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Carnobacterium maltaromaticum as bioprotective culture against spoilage bacteria in ground meat and cooked ham

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ABSTRACT

This study assessed the bioprotective effect of *Carnobacterium maltaromaticum* (CM) against *Pseudomonas fluorescens* (PF) and *Brochothrix thermosphacta* (BT) in ground beef and sliced cooked ham stored in high- and low-oxygen-modified atmospheres (66/4/30% $O_2/N_2/CO_2$ and 70/30% N_2/CO_2, respectively). Both meat products were inoculated with CM, PF, and BT individually or in combination and stored for 7 days (3 days at 4 °C + 4 days at 8 °C) for ground beef and 28 days (10 days at 4 °C + 18 days at 8 °C) for sliced cooked ham. Each food matrix was assigned to 6 treatments: NC (no bacterial inoculation, representing the indigenous bacteria of meat), CM, BT, PF, CM + BT, and CM + PF. Bacterial growth, pH, instrumental color, and headspace gas composition were assessed during storage. CM counts remained stable from inoculation and throughout the shelf-life. CM reduced the population of inoculated and indigenous spoilage bacteria, including BT, PF, and enterobacteria, and showed a negligible impact on the physicochemical quality parameters of the products. Furthermore, upon simulating the shelf-life of ground beef and cooked ham, a remarkable extension could be observed with CM. Therefore, CM could be exploited as a biopreservative in meat products to enhance quality and shelf-life.

1. Introduction

Global meat production has increased from 30 million tons in 1970 to 360 million tons (carcass weight equivalent) in 2022, of which bovine and pork meat contributed 73.9 and 124.6 million tons, respectively (FAO, 1995, 2022). This market incurs losses accounting for up to 23% of the production and that represents approximately 4% of the global food losses (Karwowska, Łaba, & Szczepański, 2021).

Meat is typically contaminated during slaughter, processing, and packaging, resulting in an initial microbial count of 2 to 3 log CFU/g (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Due to the rich nutrient content of meat, microorganisms can proliferate rapidly during storage (Iulietto, Sechi, Borgogni, & Cenci-Goga, 2015), thereby decreasing its shelf-life. The bacterial groups responsible for spoilage in chilled meat and meat products are typically *Pseudomonas* spp. and *Brochothrix thermosphacta* (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015).

Thriving in the combination of low temperatures, various atmospheres, and prolonged storage periods, these bacteria outcompete their microbial counterparts. Consequently, both bacteria, *Pseudomonas* spp. and *Brochothrix thermosphacta*, present formidable obstacles to extending the shelf-life of refrigerated meat and meat products. Their metabolic activity leads to the production of undesirable molecules, causing off-flavours, off-odors, and changes in texture, which, prompt consumers to reject the product. These economic and credibility losses significantly impact the meat and related industries (Zagorec & Champomier-Vergès, 2017).

Various techniques are employed to control microbial spoilage in meat. Among these, biopreservation offers an alternative to chemical additives to extend the shelf-life of food products by introducing specific

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microorganisms to reduce spoilage and inhibit the development of certain pathogenic bacteria. The biological agents used must be safe, non-pathogenic, and non-toxin producers. This approach reduces the reliance on chemical additives, including nitrate, nitrite, sorbates, benzoates, phosphates, and sulfites (Barcenilla, Ducic, López, Prieto, & Álvarez-Ordóñez, 2022), for controlling meat microbiota. Moreover, it proves to be a relevant strategy for preserving 'green label' products and attracting consumers (Singh, 2018).

Lactic acid bacteria (LAB) are the commonly used bacteria as biopreservatives. They possess characteristics of protective cultures and are recognized as safe (Singh, 2018). *Carnobacterium maltaromaticum* is a predominant LAB in aerobic, modified atmosphere, or vacuum-packaged meat (Laursen et al., 2005). The genus can survive in diverse conditions, including growth within a large range of pH (5.0–9.5) and temperature (−1.5 to 37 °C), and shows tolerance to NaCl concentrations as high as 5% (*w/v*) (Edima, Cailliez-Grimal, Revol-Junelles, Rondags, & Millière, 2008; Leisner, Laursen, Prévost, Drider, & Dalgaard, 2007). *C. maltaromaticum* is found in fresh short- and long-term stored meat (Laursen et al., 2005). While some strains are associated with spoilage, others are linked to the preservation of meat (Casaburi et al., 2011; Danielski et al., 2020).

