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Novel cadaverine non-invasive biosensor technology for the prediction of shelf life of modified atmosphere packed pork cutlets

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ABSTRACT

Food waste in perishable products calls for the development of cost-efficient and real-time freshness and shelf life assessment tools. The current study evaluated a newly developed cadaverine biosensor for its ability to assess the sensory freshness stage and microbial quality of modified atmosphere packed (MAP) pork cutlets under a realistic supply chain scenario.

The experiment compared the cadaverine levels measured by the biosensor to liquid chromatography - tandem mass spectrometry (LC-MS/MS) cadaverine concentrations, and associated these to the shelf life estimation and freshness states determined by sensory and microbial evaluations during an 18-day storage period (5 °C).

Results underlined the potential of cadaverine as a freshness biomarker as well as the applicability of the biosensor as a shelf life prediction tool. This is supported by the correlations obtained between sensory odour freshness evaluation and total viable counts with biosensor cadaverine levels for which the r obtained were 0.97 (<0.001) and 0.95 (<0.001), respectively.

1. Introduction

According to the UN Sustainable Development Goals (SDGs), reducing the food losses and waste along the supply chain and retail is a necessary action to decrease future demands on food production and limit the sector's environmental footprint (Godfray et al., 2010; Halloran, Clement, Kornum, Bucatariu, & Magid, 2014; UN, 2015). To this aim, optimizing shelf life prediction and date markings of food products can have a major positive impact. This is especially relevant for resource demanding, yet highly perishable food categories, such as chilled meat & fish products, for which one-third of the production that is wasted, half could have been avoided if more precise shelf life estimations were available (FAO, 2011, 2019).

Shelf life assessment and date marking for highly perishable products are defined via two important aspects, namely sensory and microbial degradation (Hazards et al., 2020). Within these frames, foods unfit for human consumption, include both foods with deteriorated sensory characteristics as well as foods that may cause risk to human health due to presence of pathogens and/or their toxins, including biogenic amines. This makes sensory evaluation of freshness along with enumeration of spoilage bacteria paramount for defining shelf life and creating the basis for decisions related to "best before labelling" (Corradini, 2018; Hazards et al., 2020). Such analyses are invasive, time consuming and expensive limiting their application to a small number of samples (Costa et al., 2020). Consequently, there is a need for rapid, cost-efficient and objective analytical tools and methods to evaluate the freshness and microbial quality of meat products in real time (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

Levels of biogenic amines have been proposed as alternative indicators of microbial spoilage and freshness quality of food products including meat (Costa et al., 2020; Halász, Barath, Simon-Sarkadi, & Holzapfel, 1994; Jairath, Singh, Dabur, Rani, & Chaudhari, 2015; Önal, 2007; Papageorgiou et al., 2018; Ruiz-Capillas & Herrero, 2019). Their detection and quantification is important also from a food safety

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perspective, since high levels of certain biogenic amines are associated with a health risk due to their toxicity (Ruiz-Capillas & Herrero, 2019; Shalaby, 1996). Novel nano biosensors for analysis of freshness biomarkers, such as biogenic amines without the requirement for sample preparation present a potential mean for this purpose (Chen, Gu, Zhang, Mao, & Tian, 2019; Costa et al., 2020; Mohammed, Bashammakh, Alsibaai, Alwael, & El-Shahawi, 2016; Wojnowski, Majchrzak, Dymerski, Gębicki, & Namieśnik, 2017).

The current study investigated the possibility of using a novel biosensor technology (micro-cantilever based prototype sensor) a mean to optimize the freshness assessment and shelf life prediction of a chilled pork product. The sensor uses a highly selective material for a biogenic biomarker, cadaverine, in the headspace of the food product. Cadaverine is a compound that accumulates in pork meat during storage, and can be correlated with increased bacterial counts (Costa et al., 2020; Custódio, Vasconcelos-Neto, Theodoro, Chisté, & Gloria, 2018). Previous work, has shown the validity of applying this technology for assessing shelf life and detecting differences in freshness states in yellowfin tuna steaks undergoing storage (Alexi et al., 2021a, 2021b).

The objectives of the present study were:

a) to assess the potential of using cadaverine as a quality marker and a shelf life indicator of pork cutlets in modified atmosphere packages (MAP) stored under refrigerated conditions (5 °C);
b) to evaluate the novel biosensor performance as a rapid and accurate freshness tool for routine analysis without the need for sample preparation.

To validate the outcomes, the cadaverine levels by the novel biosensor were compared to Liquid Chromatography- tandem mass spectrometry (LC-MS/MS) cadaverine concentrations and to the sensory and microbial freshness assessment performed on the same batch of MAP pork cutlets, stored under the same conditions. Samples originated from a conventional Danish slaughterhouse and the experimental conditions used, including packaging and storage, reflected the realistic local (Danish) supply chain of pork cutlets.

2. Materials and methods

2.1. Samples origin, production and packaging

The modified atmosphere packed (MAP: 70% O_2 + 30% CO_2) pork cutlets (in Danish: *koteletter*), were obtained from Danish Crown Foods A/S. Each package weighed approximately 200 g and contained two pork cutlets. The animals used for the cutlets production were reared in Denmark in a conventional farm (Danish pork is a mixed breed of Danish Landrace, Danish Yorkshire, and Danish Duroc) and were harvested 1–3 days prior to processing. The MAP packages were obtained directly after processing of pork meat into cutlets in the regular production line of Danish Crown Foods (Herning, DK) and were transported immediately to Aarhus University (AU-FOOD, Aarslev, DK). The packages were labelled and redistributed (<5 °C) at the same day to the Copenhagen University (KU), Danish Research Meat Institute (DMRI) and AmiNIC for microbial, cadaverine LC-MS/MS and biosensor analyses, respectively. The packages destined for sensory freshness analyses remained at the AU facilities and were stored at 5 ± 0.1 °C, until shelf life sampling.

2.2. Design of shelf life experiments

The shelf life experiments run simultaneously and under the same conditions (refrigerated storage of MAP packed pork cutlets at 5 °C) in all four experimental facilities. The packaging day was considered Day 0 (D0) of the shelf life experiments. The shelf life experiment allowed continuous sampling with 1–2 days intervals and explored changes up to and after the assigned "best before date" of the packages, which was D9 of the experiment. The specific sampling days used were D1 (initiation) with samples concurrently collected at D3, D5, D8, D11, D13, D15 and D18 of the shelf life experiments.

At each shelf life sampling date, four packages of MAP pork cutlets underwent sensory freshness evaluation. Microbial, cadaverine LC-MS/MS and sensor analyses were performed on two packages each. This accounted to a total of 10 replica packages being evaluated at each shelf life point. Within these frames, due to total high sampling per shelf life point, the two replicate packages used for certain measurements were considered as sufficient for satisfying the objective, which was to assess shelf life through different approaches in order to evaluate the potential of the sensor and volatile cadaverine as freshness tool and biomarker, respectively.

The sensory freshness evaluation, microbial and sensor analyses were performed directly after removing the packages from the 5 °C on the corresponding shelf life sampling dates. Packages destined for LC-MS/MS analyses, were transferred from 5 °C to −80 °C at each shelf life sampling date, and were stored there until further analysis.

2.3. Sensory freshness evaluation of pork cutlets

The sensory freshness evaluation concentrated on two modalities, namely the odour and the appearance changes of pork cutlets throughout shelf life. The evaluation scheme used corresponded to the odour scheme applied by the industry (Danish Crown) for internal freshness assessment, to allow valid comparison with previously gathered shelf life data for this type of meat and packaging conditions (DMRI predict curves: http://dmripredict.dk/). The appearance scheme was adapted accordingly to follow the stages of the odour scheme, which was constituted by point scale ranging from "Acceptable" (Fresh odour/appearance: 2 points), "Slightly diverging but acceptable" (Fresh with some slight off- odour/appearance: 4 points), "Diverging to an unacceptable degree" (Intense off-notes/appearance: 6 points), "Putrid, rotten" (Completely spoiled: 8 points).

