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ORIGINAL ARTICLE

Proof of concept: predicting the onset of meat spoilage by an integrated oxygen sensor spot in MAP packages

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Significance and Impact of the Study: This study demonstrates applicability of an incorporated oxygen sensor spot to non-invasively determine microbial headspace oxygen consumption in high oxygen modified atmosphere packages (MAP). Furthermore, exploitation of this technology for the prediction of the onset of meat spoilage prior to human perception is demonstrated. Thus, this study provides proof of concept for a novel rapid and easily implementable method, which can be used to reduce meat disposal as waste in retail by individual assessment of single high oxygen MAP.

Keywords
lactic acid bacteria, meat spoilage, modified atmosphere packaging, oxygen sensor spots, sensory analysis, spoilage prediction.

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Abstract

During storage of modified atmosphere packaged (MAP) meat, the initial microbiota grows to high cell numbers, resulting in perceptible spoilage after exceeding a specific threshold level. This study analyses, whether elevated oxygen consumption in the headspace of MA-packages would enable a prediction method for meat spoilage. We monitored the growth of single spoiling species inoculated on high-oxygen MAP beef and poultry, performed sensorial analysis and determined oxygen concentrations of the headspace via a non-invasive sensor spot technology. We detected microbial headspace oxygen consumption occurring prior to perceptible meat spoilage for certain species inoculated on beef steaks. However, headspace oxygen consumption and cell counts at the onset of spoilage were highly species-dependent, which resulted in a strong (*Brochothrix thermosphacta*) and moderate (*Leuconostoc gelidum* subspecies) decrease of the headspace oxygen content. No linear decrease of the headspace oxygen could be observed for *Carnobacterium divergens* and *Carnobacterium maltaromaticum* inoculated on poultry meat. We demonstrate the applicability of an incorporated oxygen sensor spot technology in MAP meat packages for detection of spoilage in individual packages prior to its perceptible onset. This enables individual package evaluation and sorting within retail, and consequently reduces meat disposal as waste.

Introduction

Beside intrinsic lipid oxidation and spontaneous enzymatic reactions, meat spoilage is caused by microbial metabolism, resulting in the production of spoilage-associated compounds (Casaburi *et al.* 2015; Pellissery *et al.* 2020). In order to inhibit microbial spoilage, meat is commonly packaged under a modified atmosphere (MA), selectively supressing O_2 and CO_2 sensitive species (Farber 1991; Lambert *et al.* 1991; Devlieghere and Debevere 2000; Ercolini *et al.* 2006). Especially red meat is commonly packaged under a high oxygen atmosphere containing 30% CO_2/70% O_2 (Eilert 2005). In contrast, white meat has been packaged under an oxygen free atmosphere for a long time (McKee 2007; Sante *et al.* 2007), however, it is now increasingly also packaged under high oxygen atmosphere due to its reported inhibitory effect on pathogens (Meredith *et al.* 2014; Rossaint *et al.* 2015). Still, meat is spoiled by bacteria adapted to those gas atmospheres, which are called "ephemeral spoilage organisms" (ESOs), (Nychas *et al.* 2008) comprising e.g., *Brochothrix* (*B.*) *thermosphacta* and lactic acid bacteria (LAB) such as *Carnobacterium* (*C.*) *divergens*, *C. maltaromaticum* or *Leuconostoc* (*L.*) *gelidum* subspecies (Lambert *et al.* 1991; Borch *et al.* 1996; Ercolini

et al. 2006; Nychas *et al.* 2007; Hilgarth *et al.* 2018). Even though all of those species are known to contribute to meat spoilage, their contribution differs on their ability to produce organoleptically perceptible metabolites called volatile organic compounds (VOCs) (Casaburi *et al.* 2015).