This genus shows great potential as bioprotective culture, including the production of metabolites such as organic acids and bacteriocins (Zhang, Gänzle, & Yang, 2019); inhibition of food pathogens to increase food safety, e.g., proven against *Listeria monocytogenes* (Brillet, Pilet, Prevost, Cardinal, & Leroi, 2005; Koné et al., 2018; Nilsson et al., 2005), and insignificant impact of most strains on sensorial characteristics of food (Casaburi et al., 2011; Laursen et al., 2005; Spanu et al., 2018). However, research regarding the biopreservative use of *C. maltaromaticum* in meat and meat products is presently limited (Evangelista et al., 2022). In a previous study, the antilisterial effect of different strains of *C. maltaromaticum* was demonstrated in sliced cooked ham (Danielski et al., 2020). Therefore, it is pertinent to investigate the potential of these strains of *C. maltaromaticum* in inhibiting relevant spoilage bacteria in meat and meat products. This study focused on assessing the bioprotective effect of a pool of *C. maltaromaticum* strains in inhibiting the growth of *B. thermosphacta* and *Pseudomonas fluorescens*, as well as investigating their influence on the physicochemical quality of ground beef and cooked ham.

2. Material and methods

2.1. Bacterial strains and culture conditions

In this study, we used a pool of strains of *C. maltaromaticum* (CM_B824, CM_B827, and CM_B289) with a previously demonstrated noteworthy antilisterial effect in sliced cooked ham (Danielski et al., 2020). These strains were selected from a group of eight *C. maltaromaticum* isolates dominant in vacuum-packed Australian bovine *longissimus thoracis et lumborum* with an extremely long shelf-life of 140 days at −1 °C. Genome sequencing revealed that the isolates belonged to three different genetic groups, all harbouring genes to produce carnobacteriocin BM1 and B2 (only CM_B824) (Imazaki et al., 2023).

We also used *Pseudomonas fluorescens* (ATCC®1355™) and a pool of strains of *B. thermosphacta*, including the strain ATCC®11509™ and indigenous isolates s109 (beef) and s153 (*jambon à l'os*) to simulate a spoilage population in meat.

Before the assays, the bacterial pool of *C. maltaromaticum* or *B. thermosphacta* strains was obtained by collecting one colony of each, previously grown in brain heart infusion (BHI) plates incubated at 25 °C for 24 h, and placing it into a tube containing BHI broth, which was incubated with the same conditions. For the experiment, 100 μL of this suspension was transferred into a new tube under the same conditions. The bacterial pellet was recovered by centrifugation (8000 rpm for 5 min) and resuspended with the same volume of NaCl 0.9%. The inoculum count was determined on plate count agar (PCA) for *C. maltaromaticum*, on STAA agar with STAA-selective supplement for *B. thermosphacta* and *Pseudomonas* agar with CFC-selective supplement for *Pseudomonas* sp. *Brochothrix thermosphacta* and *Pseudomonas* sp. plates were incubated at 25 °C for 48 h. Unless otherwise specified, all reagents listed were purchased from Oxoid (Basingstoke, Hampshire, United Kingdom).

2.2. Experimental design

2.2.1. Ground beef

Round beef (*semimembranosus* muscle) was purchased from a local butcher shop in Belgium and transported to the laboratory in an isothermal box to keep the temperature < 4 °C. Beef knuckles were ground through an 8-mm grinding plate. Six batches of ground beef were randomly assigned to six different treatments in triplicate: 1) NC – negative control inoculated only with sterile deionized water (representing meat with the indigenous bacterial population); 2) CM – *C. maltaromaticum* pool; 3) BT – *B. thermosphacta* pool; 4) PF – *P. fluorescens*; 5) CM + BT - *C. maltaromaticum* pool + *B. thermosphacta* pool; 6) CM + PF - *C. maltaromaticum* pool + *P. fluorescens*.