A sensory panel consisting of eight (8) judges with previous experience in freshness assessment was employed for the evaluation. Prior to the first evaluation the panel participated in an initial training session during which the panel leader introduced the process, scheme and point scale used to evaluate each modality. Approximately five (5) judges participated in each of the evaluation sessions that corresponded to the shelf life sampling dates. Before each session, pork cutlets originating from the four replica packages were placed in individual coded white paper plates that did not reflect light. The samples were presented at room temperature to the judges who performed the evaluation individually using a printed scheme including the two point scales corresponding to the appearance and odour modalities, as well as an overall of evaluation of whether this fillet is acceptable for consumption (YES/NO). Once the evaluation was concluded, a panel discussion regarding the consensus of the individual judges' results was performed.

2.4. Microbiological evaluation of pork cutlets

At each shelf life sampling date, two pork cutlets originating from two replica packages were analysed. For bacterial enumerations, one sample of 10 g per cutlet was taken (duplicate) and homogenized in 0.1% peptone saline (CM0982; Oxoid Ltd., United Kingdom) by use of a stomacher (Stomacher 400 Lab Blender, Seward Medical, London, United Kingdom) for 30 s and subsequent serial dilutions in 0.1% peptone saline were performed.

Total aerobic, mesophilic count (Total Viable Count: TVC) was performed by pour plating using Plate Count Agar (PCA Oxoid, 25 °C for 3 d). Psychrotrophic bacterial counts were determined by surface plating on Long and Hammer agar media (15 °C for 5 d; Van Spreekens (1974)). Counts of lactic acid bacteria (LAB) counts were determined by nitrite polymyxin (NP) medium: APT agar (Merck, Darmstadt), with added 1 mL 12% Sodium nitrite (Ampliqon A/S, Odense, Denmark) and 0.2 mL Polymyxin supplement Oxoid (SR 099) per 200 mL agar (25 °C for 3 d).

Brochothrix thermosphacta counts were determined by Streptomycin Thallous Acetate medium (Oxoid; 25 °C for 3 d). *Escherichia coli* and coliforms (violet and bluish green colonies, respectively, on Rapid *E. coli* 2 agar (Biorad); 37 °C for 1 d) and Enterobacteriaceae (VRBG agar (Oxoid); 37 °C for 1 d) were determined by pour plating.

Colonies from both PCA and NP plates were isolated at random for purification and further characterization and identification. From the 150 isolates selected, 70 originated from NP plates and 80 from PCA plates. Ten isolates were taken at each of the eight sampling days (five from each replica package), with NP agar isolates being omitted from both replicas at D1, since no colonies were observed on plates with the lowest dilution. Assessment of cellular motility and morphology were performed, and all isolates were characterized by oxidase and catalase Gram reactions, if Gram negative and positive, respectively. MALDI-TOF MS (Biomérieux, France) was used for species identification and isolates were prepared for analysis by cultivation on Tryptone Soya Agar (Oxoid) with 5% bovine blood incubated for 3 d at 15 °C. A total number of 48 isolates were not identifiable by this method. Twenty two of the unidentified isolates representing the different profiles observed for cellular motility and morphology, Gram, oxidase and catalase reactions were selected for 16S rRNA gene sequence analysis for tentative species identification. Bacterial DNA was extracted with the commercially available Maxwell RSC Cultured Cells Kit (Promega Corporation, Denmark) and the 16S rRNA genes were amplified by polymerase chain reaction (PCR) using the universal primers 8-27F (5'-AGAGTTTGATCCTGGCTNAG-3′) (Weisburg, Barns, Pelletier, & Lane, 1991), and 1390-1408R (5'-TGACGGGCGGTGTGTACAA-3′) (Lane et al., 1985). Three forward primers and three reverse primers (Table 1) were used to sequence the PCR products (~1400 bp) in fragments by Macrogen EZ-seq. Contigs of the six reads form each isolate were generated using standard trim and assembly tools in CLC Main Workbench Ver. 20.0.3 (https://digitalinsights.qiagen.com), base pairing conflicts were resolved manually. The assembled contigs were identified searching both the database at NCBI (https://www.ncbi.nlm.nih.gov/refseq/targetedloci/) and the EZbiocloud database for comparison (Sayers et al., 2019; Yoon et al., 2017).

2.5. Cadaverine detection and quantification in pork cutlets via LC-MS/MS

The stored pork samples (−80 °C) were thawed at 10 °C prior to analyses. Each sample was blended and a subsample of 2 *g* was collected in a centrifuge PP-tube with 30 mL 0.1 N $HClO_4$ for homogenization at 2000 rpm for 30 s, followed by stirring at room temperature for 15 min and centrifugation at 4500 rpm for 60 min at 4 °C.

The extraction, purification, separation and detection steps were based on the method described in detail in Alexi et al. (2021b). In short the steps included an acid extraction with perchloric acid (Lázaro et al., 2013; Leszczyńska, 2018), followed by a solid phase purification by utilizing Strata®XL-CW ion-exchange columns (Sadjadi, Misa, & Krepich, 2017). For samples with a low cadaverine content 2.5 mL column extract was concentrated by N_2 to 100 μL and added 400 μL mobile phase A. For samples with a high content of cadaverine, 200 μL column extract was added 200 μL mobile phase A. The next step was a chromatographic separation using a reverse phase column with the heptaflourobutyric acid as ion-pairing agent (Häkkinen et al., 2007; Kaufmann & Maden, 2018) and lastly detection by MS/MS by electrospray ionization (ESI). 1.7-diaminoheptane (CAS no. 646–19-5), purchased from Sigma-Aldrich (Germany), was used in the internal standard in the purification phase.

Table 1
Primers used for sequencing the 16S rRNA gene. F and R corresponds to forwards and reverse reads.

Primer no.	Name	Sequence	Reference
1	8-27F	5′-AGA GTT TGA TCC TGG CTN AG-3'	Weisburg et al. (1991)
2	519F	5'-CAG CAG CCG CGG TAA YAL-3′	Standard primer
3	785-805F	5′-GGA TTA GAT ACC CNG GTA GTC-3′	Christensen (2018)
4	519R	5′-GTR TTA CCG CGG CTG CTG-3′	Lane et al. (1985)
5	785-805R	5'-GAC TAC CNG GGT ATC TAA TCC-3′	Dewhirst, Paster, Olsen, & Fraser (1992)
6	1390-1408R	5′-TGA CGG GCG GTG TGT ACA A-3′	Lane et al. (1985)

For the low level calibration, fresh pork cutlets were spiked with cadaverine, dihydrochloride (CAS no.1476-39-7) purchased from Sigma-Aldrich (Germany), in levels 11, 18, 38, 128, 508 and 1008 μg/kg. For the high level calibration, fresh pork cutlets were spiked with cadaverine, dihydrochloride (CAS no.1476-39-7), in suitable levels in the range 38–15,008 μg/kg. The cadaverine levels were measured prior and after spiking and the calibration process was similar to the one performed for tuna samples as described in Alexi et al. (2021b). Limit of detection (LOD) and limit of quantification (LOQ) were found to be 3 μg/kg and 10 μg/kg, respectively, however, for the high level calibrations a method LOQ of 50 μg/kg was estimated.

2.6. Prototype sensor and cadaverine detection

The set-up, operation and signal processing of the prototype biosensor is described in detail in Alexi et al. (2021b). In short, it consists of a micro-cantilever, functionalized by applying a smooth thin layer of cyclam complex (a volatile cadaverine selective binder), which is mounted on a circuit board and included in a sensor handheld device. Sampling is performed by holding the nozzle of the handheld device on the surface of the tissue, sucking in the headspace air with a flowrate of 5.95 m^3/h for 60 s. The airflow reaches the functionalised cantilever with an angle of 30°. When cadaverine chemically binds, the mass change induces a shift in the cantilever impedance related to the adsorbed cadaverine mass and therefore proportional to the concentration of cadaverine in the headspace.

For the pork cutlets experiments, two cantilevers were functionalised, each one destined for the measurement of one package replica throughout shelf life. Due to malfunctioning of one of the cantilevers, no replicated measurements were taken for the 1st half (D1–9) of the storage period. The measurements were taken directly on the shelf life sampling days on fresh tissue.

2.7. Statistical analyses

Determination of significant differences ($P < 0.05$) between storage days for freshness scores (averaged across judges per package replica), microbial counts, cadaverine concentration (μg/kg) and cadaverine responses (Ohm) was achieved by ANOVA (fixed factor: sampling day; Random factor: package replica). Post hoc analysis of the results was performed by the Duncan test. ANOVA and post-hoc analyses were performed in XLSTAT® software, 2016 (Addinsoft™). Due to the absence of replicated sensor measurement for the 1st half (D1–9) of the storage period, ANOVA and post-hoc testing were only performed for the 2nd half of the storage period. All results are reported as means and standard deviation of package replicas.