Due to the diverse composition of the spoilage microbiome and synergistic spoilage, it is hard to precisely predict the timepoint of spoilage for each individual MAP meat. Thus, use-by dates are estimated with a safety margin, frequently resulting in disposal of nonspoiled but still edible meat after exceeding the expiration date (Koskela *et al.* 2015) or, *vice versa*, spoiled meat before the use-by date. Consequently, there is a need for alternative intelligent food packaging technologies, enabling a more precise prediction of the timepoint of spoilage for individual MA packages (Kuswandi *et al.* 2011). Several nondestructive approaches have been described in recent years comprising sensor technologies to predict meat spoilage based on e.g. carbon dioxide (Puligundla *et al.* 2012), hydrogen sulphide (Koskela *et al.* 2015), volatile amines (Pacquit *et al.* 2006) or pH (Rukchon *et al.* 2014). Here, we present a different approach to predict meat spoilage, based on microbial oxygen consumption in the headspace using a sensor spot technology. This is based on the fact that oxygen values of the headspace are not constant over storage time but rather decrease (Kennedy *et al.* 2004; Höll *et al.* 2016; Hilgarth *et al.* 2018). In a recent study, we have attributed this decrease to microbial growth and quantified the strain-specific oxygen uptake rate (OUR) of typical meat spoilers *in vitro* (Kolbeck *et al.* 2019). This follow-up study investigates the contribution of relevant meat spoilers to headspace oxygen consumption and spoilage of MAP meat *in situ* and further establishes a hitherto missing link of both properties to validate, whether an individual prediction of meat spoilage is feasible for food industry/retail.

Results and Discussion

In order to validate a prediction method for meat spoilage based on the oxygen content in MA-packages, microbial growth and headspace oxygen consumption was measured for typical spoilers on inoculated high oxygen packaged beef and poultry and correlated with the timepoint of perceptible meat spoilage (Fig. 1). Spoilage was determined based on a rejection of either visual or olfactory organoleptics of inoculated meat by more than 50% of our sensory panel (Fig. S1). Further details about single validation of olfactory and visual appearance of the inoculated meat pieces can be seen in Figs S2 and S3.

Species specific meat-spoilage

Spoilage of meat is considered as perceptible after exceeding a critical bacterial count. According to several studies, this threshold level is approximately at $>10^7$ CFU per cm^2 meat (Prieto *et al.* 1991; Ayres 2007; Nychas *et al.* 2008). Thus, in order to accelerate the process of species-specific spoilage we inoculated our meat with a high $\log_{10}$(CFU per cm^2) = 5–7. Similar to Nychas *et al.* (2008), but depending on the species, we determined perceptible spoilage to occur at a bacterial count of $\log_{10}$(CFU per cm^2) >6·8–8·2 (Table 1).

In detail, beef inoculated either with *B. thermosphacta* TMW2.2101, *L. gelidum* subsp. *gelidum* TMW2.1618 or *L. gelidum* subsp. *gasicomitatum* TMW2.1619 was considered as spoiled after 10 days when $\log_{10}$(CFU per cm^2) was 7·7, 7·6 and 6·8, respectively, and chicken inoculated with *C. maltaromaticum* TMW2·1581 or *C. divergens* TMW2.1577 was considered as spoiled after 7 days when $\log_{10}$(CFU per cm^2) was 7·2 and 8·2, respectively. We further identified a higher percentage of visual rejection compared to the percentage of persons, rejecting meat based on olfactory criteria for *B. thermosphacta* TMW2.2101 and *L. gelidum* subsp. *gelidum* TMW2.1618. Especially meat inoculated with latter species displayed a strong brown discolouration. Considering that the meat colour is the only apparent criteria for consumers to evaluate freshness of MAP meat in retail, prevention of meat browning is decisive for the seller. The observed brown meat colour of our experiment might be explained by a mixture of the red oxymyoglobin pigment and green sulphmyoglobin/choleglobin pigment produced by *L. gelidum* subsp. *gelidum* TMW2.1618. Production of H_2S and H_2O_2 has been described for other *L. gelidum* strains resulting in meat greening due to sulphmyoglobin pigment formation (Borch *et al.* 1996; Vihavainen and Björkroth 2007). The higher rejection of meat inoculated with *B. thermosphacta* TMW2.2101 due to visual appearance compared to olfactory development is rather surprising, as this species is commonly known to contribute more to off-odour development in air and MAP stored meat by production of VOC metabolites such as iso-butyric acid and iso-valeric acid (Dainty and Hibbard 1983; Dainty and Mackey 1992; Casaburi *et al.* 2014, 2015) The heterofermentative bacterium *C. maltaromaticum* is also known to produce high amounts of VOCs (Laursen *et al.* 2006; Casaburi *et al.* 2011) rather than discolouration, which was in accordance to our study, as the panel rejected the meat more due to olfactory than visual appearance. Concluding, we demonstrated that single bacteria contribute to different extent to spoilage characteristics, such as discolouration or off-odour production resulting in a time-shifted onset of spoilage.