In treatments inoculated with *C. maltaromaticum* (CM), ground beef (80 g) was inoculated at the concentration of 6.8 CFU/mL and aseptically mixed for 2 min, using a stand mixer (Kitchen Grand Chef, Kenwood, Hampshire, UK). For treatments involving *B. thermosphacta* or *P. fluorescens*, both bacteria were inoculated at a concentration of 3.5 CFU/mL, using the same mixing procedure as described for CM. In the case of CM + BT or CM + PF treatments, ground beef was initially inoculated with CM and mixed for 2 min. Subsequently, the respective spoilage bacteria were introduced, and the mixture was blended for an additional 2 min.

After inoculation, beef patties (80 g) ($n = 54$) were formed using a meat former with a 12-cm diameter and packaged in PP/EVOH/PP trays (Døvigen AS, Oslo, Norway). The trays were 187 × 137 × 50 mm and had an oxygen permeability of 4 $cm^3/(m^2 \times 24\ h \times bar)$ at 23 °C and 0% RH. The trays were filled with a modified atmosphere containing 66% O_2, 4% N_2, and 30% CO_2. They were sealed with PET/PP film (Wipak, Helsinki, Finland) with an oxygen permeability of 8.4 $cm^3/(m^2 \times 24\ h \times bar)$ at 23 °C and 0% RH.

The ground beef patties were stored for 7 days, initially at 4 °C for 3 days, and then at 8 °C for 4 days. Samples were withdrawn for analyses at 0, 3, and 7 days of storage. The storage conditions followed the guidelines of the French Norm NF V01–003 (AFNOR, 2004), designed to reproduce the shelf-life of chilled meat and other perishable foods (1/3 of the shelf-life initially at 4 °C, followed by 2/3 at 8 °C).

2.2.2. Cooked ham

Sliced cooked ham was purchased from a medium-scale producer in Belgium and transported to the laboratory in an isothermal box to keep the temperature < 4 °C. The ham was processed with pork muscles (*M. semimembranosus, M. semitendinosus*, and *M. biceps femoris*), injected with a brine solution (15 g brine/100 g ham meat) containing the following ingredients (%): water, 15; salt, 3; dextrose 0.54; $NaNO_2$, 0.02; sodium isoascorbate, 0.08 and condiments (onion, carrot, parsley, clove, pepper, and laurel).

Ham slices ($n = 180$) were randomly assigned to the following treatments in triplicate: (1) negative control (represents ham with the indigenous bacteria population) inoculated with saline solution (2%) to avoid dehydration (NC); (2) *C. maltaromaticum* pool (CM); (3) *B. thermosphacta* pool (BT); (4) *P. fluorescens* (PF); (5) CM + BT - *C. maltaromaticum* pool + *B. thermosphacta* pool; 6) CM + PF - *C. maltaromaticum* pool + *P. fluorescens*.

For the inoculation, slices were placed in a steel mesh basket strainer and immersed in 1000 mL of saline solution (0.9%) containing the bacterial inoculum: *C. maltaromaticum* at 6.4 CFU/mL, *B. thermosphacta* at 3.9 CFU/mL and *P. fluorescens* at 3.8 log CFU/mL. In treatments with a

single bacterial inoculum (CM, BT, and PF), the slices were maintained in contact with the inoculum suspension for 10 min to facilitate cell attachment to the product surface. In the case of CM + BT or CM + PF treatments, ham slices were initially inoculated with CM following the same procedure as previously described. Subsequently, slices were inoculated with the respective spoilage bacteria by immersion for 10 min. After inoculation, the slices were suspended outside the inoculum solution for 10 min to allow the liquid to dry.