Dynamic microbial growth models were calculated according to Baranyi & Roberts (1994) using the DMFit (version 3.5) add-in in Microsoft Excel software, 2016. Furthermore, to study the linear correlation between different dataset measurements across shelf life sampling dates, simple linear regression models were calculated using Microsoft Excel software, 2016. Analysed relationships were evaluated by an F-test with a significance level of 5%, and are stated as correlation coefficients (r) accompanied with their respective *P*-values (*P* r).

3. Results

3.1. Sensory freshness of pork cutlets during storage

The odour scores showed a linear increase ($R^2 = 0.97$) during shelf life, ranging from "acceptable" on D1 to "Diverging to an unacceptable degree" on D18 of storage (Fig. 1). This increase was found significant according to the ANOVA model ($P < 0.001$). The post-hoc results of the freshness odour scores revealed a clear discrimination between D11 and D13 of storage, which coincided with the transition of acceptable to unacceptable for consumption according to the judges' assessment.

Appearance scores did not diverge from "acceptable" until D11 of storage, which was evaluated as last acceptable day for consumption of the cutlets by the freshness sensory judges (Fig. 2). From D13 of storage the appearance scores increased gradually, to ranges corresponding to "slightly diverging, but acceptable" for D13-D15 and "Diverging to an unacceptable degree" for D18. The appearance scores' increase during shelf life was found significant according to the ANOVA model ($P < 0.001$).

The largest variations (SD) in the freshness quality of replica packages of the same storage day were found in transitioning freshness periods and were most commonly located towards the end of the cutlets shelf life.

3.2. Microbial composition of pork cutlets during storage

Total viable counts (TVC) of aerobic bacteria increased significantly ($P < 0.001$) from log 3.5 CFU/g on D1, reaching approximately log seven CFU/g on D13–18 of storage (Fig. 3A). As indicated by the dynamic growth model the TVC increase rate was at log 0.27 CFU/g per day, reaching a final population of 7.1 CFU/g (plateau), with an R^2 of 0.94 (standard error, SE of fit: log 0.33 CFU/g). Lactic acid bacteria (LAB) counts were initially lower than the TVC but reached the same or slightly higher maximum level after an increase of log five CFU/g (Fig. 3B). This is also reflected in the higher increase rate of the dynamic growth model for LAB, which was log 0.60 CFU/g per day, reaching a final population of 7.2 CFU/g (plateau), with an R^2 of 0.97 (SE of fit: log 0.35 CFU/g). Similarly, to TVC, LAB counts exhibited a significant ($P < 0.001$) increase with shelf life, with counts of D13 (and onwards) being found significantly higher than D11, which coincided with the transition of pork cutlets from acceptable to unacceptable for consumption according to the freshness sensory evaluation (Fig. 3A and B). *B. thermosphacta* counts varied significantly ($P = 0.029$) during storage, with levels ranging between log two (D1-D5) and three (D8-D13) CFU/g for the initial 13 days of storage, but increased to log five CFU/g on D15 (Fig. 3C). The *B. thermosphacta* dynamic growth model, indicated an increase rate of log 0.21 CFU/g per day, whereas final population (plateau) was not reached within the storage period examined (R^2: 0.59; SE of fit 0.98). The total number of psychrophilic/psychrotrophic bacteria varied between log three and log five CFU/g throughout storage but increased to log seven CFU/g at D18 for one package replica (Fig. 3D). However, this increase was not validated by the ANOVA model ($P = 0.154$) due to the significant variance between package replicas ($P = 0.015$) identified from D8 of storage and onwards. According to the dynamic growth model, an increase rate of log 0.11 CFU/g per day, was found however with an R^2: 0.21 (SE of fit 1.09), whereas final population (plateau) was not reached within the storage period examined. Counts of coliforms as determined by Rapid *E. coli* 2 agar and Enterobacteriaceae as determined by VRBG remained below log two CFU/g until D18 at which time concentrations increased to log 4.0 and log 3.0 CFU/g, respectively, for one of the replicas, but not the other.

TVC ($r = 0.97$; $Pr < 0.001$), LAB ($r = 0.90$; $Pr = 0.002$) and *B. thermosphacta* ($r = 0.85$; $Pr = 0.007$) counts were all found to correlate to the odour freshness assessment scores, with TVC showing the strongest relationship. No correlations were identified between freshness odour scores and psychrophilic/psychrotrophic counts or Enterobacteriaceae counts.

Identification of the predominant microbial flora as assessed by PCA counts showed a predominance of the LAB species *Leuconostoc carnosum*, especially from D11 to D15 of storage (Table 2). Notably, there was a higher degree of variety from D1 to D8 and again at D18. Other taxons encountered more than sporadically from D1 to D8 included *Acinetobacter* spp., *Bacillus pumilus*, *Chryseobacterium* spp. and *Pseudomonas* spp. It is also of interest to note the observation of *B. thermosphacta* in one package at the end of storage (D18), which agrees with higher specific counts for this species at this shelf life stage (Fig. 3C).

Identification of the LAB flora as assessed by NP agar counts demonstrated again the predominance of L. *carnosum* throughout storage (Table 3). *Weissella* spp. were also encountered throughout storage, whereas *Latilactobacillus* spp. were observed initially and *Carnobacterium* spp. at the end of storage.

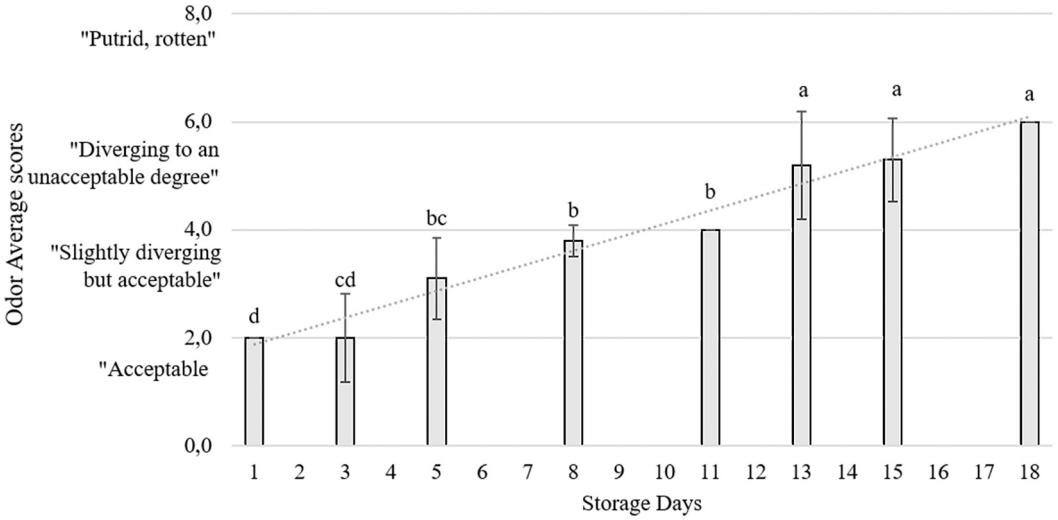

Fig. 1. Average odour freshness scores corresponding to four cutlet packages per day of storage. Bars represent the standard deviation between package replicates. Lowercase letters indicate post-hoc groupings (Duncan). Cutlets were found unacceptable for consumption from D13 of shelf life and onwards. A linear relationship $R^2 = 0.97$ was identified between odour scores and storage time.