Species-specific headspace oxygen consumption

In this study, we demonstrated that the sensor spot technology is a powerful tool to non-invasively monitor the

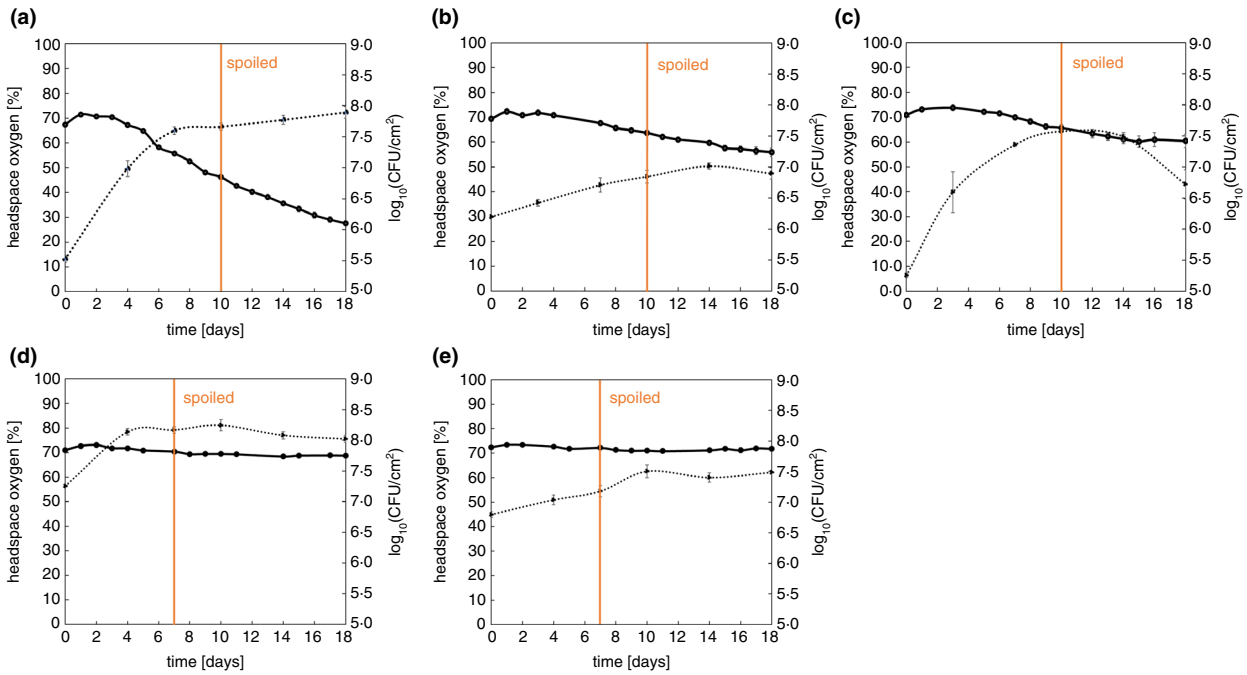

Figure 1 Growth and headspace oxygen consumption. Growth (grey dotted lines) and head space oxygen consumption (black solid lines) monitored over 18 days for the meat-spoiling bacteria (a) *Brochothrix thermosphacta* TMW2.2101, (b) *Leuconostoc gelidum* subsp. *gasicomitatum* TMW2.1619, (c) *Leuconostoc gelidum* subsp. *gelidum* TMW2.1618, (d) *Carnobacterium divergens* TMW2.1577 and (e) *Carnobacterium maltaromaticum* TMW2.1581. Error bars represent the standard error of three independent replicates. Orange lines indicate the time point of meat spoilage, based on sensorial rejection of more than 50% of the panel to either visual or olfactory validation. [Colour figure can be viewed at wile yonlinelibrary.com]

Table 1 Correlating the microbial oxygen consumption and meat spoilage. The bacterial count at the timepoint of first O_2 decrease and perceptible meat spoilage was determined for all species inoculated on the corresponding meat type. Furthermore, the headspace oxygen content at the timepoint of perceptible meat spoilage is given.