The slices were packaged in PP/EVOH/PP trays (Døvigen AS, Oslo, Norway) with dimensions of 187 × 137 × 50 mm, with an oxygen permeability of 4 $cm^3/(m^2 \times 24\ h \times bar)$ at 23 °C and 0% RH, in modified atmosphere, containing 70% N_2 and 30% CO_2. They were sealed with PET/PP film (Wipak, Helsinki, Finland) with an oxygen permeability of 8.4 $cm^3/(m^2 \times 24\ h \times bar)$ at 23 °C and 0% RH. The samples were stored for 10 days at 4 °C, followed by 18 days at 8 °C, following the French Norm NF V01–003 (AFNOR, 2004) to simulate the shelf-life of chilled meat and other perishable foods (1/3 of the shelf-life initially at 4 °C, followed by 2/3 at 8 °C). Analyses were conducted at 0, 5, 10, 19, and 28 days of storage.

2.3. Microbiological analyses

Ground beef patties and sliced cooked ham samples (25 g) were placed in sterile stomacher bags containing 225 mL of saline solution (0.9% NaCl). The samples were homogenized using a stomacher (Masticator Basic 2000, IUL, Barcelona, Spain) for 120 s, serially diluted in the same dilutant, and plated using a spiral plater (EasySpital® Pro, Interscience, France). The microbiological counts were carried out using the following culture media and incubation conditions: total viable count (TVC) (PCA plates incubated at 25 °C for 48 h); Lactic acid bacteria (LAB) (MRS plates incubated at 25 °C for 72 h); Enterobacteriaceae (violet red bile glucose (VRBG) plates incubated at 37 °C for 24 h, BIORAD, Hercules, California, USA); *B. thermosphacta* (STAA plates incubated at 25 °C for 48 h followed by an oxidase test confirmation to differentiate *Brochothrix* from *Pseudomonas*, according to ISO 13722:1996); *Pseudomonas* sp. (CFC plates incubated at 25 °C for 48 h followed by an oxidase test and growth on Klinger Iron Agar confirmation, ISO 13720:1995). *Carnobacterium maltaromaticum* population was calculated as the difference between counts on PCA and the other culture media (Danielski et al., 2020).

2.4. Physicochemical analyses

2.4.1. pH

The pH was measured in triplicate using a pH meter (Model 104,063,123, Ingold, Houston, USA) according to the International Organisation for Standardisation 2971 (ISO, 1999).

2.4.2. Instrumental color

The instrumental color was measured using a spectrophotometer (CM-600D, Konica-Minolta, Tokyo, Japan), set to D65 illuminant with an aperture diameter of 8 mm and 10° angle of observation and color coordinates CIE L*, a*, b*. Data were collected from 5 different spots on the surface of the meat product. The color disparities (ΔE) were calculated using the formula $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Hunt et al., 2012).

2.4.3. Headspace gas composition

Changes in the gas composition inside the packages (CO_2, O_2, and N_2%) during storage were evaluated with a gas analyzer (Checkmate 3, Dansensor, Ringsted, Denmark) using a sterile needle for collection through a 15 mm diameter *septum* attached to the package (Spanu et al., 2018).

2.5. Statistical analysis

Each meat matrix assay was performed two times independently. The microbiological analyses ($n = 4$) were conducted in duplicate, and the physicochemical analyses ($n = 6$) were in triplicate. The data were expressed in mean ± SE (standard error) and analyzed using a random block design, considering a mixed linear model including treatment and storage time as fixed effects and replication as a random effect. Means were compared by Tukey's test, $P < 0.05$ indicating a significant difference between pairs of means. The analyses were performed using the software Statgraphics® Centurion XVI version 16.1.11 (Statpoint Technologies, Warrenton, Virginia, USA).

To estimate the theoretical shelf-life of the products, the bacterial count data were analyzed with a dose-response curve model using the software Table Curve 2D (version 3; Systat Software Inc., Richmond California, USA), and a logistic regression model was used to predict the product storage time (days) considering the spoilage threshold based on the observed microbial growth using the following equation:

$Y = a + b/(1 + (x/c)^d)$.

Where y is the microbial population count ($Y=F(x)_\infty$), x is the storage time (days), and a, b, c, and d are independent variables. The spoilage threshold used for *B. thermosphacta* was 6 log CFU/g and for *Pseudomonas* sp. was 7 log CFU/g (Mills, Donnison, & Brightwell, 2014; Pellissery, Vinayamohan, Amalaradjou, & Venkitanarayanan, 2020).