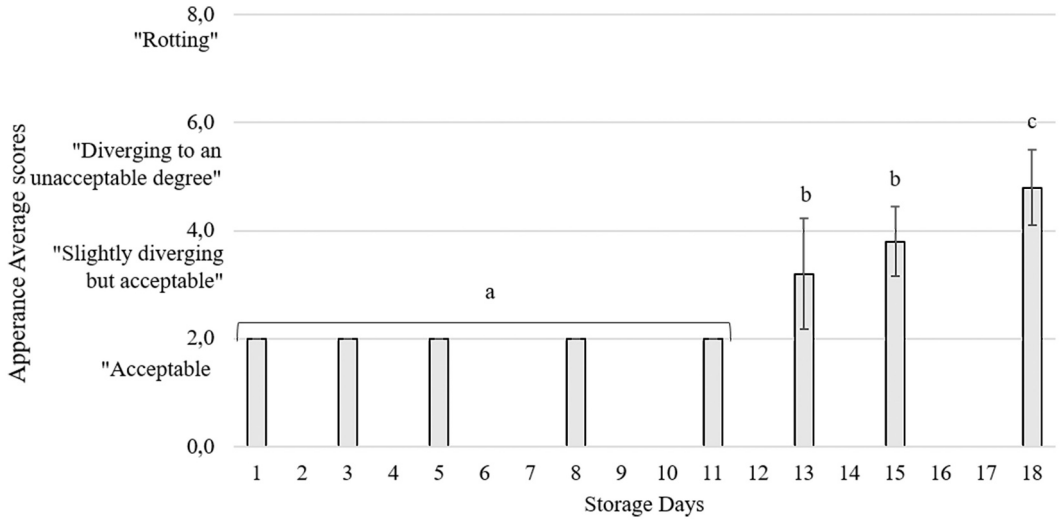

Fig. 2. Average appearance freshness scores corresponding to four MAP cutlet packages per day of storage (5 °C). Bars represent the standard deviation between package replicates. Lower case letters indicate post-hoc groupings (Duncan). Cutlets were found unacceptable for consumption from D13 of shelf life and onwards.

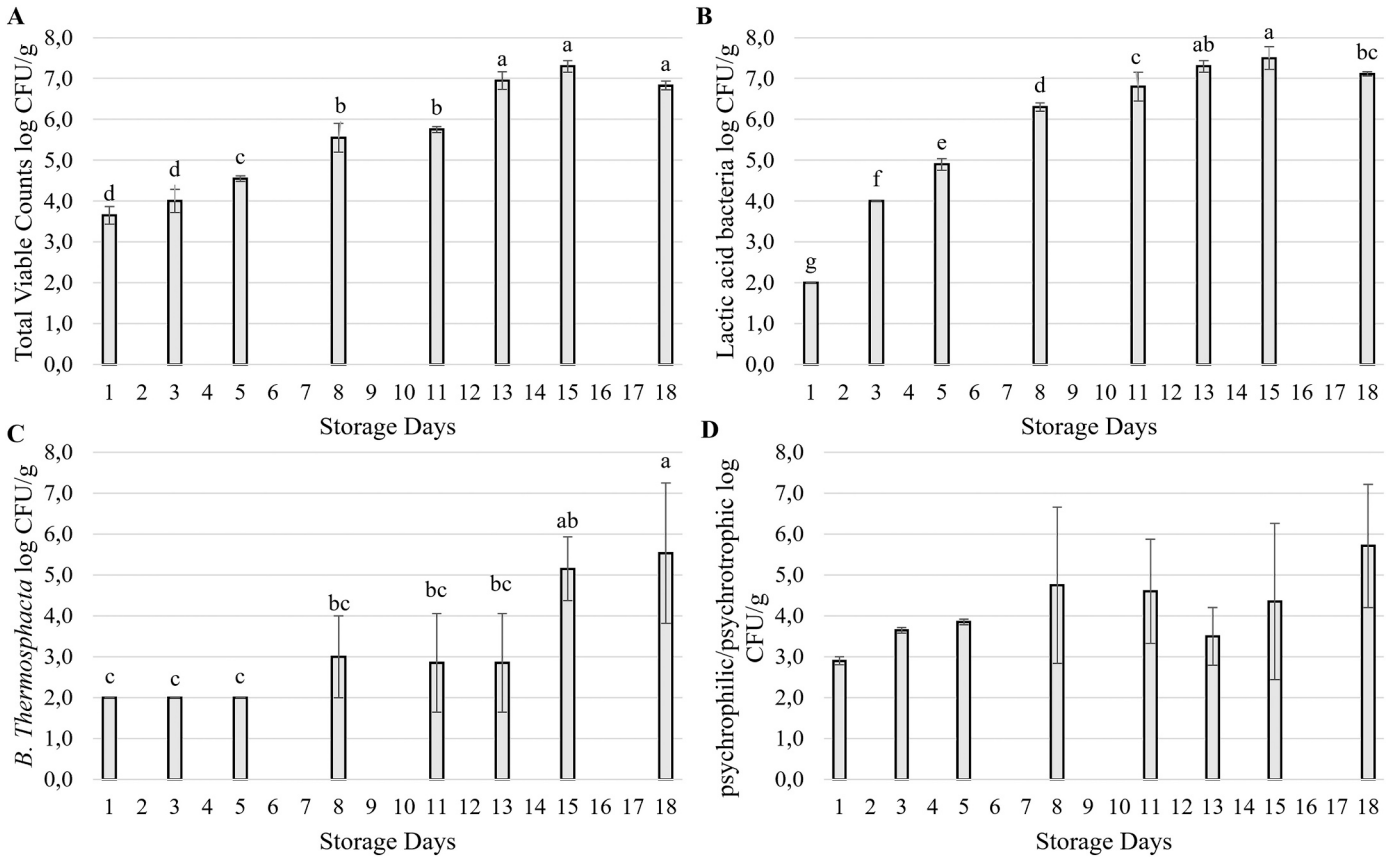

Fig. 3. A) TVC (Total Viable Counts); B) Lactic acid bacteria counts; C) *Brochothrix thermosphacta* counts and D) Total psychrophilic/psychrotrophic counts, per day of storage (5 °C) of MAP cutlet packages. Bars represent the standard deviation between package replicates. Lower case letters indicate post-hoc groupings (Duncan).

3.3. Cadaverine concentration in pork cutlet tissue during storage

The concentration of tissue cadaverine as defined via LC-MS/MS increased significantly ($P = 0.037$) during from D1 to D18 of storage. The average cadaverine levels gradually increased from 24 μg /Kg on D1 to 201 μg /Kg on D13, whereas a very fast accumulation was noted thereafter, with cadaverine reaching an average level of 2790 μg /Kg and 5817 μg /Kg on D15 and D18 of storage, respectively (Fig. 4).

Examining separately the period up to D13, which signified the transition of acceptable (up to D11) to unacceptable (D13) for consumption according to the sensory freshness assessment, a significant increase in the levels of cadaverine was noted ($P = 0.026$). This was described by significant increase from D1 to D3-D8 of storage, where cadaverine levels remained approximately stable. Thereafter, a gradual increase accounting to approximately 60% of the previous storage day's concentration was noted, with average cadaverine levels reaching on

Table 2
Identification of microorganisms isolated from the PCA-agar by MALDI-TOF or 16S rRNA gene (*) analysis. P1 and P2 corresponds to Package 1 and Package 2 pork cutlet replicas, respectively. Isolates with confidence below 75% match using the MALDI-TOF and below 99% similarity using 16S rRNA gene analysis in identification, are reported at genus level.

Taxon	Total	Day 1		Day 3		Day 5		Day 8		Day 11		Day 13		Day 15		Day 18	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Acinetobacter iwoffi	2				1		1										
Acinetobacter johnsonii	4		1			1			2								
Bacillus pumilus	5	2	1	1					1								
Bacillus amyloliquefaciens	1																1
*Brevundimonas intermedia**	1				1												
Brevundimonas sp.*	1				1												
Brochothrix thermosphacta	2															2	
Chryseobacterium spp.*	4		1	2			1										
Cronobacter muytjensii	1															1	
*Galactobacter caseinivorans**	1			1													
Gordonia polyisoprenivorans	1	1															
*Lactococcus carnosus**	1															1	
*Lactococcus piscium**	1				1												
Leuconostoc carnosum	24		1				2			5	5	4	3		4		
*Leuconostoc gelidum**	2											1	1				
Micrococus luteus	1				1												
Pseudomonas fluorescens	3					3											
Pseudomonas fragi	6		1	1		1		2						1			
Rhodococcus erythropolis	2	2															
Serratia protamaculans	1							1									
Cryptococcus curvatus	1								1								
Pseudallescheria boydii	1							1									
Not identified	14						1	1[1]	1				1[1]	4[1]	1[1]	1[1]	4[1]
total	80	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[1] Phenotypic similar to identified *Leuconostoc gelidum* isolates identified by 16S rRNA gene analysis.