		$\log_{10}$(CFU per cm^2)		O_2 content
	TMW	O_2 decrease	Meat spoilage	Meat spoilage
Brochothrix thermosphacta	2·2101	7·0 ± 0·13	7·7 ± 0·06	46%
Leuconostoc gelidum subsp. *gasicomitatum*	2·1619	6·4 ± 0·05	6·8 ± 0·10	63·7%
Leuconostoc gelidum subsp. *gelidum*	2·1618	7·4 ± 0·03	7·6 ± 0·03	65·7%
Carnobacterium divergens	2·1577	–	8·2 ± 0·05	70·4%
Carnobacterium maltaromaticum	2·1581	–	7·2 ± 0·09	72·2%

headspace oxygen within MAP packages. In this manner, we observed a decrease in headspace oxygen for packages inoculated with both *L. gelidum* subspecies and *B. thermosphacta* TMW2.2101 at cell counts of 6·4–7·4 (Fig. 1, Table 1). The highest decrease in oxygen content (to 28% within 18 days) could be identified for packages containing meat inoculated with *B. thermosphacta* TMW2.2101. These observations fit to our previous study, were we determined a 31-times higher OUR in 60 h per single cell for *B. thermosphacta* TMW2.2101 compared to the other LAB (Kolbeck *et al.* 2019). Furthermore, in our previous study, the determined OUR of *Carnobacteria* was comparable to that of both *Leuconostoc* species. Comparatively, in this study we observe almost no oxygen consumption for both *Carnobacteria*. This might be due to the fact that respiration of *Carnobacteria* obligately relies on exogenous heme (Lechardeur *et al.* 2011; Kolbeck *et al.* 2019). Heme was available in our previous study performed in a synthetic media, whereas in this study, both *Carnobacteria* were inoculated on chicken meat, in which low amounts of heme may limit their respiration (Han *et al.* 2006). Thus, prediction of spoilage by microbial oxygen consumption in the headspace of MAP packages of white meat might be limited to spoilage cases,

which are dominated by bacteria able to synthesize heme by themselves. We also inoculated our *Carnobacteria* strains on beef steaks, but no significant growth could be detected for this species (data not shown). Strains used in this study have been isolated from chicken and appear adapted to that environment (Höll *et al.* 2016), where they are dominating the spoilage microbiome. In comparison, on beef, they represent only a minor part of the spoilage microbiome.

Correlation of spoilage and oxygen consumption

We further probed to correlate onset of spoilage with microbial headspace oxygen consumption, which would provide retailers with a powerful tool for predicting individual spoilage for single packages before the actual onset. The applied sensor spot technology used in this study enabled to detect microbial oxygen consumption in the headspace of inoculated MAP beef, before critical cell numbers for spoilage were reached and before meat was perceived as spoiled. This could be demonstrated for high oxygen MAP inoculated with *B. thermosphacta* TMW2.2101, *L. gelidum* subsp. *gasicomitatum* TMW2.1619 and *L. gelidum* subsp. *gelidum* TMW2.1618 where the oxygen decreases at a $\log_{10}$(CFU per cm^2) of 7·0, 6·4 and 7·4 and perceptible spoilage occurs at a $\log_{10}$(CFU per cm^2) of 7·7, 6·8 and 7·6 (Table 1). Furthermore, considering that the ratio of headspace volume to meat volume was increased compared to industrial standards (1 : 8 for our study, 1 : 3 for industry) (Gill and Gill 2005; Blakistone 2012), the oxygen consumption should lead to a faster relative headspace-oxygen drop in an industrial MAP system compared to our study. Thus, we conclude that a prediction of meat spoilage based on headspace oxygen content is feasible for high oxygen MAP red meat. Based on our data obtained for *Carnobacteria* on meat, we predict this method to be also applicable on white MAP meat products, as long as bacteria able to respire without exogenous heme dominate the initial microbiota.

Concluding, this study investigates the headspace oxygen consumption of single spoilers on high oxygen MAP beef and chicken and further determines the onset of spoilage based on the organoleptic criteria visual appearance and olfactory. We detected a decrease in headspace oxygen even before the actual onset of perceptible meat spoilage in MAP beef steaks. This correlation functions as a signal of impending spoilage before the actual onset and can be used to take action at retail level e.g. marking respective packages with a limited residual shelf life with reduced price. Consequently, we suggest that this correlational signal is used to predict meat spoilage in individual meat packages by employing an incorporated noninvasive sensor spot technology as applied in this study. Further data on the critical signal oxygen concentration as well as a ring trial applications should be investigated in future studies.