3. Results and discussion

3.1. Microbiological counts

A significant interaction between storage time and treatment was observed for all microbiological counts (TVC, LAB, Enterobacteriacea, *B. thermosphacta*, *P. fluorescens,* and *Carnobacterium*) in ground beef and sliced cooked ham. The counts increased during storage in all treatments ($P < 0.05$) (Figs. 1 and 2). The comprehensive microbiological counts can be found in the Supplementary Material.

The pool of *C. maltaromaticum* inhibited the growth of TVC in both ground beef and cooked ham. In ground beef, TVC increased by 3.4 log CFU/g in the non-treated sample (NC), while in the samples treated with *Carnobacterium* (CM, CM + BT, and CM + PF), the increase was lower at 1.1, 1.6, and 0.8 log CFU/g, respectively (Fig. 1) over 7 days. For cooked ham, the TVC population rose by 5.7 log CFU/g in NC, whereas in the CM, CM + BT, and CM + PF, the increases were much lower at 0.3, 1.1, and 0.3 log CFU/g, respectively (Fig. 2). Notably, LAB counts showed similar increases across all treatments in both products (Figs. 1 and 2).

The pool of *C. maltaromaticum* also inhibited the growth of Enterobacteriaceae. The treatments containing *Carnobacterium* (CM, CM + BT, and CM + PF) showed a lower rise in Enterobacteriaceae population when compared to NC. During 7 days in ground beef, the Enterobacteriaceae population increased by 2.4 log CFU/g in NC, whereas it increased by 1.4, 1.7, and 1.9 log CFU/g in CM + BT, CM, and CM + PF, respectively (Fig. 1). In ham, during 28 days of storage, the rise in Enterobacteriaceae population was 4.9 log CFU/g in NC, and 2.5, 3.4, and 3.5 log CFU/g in CM + BT, CM + PF, and CM, respectively (Fig. 2). These findings corroborate the results of a previous study by Imazaki et al. (2023), which demonstrated an inhibitory effect of the same *C. maltaromaticum* isolated strains on Enterobacteriaceae in beef stored in a low-oxygen atmosphere. The inhibition of this group of bacteria was also reported for *Carnobacterium* spp. in vitro (Zhang, Baranyi, & Tamplin, 2015) and in meat simulated medium (Héquet et al., 2009), and for *C. divergens V41* in shrimp (Saraoui et al., 2017) and in cold smoked salmon (Brillet et al., 2005). Besides, in our study, the inhibitory effect of *C. maltaromaticum* on Enterobacteriaceae did not seem to be influenced by the presence of both inoculated *P. fluorescens* or *B. thermosphacta*. Tshabalala, de Kock, and Buys (2012) also report that other LAB species (*Lactiplantibacillus plantarum*) could impact the growth of *Escherichia coli* O157:H7 in fresh beef stored aerobically and under vacuum storage,