Table 3
Identification of bacteria isolated from the NP-agar by MALDI-TOF or 16S rRNA gene (*) analysis. P1 and P2 corresponds to Package 1 and Package 2 pork cutlet replicas, respectively. Isolates with confidence below 75% match using the MALDI-TOF and below 99% similarity using 16S rRNA gene analysis, are reported at genus level.

Bacterial isolates	Total	Day 1		Day 3		Day 5		Day 8		Day 11		Day 13		Day 15		Day 18	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Carnobacterium maltaromaticum	3															3	
Carnobacterium divergens	1															1	
Latilactobacillus sakei	5					3	1								1		
Latilactobacillus curvatus	1				1												
Leuconostoc carnosum	35			2	2		2	1	5	2	2	5	2	4	3	1	4
Leuconostoc gelidum *	1									1							
Leuconostoc sp.*	1										1						
*Weissella cryptocerci**	5			1	1		1	1							1		
Weissella spp.*	4					1		1			1			1			
Fusarium chlamydosporum	1										1						
Not identified	13			2[1&2]	1[2]	1[2]	1[1]	2[2]		2[1]			3[1]				1[1]
Total N	70	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[1] Phenotype similar to identified *Leuconostoc* spp. isolates identified by 16S rRNA gene analysis.
[2] Phenotype similar to *Weissella* spp. isolates identified by 16S rRNA gene analysis.

average 124 and 201 μg /Kg in D11 and D13, respectively (Fig. 4B).

Both throughout the whole storage period examined (D1-D18) and the critical storage period (D1-D13), an order 3 polynomial relationship with a R^2 of 0.975 and 0.999, respectively, was identified between cadaverine and storage time. For the same storage periods, the R^2 of an exponential relationship between cadaverine and storage time was 0.831 and 0.909 for D1-D18 and D1-D13, respectively.

The cadaverine tissue concentration showed a strong correlation to the *B. thermosphacta* counts ($r = 0.91$; $Pr = 0.006$). No other significant correlation was identified between cadaverine concentration and microbial counts. A significant but not as strong correlation ($r = 0.72$; $Pr = 0.046$) was identified between cadaverine concentration and odour freshness assessment scores.

3.4. Sensor responses to volatile cadaverine during storage of pork cutlet tissue

The sensor response showed an increase in volatile cadaverine levels measured from pork cutlets, with shifts in impedance ranging from approximately 6×10^5 Ohms on D1 to 16×10^5 Ohms on D18 (Fig. 5). This increase was validated by the Duncan post-hoc test indicating that D11 and D18 belong in significantly different response groups, however for D1-D8 due to absence of duplicated measurement (cantilever No 2 malfunctioning) no validation was possible. The biggest change in average cadaverine responses between subsequent storage days was noted between D11 to D13 of storage, with levels accounting to approximately 10 and 14×10^5 Ohms, respectively. Similarly to sensory, for microbial and tissue cadaverine concentration data, the largest variations between replicate packages of the same storage day were identified in transitioning freshness periods, such as D13, and were most

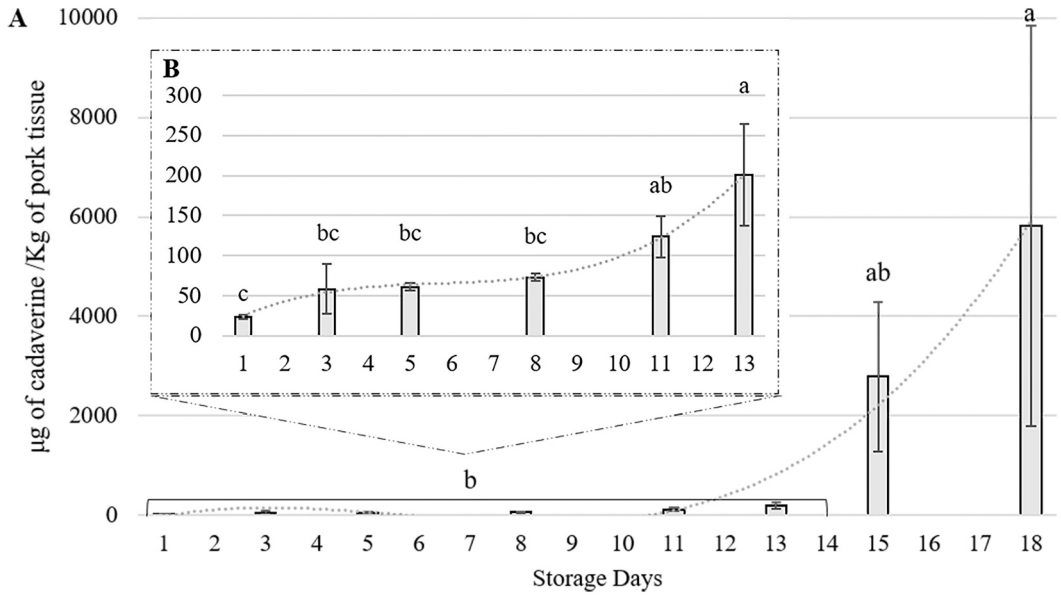

Fig. 4. Cadaverine concentration in μg /Kg of pork cutlet tissue stored at 5 °C as identified via LC-MS/MS for: A) the total storage period, D1-D18; B) up to D13, which signified the transition of acceptable (up D11) to unacceptable (D13) for consumption according to the sensory freshness assessment. Lower case letters indicate post-hoc groupings according to the Duncan test performed on D1-D13 and D1-D18 data for A and B, respectively. Dotted lines represent an order 3 polynomial relationship between cadaverine and storage time with a R^2 of 0.975 and 0.999, for A and B, respectively. The R^2 of an exponential relationship between cadaverine and storage time was 0.831 and 0.909 for A and B, respectively.

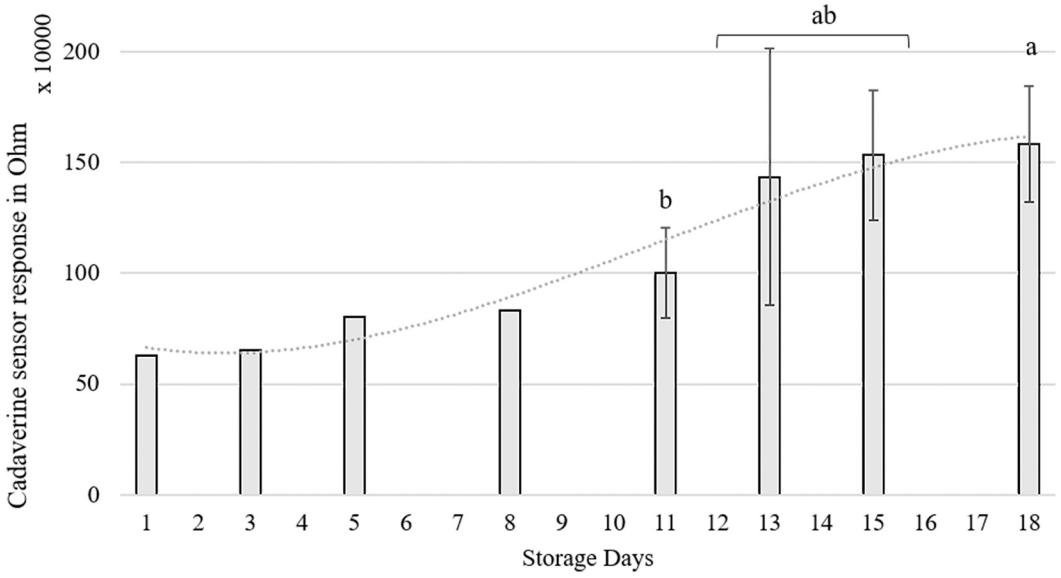

Fig. 5. Headspace cadaverine response in Ohms of pork cutlet tissue stored at 5 °C as identified via the sensor for the storage period of D1-D18. Lower case letters indicate post-hoc groupings according to the Duncan test (For D1 to D8 data included originate only from cantilever No 1, due to malfunctioning of cantilever No 2). Dotted lines represent an order 3 polynomial relationship between cadaverine and storage time with a R^2 of 0.952 (D1 to D13, $R^2 = 0.978$). The R^2 of an exponential relationship between cadaverine and storage time was 0.944 (D1 to D13, $R^2 = 0.904$).

commonly located towards the end of the cutlets shelf life.