In conclusion, we could prove the principal concept of a rapid, easily implementable and physically method (supposedly conducted by a hand-held device), which can be used to reduce the avoidable meat waste at the retail level.

Material and Methods

Meat processing and inoculation

Fresh MA packaged (30% CO_2 and 70% O_2), nonmarinated chicken breast filets and beef steaks were bought form a local discounter with an use-by date assigned to >4 days. Chicken breast was cut into squared (45·08 cm^2) and beef steak into round pieces (52·8 cm^2) all exhibiting equal thickness. *C. divergens* TMW2.1577 and *C. maltaromaticum* TMW2.1581 were inoculated on chicken meat and *L. gelidum* subsp. *gelidum* TMW2.1618, *L. gelidum* subsp. *gasicomitatum* TMW2.1619 as well as *B. thermosphacta* TMW2.2101 on beef steak, corresponding to their isolation source (Höll *et al.* 2016; Hilgarth *et al.* 2018, 2019). Each meat piece was inoculated on both sides with 100 µl of the previously prepared cold-adapted and washed overnight cultures adjusted to an optical density $(OD)_{600\ nm} = 10$ with quarter-strength ringer solution. Cells were homogeneously distributed over the meat surface using a sterile spatula. For each MAP meat tray, representing one replicate, four round beef pieces or five squared chicken pieces were inoculated.

Packaging and sensor spot technology

Inoculated meat pieces were placed into polypropylene (PP) trays coated with an ethylene-vinylalcohol (EVOH) barrier film (volumetric permeation rate 0·25 cc.20μ m^{-2}.day.atm) (ES-Plastic, Hutthurm, Germany). The trays were equipped with a self-made polyvinylchloride (PVC) insert, shaped with a PVC-laser cutter, to reduce the headspace volume comparable to the industrial standards (1 : 8 volume meat to volume headspace or 1 : 2 surface meat to volume headspace). At the inner part of one corner of each tray, we fixed a SP-PSt3 oxygen sensor spot (PreSens, Regensburg, Germany), enabling noninvasive, fast and easy monitoring of the oxygen content within each package by a physically fluorescence signal. The sensor spots were read out using a portable and easy to handle OXY-1 SMA oxygen meter (PreSens). A twopoint calibration of the sensor spots was performed according to the manufacturer's validated

protocol for calibration. Therefore, an unpackaged tray was equipped with an oxygen sensor spot and calibration of cal100 was performed at 21% O_2 (100% air saturation). Second, the same tray was packaged under 100% nitrogen atmosphere and calibration of cal0 was performed at 0% O_2. All trays were packaged under a MA of 30% CO_2 and 70% O_2 using a packaging machine Rotarius VG (VarioVAC, Zarrentin, Germany) and an oxygen impermeable PP-EVOH sealing foil. Trays were stored at 4°C for a total of 18 days.

Sampling procedure

Oxygen was monitored once per day for all trays by sensor spot technology. After 3 or 4, 7, 10, 14 and 18 days, three trays were used for sampling (triplicates). Therefore, two meat pieces of each tray were used to determine the colony forming units (CFU) and one meat piece was used for sensory analysis. A summary of the sampling procedure is provided in Fig. S4. At day 0, three meat pieces were used for sensorial analysis and other three for CFU determination directly after inoculation (without packaging). CFUs were determined by transferring meat pieces into sterile sampling bags with a lateral filter (VWR, Darmstadt, Germany) containing 10 ml of quarter-strength ringer solution and homogenized for two minutes using a bag mixer (Interscience, Paris, France). Afterwards a serial dilution of the cell homogenisate was performed and each dilution was plated on BHI agar. After incubation of the plates at 25°C for 48 h, the CFU of plates containing single countable colonies was determined. An average and standard error value was calculated for each timepoint based on six replicates. To prove the recovery/identity of the inoculated strains on meat, 12 colonies of each replicate were checked after 18 days by random amplified polymorphic DNA (RAPD) PCR (Hilgarth *et al.* 2018) using the M13V primers designed by Ehrmann *et al.* (2003). Sensorial validation was performed by an untrained panel of 10 persons validating the olfactory and visual appearance of meat, attributing one of the following options: "Fresh", "distinguishable from fresh, but still edible" and "spoiled" and thereby mimicking customer decisions. Meat was considered as spoiled as soon as one of the two organoleptic criteria were rejected as "spoiled" by at least 50% of the panel.

