mozzarella odor scores in air pack compared to vacuum packs. Based on the presented results, under anaerobic storage condition we can assume that *Carnobacterium* spp. can cause spoilage when the TVC are around 8 log CFU/cm_2.

The species *B. thermosphacta* is previously reported as commonly associated with meat spoilage and to dominate during aerobic, anaerobic storage and under CO_2 enriched atmosphere (Doulgeraki et al., 2012). Still, to our knowledge, the spoilage potential under different anaerobic packaging atmospheres is not defined. In the presence of high-oxygen levels in modified atmosphere, which are used in many countries, an increased spoilage potential is reported for *Brochothrix* spp. and lactic acid bacteria due to the formation of acetoin (3-hydroxy-2-butanone) (Casaburi et al., 2014; Rossaint et al., 2015) and acetic

acid, but to a lesser extent under anaerobically MA-packaged meat (Pin et al., 2002). Neither acetoin nor acetic acid were detected in the present study under any of the anaerobic storage conditions. However, significantly higher levels of TMA were found in fillets where *Brochothrix* had reached the stationary phase in a 100% N_2 atmosphere compared to CO_2.

Neither the fillets inoculated with *Carnobacterium* spp. nor with *Brochothrix* spp. showed any differences in residual O_2 content between the packaging gases, which indicates unaffected O_2 consumption for the 2 atm. Studies of residual O_2 have been mostly performed in studies of meat color preservation (Sorheim et al., 2017), and is less common in bacteriological research. A few studies have already pointed out that *Carnobacterium* spp. specifically (Holck et al., 2014; Höll et al., 2019) and *B. thermosphacta* (Chouliara et al., 2008; Höll et al., 2019) as specific spoilage organisms, and our presented study confirmed these findings.

Under the CO_2-containing atmosphere, we found *Serratia* spp. to develop an odor of alcohol, but with very low intensity and probably not sufficient to be associated with spoilage. Under an anaerobic CO_2 containing atmosphere, *Serratia* spp. was previously detected as part of a total bacteriota at the end of refrigerated storage of skinless chicken fillets together with *Carnobacterium* spp., *Lactobacillus* spp. (Holck et al., 2014; Höll et al., 2016; Rossaint et al., 2015; Sade et al., 2013).

Shewanella spp. did not develop off odors under the CO_2 atmosphere and developed lower levels of 3-methyl-1-butanol compared to storage under the N_2 atmosphere. Still, *Shewanella* spp. grew to similar levels of TVC as for the N_2 stored fillets, but with approximately 2–3 days of delay.

4.4. Future perspectives of conditions affecting spoilage bacteriota

The storage condition very much decides the nature of the spoilage as seen in the presented study. Additionally, the initial bacteriota can differ between processing plants or batches, as we found for the two batches from Plant 1 (relatively high diversity vs. dominated by *Shewanella* spp.). The packaging gas mixture and storage conditions seemed to further determine the bacteriota during storage, and that some species can be outcompeted by others, that is also found by (Höll et al., 2016; Rudi et al., 2004), and as shown in| the presented multi-genera cocktail.

The presented study does not show metabolic pathways, only the volatile organic compounds and odor attributes. Future studies should involve all this and compare it to the commonly used high-O_2 atmosphere. However, high-O_2 is not used by Norwegian producers, and is therefore not focused in this study. Nevertheless, Sarfraz et al. (2021) have shown higher intensities of negative associated odor attributes after 14 days of storage of chicken breast fillets under high O_2 atmosphere compared to an anaerobic atmosphere. Regarding VOCs, there is a lack of knowledge in VOCs on raw meat (Casaburi et al., 2015; Ercolini et al., 2009). As the development of the different VOCs probably is related to both the storage conditions, the nature of the meat product and the bacterial contamination (Casaburi et al., 2015; Rodbotten et al., 2004), the complexity of the biochemistry of chicken should be more studied.

5. Conclusions

A selection of potential spoilage bacteria has been studied on skinless chicken fillets, and to which degree they develop off odor attributes. We found that sulfur odor is developed by *Shewanella* and *Hafnia* under 100% N_2, and by *Carnobacterium* and *Brochothrix* under CO_2/N_2 atmosphere. The attributes cloying, fermented/sour and yeast odors were also prominent. The novelty of the presented study was, nevertheless, the presence of higher intensity of off odors for *Brochothrix* spp. and *Shewanella* spp. under 100% N_2 compared to 60% CO_2/40% N_2, at similar TVC numbers at early and late in the stationary phase. We have also given descriptions of different odor attributes for strains within the genera *Pseudomonas*, *Hafnia*, *Serratia*, *Shewanella*, *Brochothrix* and *Carnobacterium* under anaerobic atmospheres with or without CO_2.

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CRediT authorship contribution statement

Anlaug Ådland Hansen: Conceptualization, Methodology, Project administration, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Solveig Langsrud:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Mats Carlehög:** data analysis, Writing – original draft. **John-Erik Haugen:** data analysis, Writing – original draft. **Birgitte Moen:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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