An order 3 polynomial relationship between cadaverine and storage time was identified with a R^2 of 0.952 and 0.978, for D1-D18 and D1-D13, respectively. For the same storage periods, the R^2 of an exponential relationship between cadaverine and storage time was 0.944 and 0.904 for D1-D18 and D1-D13, respectively.

The strongest correlations between sensor responses and other relevant shelf life data were found with the sensory freshness odour scores ($r = 0.97$; $Pr < 0.001$) as well as TVC counts ($r = 0.95$; $Pr < 0.001$). LAB ($r = 0.83$; $Pr = 0.012$) and *B. thermosphacta* ($r = 0.86$; $Pr = 0.006$) counts as well as tissue cadaverine concentration ($r = 0.74$; $Pr = 0.035$), also significantly correlated to the sensor responses, yet not as strongly as for freshness odour scores and TVC counts.

4. Discussion

4.1. Pork cutlets quality changes and shelf life estimation during MAP storage at 5 °C

The agreement noted between the sensory freshness and microbial

quality results indicate that the end of shelf life of the MAP pork cutlet's batch, investigated herein, is located between D11 and D13 of storage at 5 °C. These results coincide with the predictive DMRI meat models, according to which an odour score of 4 ("acceptable"), is expected to occur on D11 of storage for the same meat cuts, packaging and storage conditions (initial psychrotrophic count log of 2.95 CFU used for model creation). Therefore, according to the date marking assigned to this product batch corresponding to D9 of storage, the assigned shelf life could have been extended by approximately 2–3 days, without any major changes in the cutlets quality.

Examining the sensory and microbial results, not all aspects measured were equally important for defining the shelf life of pork cuts. Muscle colour has been found as important aspect of acceptance and expected freshness upon purchase for pork products (Brewer, 2001; Brewer, Jensen, Prestat, Zhu, & Mckeith, 2002). However, appearance did not play a role in defining shelf life in this study, since changes occurred after D11 of storage.

With respect to microbial counts, TVC and LAB growth were more discriminative for shelf life evaluation. The growth of the microbial flora, as assessed by overall and specific counts, agrees with maximum counts for MAP pork chops observed by Sørheim, Nissen, and Nesbakken (1999). Moreover, the initial microbial flora showed a diversity mostly typical for pork carcasses including the presence of *Acinetobacter* spp., *Brevundimonas* spp., *Carnobacterium* spp., *Chryseobacterium* spp., *Leuconostoc* spp., *Pseudomonas* spp. but excluding e.g. *Staphylococcus* spp. (Peruzy et al., 2021). *L. carnosum*, predominant during most of the storage time, is commonly associated with raw and processed meat products (Audenaert et al., 2010; Candeliere et al., 2020; Laursen, Byrne, Kirkegaard, & Leisner, 2009; Raimondi et al., 2019; Shaw & Harding, 1989; Vasilopoulos et al., 2008). This species is, however, not prevalent on pork meat at all instances (Cauchie et al., 2020; McMullen & Stiles, 1993). In overall, the microbial composition of MAP pork cutlets observed in this study appear to be representative for this type of products.

4.2. Microbial production of cadaverine in pork cutlets during MAP storage at 5 °C

Cadaverine is a commonly found in pork meat (Halász et al., 1994; Ngapo & Vachon, 2017), coinciding with the results of the current study which show an accumulation of this compound pork cutlet tissue with storage.

Strains of L. *carnosum* produces tyramine but not cadaverine (Bover-Cid & Holzapfel, 1999). Neither do tested strains of *Carnobacterium* spp. and *Weisella* spp., including *Weisella cryptocerci,* whereas *Latilactobacillus curvatus* may produce cadaverine (Barbieri, Montanari, Gardini, & Tabanelli, 2019; Bover-Cid & Holzapfel, 1999; Heo et al., 2019). Presumably, this is not relevant for the product examined in this study, since *Latilactobacillus curvatus* was observed only initially, whereas cadaverine accumulated at the end of storage. The possibility remains, however, that this species was present throughout storage in numbers more than one log unit lower than the overall PCA and NP counts.

Among the non-LAB taxons, species belonging to Enterobacterales are commonly suggested as sources of biogenic amines including cadaverine in meat products (Bover-Cid, Hernández-Jover, Miguélez-Arrizado, & Vidal-Carou, 2003; Durlu-Özkaya, Ayhan, & Vural, 2001; Li et al., 2014a; Pircher, Bauer, & Paulsen, 2007). We only found a few taxons belonging to this order sporadically during storage by use of PCA, whereas counts were below detection limits on media specific for Enterobacteriaceae except for an increase in counts at D18. Enterobacterales colonies on PCA could be overlooked, if present in numbers more than tenfold lower than the predominant PCA isolates of LAB flora at most sampling times. The sporadic observation of *Serratia protamaculans* at D8 and *Cronobacter muytjensii* on PCA at D18 in one package replica, points to this possibility.

Among the other non-LAB, *Chryseobacterium* spp., belonging to the Flavobacteriaecea family observed initially during storage has potential for production of cadaverine at 15 °C but not, or only weakly, at 7 °C limiting the likelihood that this genus was the source of this compound (Mielmann, Hugo, & Jooste, 2011). The relatively low numbers of psychrotrophs/ psychrophile bacteria also limited the probability that they served as a major source of the observed cadaverine. Interestingly, while *B. thermosphacta* counts correlated significantly with cadaverine concentrations, this species has not been reported to produce cadaverine. However, it has been shown that *B. thermosphacta* can promote the production of cadaverine by *E. coli*, which can explain partly the results observed herein (Nowak & Czyzowska, 2011). Strains belonging to *P. fluorescens* have the potential for producing cadaverine, but this species was generally not observed towards the end of the storage period. All in all, whereas some indications exist, the precise bacterial producers of cadaverine late in the storage period remains to be elucidated in the product examined herein.

4.3. Cadaverine as a shelf life biomarker and freshness assessment by the sensor

Cadaverine concentrations observed in this study were below the tolerable levels suggested for other food products (e.g. fish and fish products: 510 mg/kg) by Rauscher-Gabernig et al. (2012). The sudden increase in cadaverine at D11 and onwards, after a plateau observed in the concentration between D3-D8, reflected in overall the changes in the sensory and microbial quality that rendered the pork cutlets unacceptable for consumption at D13. This is supported by the correlation of the cadaverine concentrations to the odour freshness assessment and *B. thermosphacta* counts that has been associated to pork spoilage and off-odour development under the given storage conditions (Koutsoumanis, Geornaras, & Sofos, 2006; Liu, Yang, & Li, 2006). Indeed, cadaverine has been shown to be a useful measure for monitoring spoilage in chilled pork, underlining the usage of cadaverine as a shelf_life biomarker for chilled MAP pork cutlets (Costa et al., 2020; Li et al., 2014b). Considering that the MAP pork loins' shelf life can vary great in retail, applying cadaverine as a real-time measure of freshness and shelf life potential indicator can create a solution for reducing the losses observed at the consumption level (Livingston, Susan Brewer, Killifer, Bidner, & McKeith, 2004).

Examining the sensor applicability for freshness assessment, significant correlations were found between the headspace cadaverine levels to all sensory (odour) and microbial aspects (TVC, LAB and *B. thermosphacta* counts) determining the shelf life of the MAP pork cutlets. Moreover, a significant yet not as high correlation was obtained between the headspace and tissue cadaverine measured by the sensor and LC-MS/MS, respectively. Within these frames, it seems that the headspace concentration of cadaverine (sensor) is more representative of freshness quality measurements (sensory odour & microbial) when compared to tissue concentrations (LC-MS/MS). We propose that this is related to the differential pattern of increase across the different types of measurements. In specific, tissue cadaverine is accumulating very fast from D15 and onwards (a pattern not observed in the headspace cadaverine), whereas at this point microbial growth (TVC, LAB) reach a plateau associated to the end of shelf life. On the other hand, this does not apply to *B. thermosphacta*, which is still showing an increase in log CFU counts even after D15, explaining therefore the significant correlation to cadaverine tissue concentration. Since the sensory freshness odour scores show a relative –but not as intense- increase they correlate to both tissue and headspace cadaverine, yet still better to the latter. These results are supported by our previous work examining the applicability of the sensor for evaluation of the freshness of chilled tuna steaks, where similar patterns of correlations among different types of measurements were observed (Alexi et al., 2021b).