Conflict of Interest

No conflict of interest is declared by the authors.

Author contributions

SK design the study, performed the experiments and data evaluation and wrote the first draft of the manuscript. MH helped to interpret the data, draft of the manuscript and supervised the work of SK. RV initiated the project and supervised the work of SK. All authors read and approved the final manuscript.

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References

Ayres, J. (2007) Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. *J Food Sci* **25**, 1–18.

Blakistone, B.A. (2012) *Principles and Applications of Modified Atmosphere Packaging of Foods*. 1st edn. New York, NY: Springer.

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

Casaburi, A., De Filippis, F., Villani, F. and Ercolini, D. (2014) Activities of strains of *Brochothrix thermosphacta* in vitro and in meat. *Food Res Int* **62**, 366–374.

Casaburi, A., Nasi, A., Ferrocino, I., Di Monaco, R., Mauriello, G., Villani, F. and Ercolini, D. (2011) Spoilage-related activity of *Carnobacterium maltaromaticum* strains in air-stored and vacuum-packed meat. *Appl Environ Microbiol* **77**, 7382–7393.

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

Dainty, R.H. and Hibbard, C.M. (1983) Precursors of the major end products of aerobic metabolism of *Brochothrix thermosphacta*. *J Appl Bacteriol* **55**, 127–133.

Dainty, R.H. and Mackey, B.M. (1992) The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J Appl Bacteriol* **73**, 103s–114s.

Devlieghere, F. and Debevere, J. (2000) Influence of dissolved carbon dioxide on the growth of spoilage bacteria. *LWT – Food Sci Technol* **33**, 531–537.

Ehrmann, M.A., Müller, M.R.A. and Vogel, R.F. (2003) Molecular analysis of sourdough reveals *Lactobacillus mindensis* sp. nov. *Int J Syst Evol Microbiol* **53**, 7–13.

Eilert, S.J. (2005) New packaging technologies for the 21st century. *Meat Sci* **71**, 122–127.

Ercolini, D., Russo, F., Torrieri, E., Masi, P. and Villani, F. (2006) Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Appl Environ Microbiol* **72**, 4663–4671.

Farber, J.M. (1991) Microbiological aspects of modified-atmosphere packaging technology – a review. *J Food Prot* **54**, 58–70.

Gill, A.O. and Gill, C.O. (2005) Preservative packaging for fresh meats, poultry, and fin fish. In *Innovations in Food Packaging* ed. Han, J.H. pp. 204–226. London: Academic Press.

Han, D., McMillin, K. and Godber, J. (2006) Hemoglobin, myoglobin, and total pigments in beef and chicken muscles: chromatographic determination. *J Food Sci* **59**, 1279–1282.

Hilgarth, M., Behr, J. and Vogel, R.F. (2018) Monitoring of spoilage-associated microbiota on modified atmosphere packaged beef and differentiation of psychrophilic and psychrotrophic strains. *J Appl Microbiol* **124**, 740–753.

Hilgarth, M., Lehner, E.M., Behr, J. and Vogel, R.F. (2019) Diversity and anaerobic growth of *Pseudomonas* spp. isolated from modified atmosphere packaged minced beef. *J Appl Microbiol* **127**, 159–174.

Höll, L., Behr, J. and Vogel, R.F. (2016) Identification and growth dynamics of meat spoilage microorganisms in modified atmosphere packaged poultry meat by MALDI-TOF MS. *Food Microbiol* **60**, 84–91.

Kennedy, C., Buckley, D.J. and Kerry, J.P. (2004) Display life of sheep meats retail packaged under atmospheres of various volumes and compositions. *Meat Sci* **68**, 649–658.

Kolbeck, S., Reetz, L., Hilgarth, M. and Vogel, R.F. (2019) Quantitative oxygen consumption and respiratory activity of meat spoiling bacteria upon high oxygen modified atmosphere. *Front Microbiol* **10**, 2398.