This outcome underlines that the sensor has the potential to become cheap and non-invasive measure the assess freshness of chilled MAP pork cutlets as shown for tuna loins at our previous work (Alexi et al.,

2021b). However, more experiments are required to identify the specific sensor cut-off points that define the transition from acceptable to unacceptable for consumption for MAP pork cutlets; since the variance in the cadaverine levels observed between different packages at D13, does not allow for a significant cut off point to be determined. Moreover, it would be of interest to examine the appropriateness of the sensor as well as volatile cadaverine as a freshness biomarker in different temperature conditions, including lower (0–4 °C) and abuse (8–10 °C) temperatures, commonly encountered in the supply chain of fresh meat products, such as retail and consumer households (Kennedy et al., 2005; Koutsoumanis, Stamatiou, Skandamis, & Nychas, 2006; Limbo, Torri, Sinelli, Franzetti, & Casiraghi, 2010). Considering our previous results in tuna, examining the biosensor potential in lower storage temperatures (~2 °C) and literature indicating an increased production of cadaverine and as a function of elevated storage temperatures (Alexi et al., 2021b; De Filippis et al., 2013; Komitopoulou, 2012), we expect that the sensor and biomarker can be used as freshness indicators under variable temperature storage conditions.

Ethical statement

The current work does not include experimentation with living animals, since the tissue used was obtained by a commercial distributor (Danish Crown Foods). Therefore, no ethical issues were raised in regard with animal experiments and no respective approval was required by an ethical committee.

CRediT authorship contribution statement

Niki Alexi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Kristian Thamsborg:** Methodology, Validation, Investigation, Writing – original draft, Writing – review & editing. **Jeanette Hvam:** Conceptualization, Methodology, Resources, Writing – review & editing, Project administration, Funding acquisition. **Birgitte W. Lund:** Validation, Investigation, Writing – review & editing. **Lawrence Nsubuga:** Software, Validation, Investigation, Data curation, Writing – review & editing. **Roana Melina de Oliveira Hansen:** Data curation, Writing – review & editing, Supervision. **Derek V. Byrne:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Jørgen J. Leisner:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

None.

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References

Alexi, N., Hvam, J., Lund, B. W., Nsubuga, L., de Oliveira Hansen, R. M., Thamsborg, K., … Leisner, J. J. (2021a). Corrigendum to 'Potential of novel cadaverine biosensor technology to predict shelf life of chilled yellowfin tuna (*Thunnus albacares*). *Food Control, 125*, 107958. https://doi.org/10.1016/j.foodcont.2020.107458

Alexi, N., Hvam, J., Lund, B. W., Nsubuga, L., de Oliveira Hansen, R. M., Thamsborg, K., … Leisner, J. J. (2021b). Potential of novel cadaverine biosensor technology to predict shelf life of chilled yellowfin tuna (Thunnus albacares). *Food Control, 119*, Article 107458.

Audenaert, K., D'Haene, K., Messens, K., Ruyssen, T., Vandamme, P., & Huys, G. (2010). Diversity of lactic acid bacteria from modified atmosphere packaged sliced cooked meat products at sell-by date assessed by PCR-denaturing gradient gel electrophoresis. *Food Microbiology, 27*(1), 12–18.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology, 23*(3–4), 277–294.

Barbieri, F., Montanari, C., Gardini, F., & Tabanelli, G. (2019). Biogenic amine production by lactic acid bacteria: A review. *Foods, 8*(1), 17.

Bover-Cid, S., Hernández-Jover, T., Miguélez-Arrizado, M. J., & Vidal-Carou, M. C. (2003). Contribution of contaminant enterobacteria and lactic acid bacteria to biogenic amine accumulation in spontaneous fermentation of pork sausages. *European Food Research and Technology, 216*(6), 477–482.

Bover-Cid, S., & Holzapfel, W. H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology, 53*(1), 33–41.

Brewer, M. S. (2001). Consumer attitudes towards color and marbling of fresh pork. In *Facts: Pork Quality* (p. 4520). National Pork Board.

Brewer, M. S., Jensen, J., Prestat, C., Zhu, L. G., & Mckeith, F. K. (2002). Visual acceptability and consumer purchase intent of enhanced pork loin roasts. *Journal of Muscle Foods, 13*(1), 53–68. https://doi.org/10.1111/j.1745-4573.2002.tb00320.x

Candeliere, F., Raimondi, S., Spampinato, G., Tay, M. Y. F., Amaretti, A., Schlundt, J., & Rossi, M. (2020). Comparative genomics of *Leuconostoc carnosum*. *Frontiers in Microbiology, 11*.

Cauchie, E., Delhalle, L., Taminiau, B., Tahiri, A., Korsak, N., Burteau, S., … Daube, G. (2020). Assessment of spoilage bacterial communities in food wrap and modified atmospheres-packed minced pork meat samples by 16S rDNA metagenetic analysis. *Frontiers in Microbiology, 10*, 3074.

Chen, J., Gu, J., Zhang, R., Mao, Y., & Tian, S. (2019). Freshness evaluation of three kinds of meats based on the electronic nose. *Sensors, 19*(3), 605.

Christensen, H. (2018). Introduction. In H. Christensen (Ed.), *Introduction to bioinformatics in microbiology* (pp. 1–8). Cham: Springer International Publishing.

Corradini, M. G. (2018). Shelf life of food products: From open labeling to real-time measurements. *Annual Review of Food Science and Technology, 9*, 251–269.

Costa, C. A. B., Grazhdan, D., Fiutowski, J., Nebling, E., Blohm, L., Lofink, F., … de Oliveira Hansen, R. (2020). Meat and fish freshness evaluation by functionalized cantilever-based biosensors. *Microsystem Technologies, 26*(3), 867–871. https://doi.org/10.1007/s00542-019-04598-7

Custódio, F. B., Vasconcelos-Neto, M. C., Theodoro, K. H., Chisté, R. C., & Gloria, M. B. A. (2018). Assessment of the quality of refrigerated and frozen pork by multivariate exploratory techniques. *Meat Science, 139*, 7–14.

De Filippis, F., Pennacchia, C., Di Pasqua, R., Fiore, A., Fogliano, V., Villani, F., & Ercolini, D. (2013). Decarboxylase gene expression and cadaverine and putrescine production by *Serratia proteamaculans* in vitro and in beef. *International Journal of Food Microbiology, 165*(3), 332–338.

Dewhirst, F. E., Paster, B. J., Olsen, I., & Fraser, G. J. (1992). Phylogeny of 54 representative strains of species in the family Pasteurellaceae as determined by comparison of 16S rRNA sequences. *Journal of Bacteriology, 174*(6), 2002–2013.

Durlu-Özkaya, F., Ayhan, K., & Vural, N. (2001). Biogenic amines produced by Enterobacteriaceae isolated from meat products. *Meat Science, 58*(2), 163–166.

FAO. (2011). *Global food losses and food waste - extent, causes and prevention*. Italy: FAO Rome.

FAO. (2019). The state of food and agriculture 2019. In *Moving Forward on Food Loss and Waste Reduction*. FAO Rome, Italy.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., … Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science, 327*(5967), 812–818.

Häkkinen, M. R., Keinänen, T. A., Vepsäläinen, J., Khomutov, A. R., Alhonen, L., Jänne, J., & Auriola, S. (2007). Analysis of underivatized polyamines by reversed phase liquid chromatography with electrospray tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis, 45*(4), 625–634. https://doi.org/10.1016/j.jpba.2007.09.001

Halász, A., Barath, A., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology, 5*(2), 42–49.

Halloran, A., Clement, J., Kornum, N., Bucatariu, C., & Magid, J. (2014). Addressing food waste reduction in Denmark. *Food Policy, 49*, 294–301.

Hazards, E. P., Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., … Herman, L. (2020). Guidance on date marking and related food information: Part 1 (date marking). *EFSA Journal, 18*(12), Article e06306.

Heo, J., Hamada, M., Cho, H., Weon, H.-Y., Kim, J.-S., Hong, S.-B., … Kwon, S.-W. (2019). *Weissella cryptocerci* sp. nov., isolated from gut of the insect *Cryptocercus kyebangensis*. *International Journal of Systematic and Evolutionary Microbiology, 69*(9), 2801–2806.

Jairath, G., Singh, P. K., Dabur, R. S., Rani, M., & Chaudhari, M. (2015). Biogenic amines in meat and meat products and its public health significance: A review. *Journal of Food Science and Technology, 52*(11), 6835–6846.

Kaufmann, A., & Maden, K. (2018). Easy and fast method for the determination of biogenic amines in fish and fish products with liquid chromatography coupled to orbitrap tandem mass spectrometry. *Journal of AOAC International, 101*(2), 336–341. https://doi.org/10.5740/jaoacint.17-0407

Kennedy, J., Jackson, V., Blair, I., McDowell, D., Cowan, C., & Bolton, D. (2005). Food safety knowledge of consumers and the microbiological and temperature status of their refrigerators. *Journal of Food Protection, 68*(7), 1421–1430.

Komitopoulou, E. (2012). Microbial and chemical markers of meat spoilage. *International Food & Meat Topics, 3*, 23–25.

Koutsoumanis, K., Stamatiou, A., Skandamis, P., & Nychas, G.-J. (2006). Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. *Applied and Environmental Microbiology, 72*(1), 124–134.

Koutsoumanis, K., Geornaras, I., & Sofos, J. N. (2006). Microbiology of land muscle foods, Handbook of Food Science, Technology and Engineering. *Taylor & Francis, 1*, 52.51-52.43.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences, 82*(20), 6955–6959.

Laursen, B. G., Byrne, D. V., Kirkegaard, J. B., & Leisner, J. J. (2009). Lactic acid bacteria associated with a heat-processed pork product and sources of variation affecting chemical indices of spoilage and sensory characteristics. *Journal of Applied Microbiology, 106*(2), 543–553.

Lázaro, C. A., Conte-Júnior, C. A., Cunha, F. L., Mársico, E. T., Mano, S. B., & Franco, R. M. (2013). Validation of an HPLC methodology for the identification and quantification of biogenic amines in chicken meat. *Food Analytical Methods, 6*(4), 1024–1032.

Leszczyńska, A. C. M. J. (2018). Liquid chromatography–single-quadrupole mass spectrometry as a responsive tool for determination of biogenic amines in readyto-eat baby foods. *Chromatographia, 81*, 901–910.

Li, M., Tian, L., Zhao, G., Zhang, Q., Gao, X., Huang, X., & Sun, L. (2014a). Formation of biogenic amines and growth of spoilage-related microorganisms in pork stored under different packaging conditions applying PCA. *Meat Science, 96*(2), 843–848.

Li, M., Tian, L., Zhao, G., Zhang, Q., Gao, X., Huang, X., & Sun, L. (2014b). Formation of biogenic amines and growth of spoilage-related microorganisms in pork stored under different packaging conditions applying PCA. *Meat Science, 96*(2, Part A), 843–848. https://doi.org/10.1016/j.meatsci.2013.09.023

Limbo, S., Torri, L., Sinelli, N., Franzetti, L., & Casiraghi, E. (2010). Evaluation and predictive modeling of shelf life of minced beef stored in high-oxygen modified atmosphere packaging at different temperatures. *Meat Science, 84*(1), 129–136.

Liu, F., Yang, R.-Q., & Li, Y.-F. (2006). Correlations between growth parameters of spoilage micro-organisms and shelf-life of pork stored under air and modified atmosphere at −2, 4 and 10°C. *Food Microbiology, 23*(6), 578–583. https://doi.org/10.1016/j.fm.2005.10.002

Livingston, M., Susan Brewer, M., Killifer, J., Bidner, B., & McKeith, F. (2004). Shelf life characteristics of enhanced modified atmosphere packaged pork. *Meat Science, 68*(1), 115–122. https://doi.org/10.1016/j.meatsci.2003.10.020

McMullen, L. M., & Stiles, M. E. (1993). Microbial ecology of fresh pork stored under modified atmosphere at− 1, 4.4 and 10° C. *International Journal of Food Microbiology, 18*(1), 1–14.

Mielmann, A., Hugo, C. J., & Jooste, P. J. (2011). The potential of Chryseobacterium species to produce biogenic amines. *Journal of Food Safety, 31*(1), 75–80.

Mohammed, G., Bashammakh, A., Alsibaai, A., Alwael, H., & El-Shahawi, M. (2016). A critical overview on the chemistry, clean-up and recent advances in analysis of biogenic amines in foodstuffs. *TrAC Trends in Analytical Chemistry, 78*, 84–94.

Ngapo, T. M., & Vachon, L. (2017). Biogenic amine concentrations and evolution in "chilled" Canadian pork for the Japanese market. *Food Chemistry, 233*, 500–506.

Nowak, A., & Czyzowska, A. (2011). In vitro synthesis of biogenic amines by *Brochothrix thermosphacta* isolates from meat and meat products and the influence of other microorganisms. *Meat Science, 88*(3), 571–574.

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

Önal, A. (2007). A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry, 103*(4), 1475–1486.

Papageorgiou, M., Lambropoulou, D., Morrison, C., Kłodzińska, E., Namieśnik, J., & Płotka-Wasylka, J. (2018). Literature update of analytical methods for biogenic amines determination in food and beverages. *TrAC Trends in Analytical Chemistry, 98*, 128–142.

Peruzy, M. F., Houf, K., Joossens, M., Yu, Z., Proroga, Y. T. R., & Murru, N. (2021). Evaluation of microbial contamination of different pork carcass areas through culture-dependent and independent methods in small-scale slaughterhouses. *International Journal of Food Microbiology, 336*, Article 108902.

Pircher, A., Bauer, F., & Paulsen, P. (2007). Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. *European Food Research and Technology, 226*(1), 225–231.

Raimondi, S., Luciani, R., Sirangelo, T. M., Amaretti, A., Leonardi, A., Ulrici, A., … Zuliani, V. (2019). Microbiota of sliced cooked ham packaged in modified atmosphere throughout the shelf life: Microbiota of sliced cooked ham in MAP. *International Journal of Food Microbiology, 289*, 200–208.

Rauscher-Gabernig, E., Gabernig, R., Brueller, W., Grossgut, R., Bauer, F., & Paulsen, P. (2012). Dietary exposure assessment of putrescine and cadaverine and derivation of tolerable levels in selected foods consumed in Austria. *European Food Research and Technology, 235*(2), 209–220.

Ruiz-Capillas, C., & Herrero, A. M. (2019). Impact of biogenic amines on food quality and safety. *Foods, 8*(2), 62.

Sadjadi, S. A., Misa, A., & Krepich, S. (2017). A new twist to ion-pairing chromatography: In-sample addition of ion-pairing reagent. *LCGC North America, 35*(11), 824–831.

Sayers, E. W., Agarwala, R., Bolton, E. E., Brister, J. R., Canese, K., Clark, K., … Hefferon, T. (2019). Database resources of the national center for biotechnology information. *Nucleic Acids Research, 47*(Database issue), D23.

Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International, 29*(7), 675–690.

Shaw, B., & Harding, C. (1989). *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. *International Journal of Systematic and Evolutionary Microbiology, 39*(3), 217–223.

Sørheim, O., Nissen, H., & Nesbakken, T. (1999). The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. *Meat Science, 52*(2), 157–164.

UN. (2015). *Transforming our world: The 2030 agenda for sustainable development*. New York, NY, USA: United Nations.

Van Spreekens, K. J. A. (1974). The suitability of a modification of long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Archiv für Lebensmittelhygiene*.

Vasilopoulos, C., Ravyts, F., De Maere, H., De Mey, E., Paelinck, H., De Vuyst, L., & Leroy, F. (2008). Evaluation of the spoilage lactic acid bacteria in modified-atmosphere-packaged artisan-type cooked ham using culture-dependent and culture-independent approaches. *Journal of Applied Microbiology, 104*(5), 1341–1353.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology, 173*(2), 697–703.

Wojnowski, W., Majchrzak, T., Dymerski, T., Gębicki, J., & Namieśnik, J. (2017). Electronic noses: Powerful tools in meat quality assessment. *Meat Science, 131*, 119–131.

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology, 67*(5), 1613.