Koskela, J., Sarfraz, J., Ihalainen, P., Määttänen, A., Pulkkinen, P., Tenhu, H., Nieminen, T., Kilpelä, A. *et al.* (2015) Monitoring the quality of raw poultry by detecting hydrogen sulfide with printed sensors. *Sens Actuators B* **218**, 89–96.

Kuswandi, B., Wicaksono, Y., Jayus Abdullah, A., Heng, L.Y. and Ahmad, M. (2011) Smart packaging: sensors for monitoring of food quality and safety. *Sens Instrum Food Qual Saf* **5**, 137–146.

Lambert, A.D., Smith, J.P. and Dodds, K.L. (1991) Shelf life extension and microbiological safety of fresh meat — a review. *Food Microbiol* **8**, 267–297.

Laursen, B.G., Leisner, J.J. and Dalgaard, P. (2006) *Carnobacterium* species: effect of metabolic activity and interaction with *Brochothrix thermosphacta* on sensory characteristics of modified atmosphere packed shrimp. *J Agricult Food Chem* **54**, 3604–3611.

Lechardeur, D., Cesselin, B., Fernandez, A., Lamberet, G., Garrigues, C., Pedersen, M., Gaudu, P. and Gruss, A. (2011) Using heme as an energy boost for lactic acid bacteria. *Curr Opin Biotechnol* **22**, 143–149.

McKee, L. (2007) Microbiological and sensory properties of fresh and frozen pork products. In *Handbook of Meat, Poultry and Seafood Quality*, ed. Nollet, L. M. L. pp. 395–404. NJ: Blackwell Publishing.

Meredith, H., Valdramidis, V., Rotabakk, B.T., Sivertsvik, M., McDowell, D. and Bolton, D.J. (2014) Effect of different modified atmospheric packaging (MAP) gaseous combinations on *Campylobacter* and the shelf-life of chilled poultry fillets. *Food Microbiol* **44**, 196–203.

Nychas, G.-J.-E., Marshall, D.L. and Sofos, J.N. (2007) Meat, poultry, and seafood. *Food Microbiology: Fundamentals and Frontiers*, 3rd edn. American Society of Microbiology.

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

Pacquit, A., Lau, K.T., McLaughlin, H., Frisby, J., Quilty, B. and Diamond, D. (2006) Development of a volatile amine sensor for the monitoring of fish spoilage. *Talanta* **69**, 515–520.

Pellissery, A.J., Vinayamohan, P.G., Amalaradjou, M.A.R. and Venkitanarayanan, K. (2020) Spoilage bacteria and meat quality. In *Meat Quality Analysis*, ed. Biswas, A.K. and Mandal, P.K. pp. 307–334. New York, NY: Academic Press.

Prieto, M., García, M.L., García, M.R., Otero, A. and Moreno, B. (1991) Distribution and evolution of bacteria on lamb carcasses during aerobic storage. *J Food Prot* **54**, 945–949.

Puligundla, P., Jung, J. and Ko, S. (2012) Carbon dioxide sensors for intelligent food packaging applications. *Food Control* **25**, 328–333.

Rossaint, S., Klausmann, S. and Kreyenschmidt, J. (2015) Effect of high-oxygen and oxygen-free modified atmosphere packaging on the spoilage process of poultry breast fillets. *Poult Sci* **94**, 96–103.

Rukchon, C., Nopwinyuwong, A., Trevanich, S., Jinkarn, T. and Suppakul, P. (2014) Development of a food spoilage indicator for monitoring freshness of skinless chicken breast. *Talanta* **130**, 547–554.

Sante, V., Renerre, M. and Lacourt, A. (2007) Effect of modified atmosphere packaging on color stability and microbiology of turkey breast meat. *J Food Qual* **17**, 177–195.

Vihavainen, E.J. and Björkroth, K.J. (2007) Spoilage of value-added, high-oxygen modified-atmosphere packaged raw beef steaks by *Leuconostoc gasicomitatum* and *Leuconostoc gelidum*. *Int J Food Microbiol* **119**, 340–345.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Summary of the visual and olfactory validation of inoculated meat pieces.

Figure S2. Sensorial validation of the olfactory of meat pieces.

Figure S3. Sensorial validation of the visual apperance of meat pieces.

Figure S4. Illustration of the experimental setup performed for all of the five meat-spoiling bacteria.