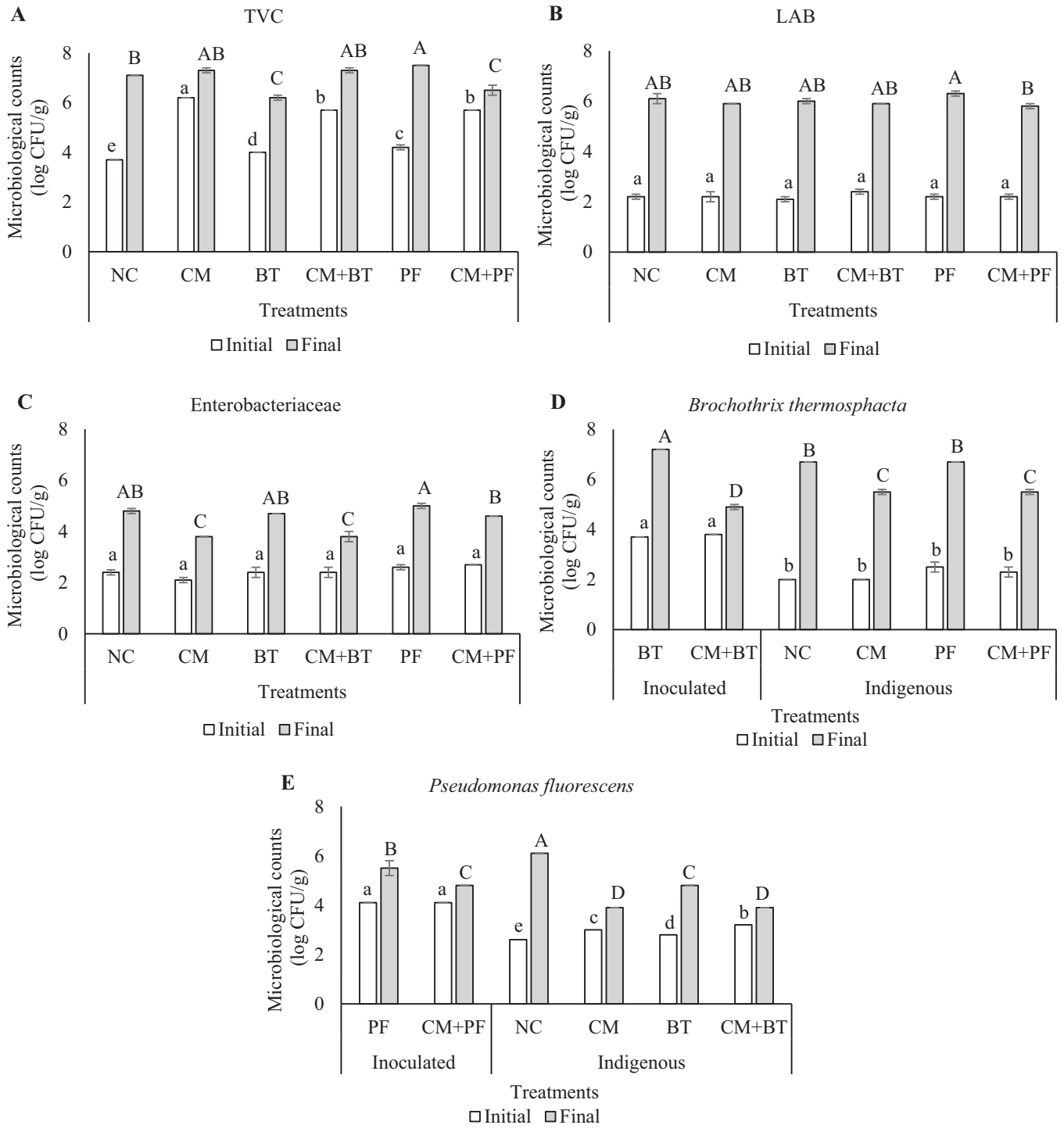

Fig. 1. Effect of *C. maltaromaticum* on the microbiological count in ground beef stored in MAP (66/30/4% $O_2/CO_2/N_2$) for 7 days (3 days at 4 °C and 4 days at 8 °C). Treatments: NC = negative control; CM = pool of C. maltaromaticum; BT = pool of B. thermosphacta; PF = P. fluorescens; CM+BT = pool of C. maltaromaticum + pool of B. thermosphacta; CM+PF = pool of C. maltaromaticum + P. fluorescens. A = Total viable counts; B = Lactic acid bacteria counts; C = Enterobacteriaceae counts, D = inoculated B. thermosphacta counts are shown in BT and CM+BT and indigenous B. thermosphacta counts are shown in NC, CM, PF, and CM+PF; E = inoculated P. fluorescens counts are shown in PF and CM+PF and indigenous P. fluorescens counts are shown in NC, CM, BT, and CM+BT. Storage time: initial = 0 days; final = 7 days. Values are expressed as mean ± SE. Different lowercase letters show significant differences among microbial counts in samples on the first day of storage ($P < 0.05$). Different uppercase letters show significant differences among microbial counts in samples on the final day of storage ($P < 0.05$).

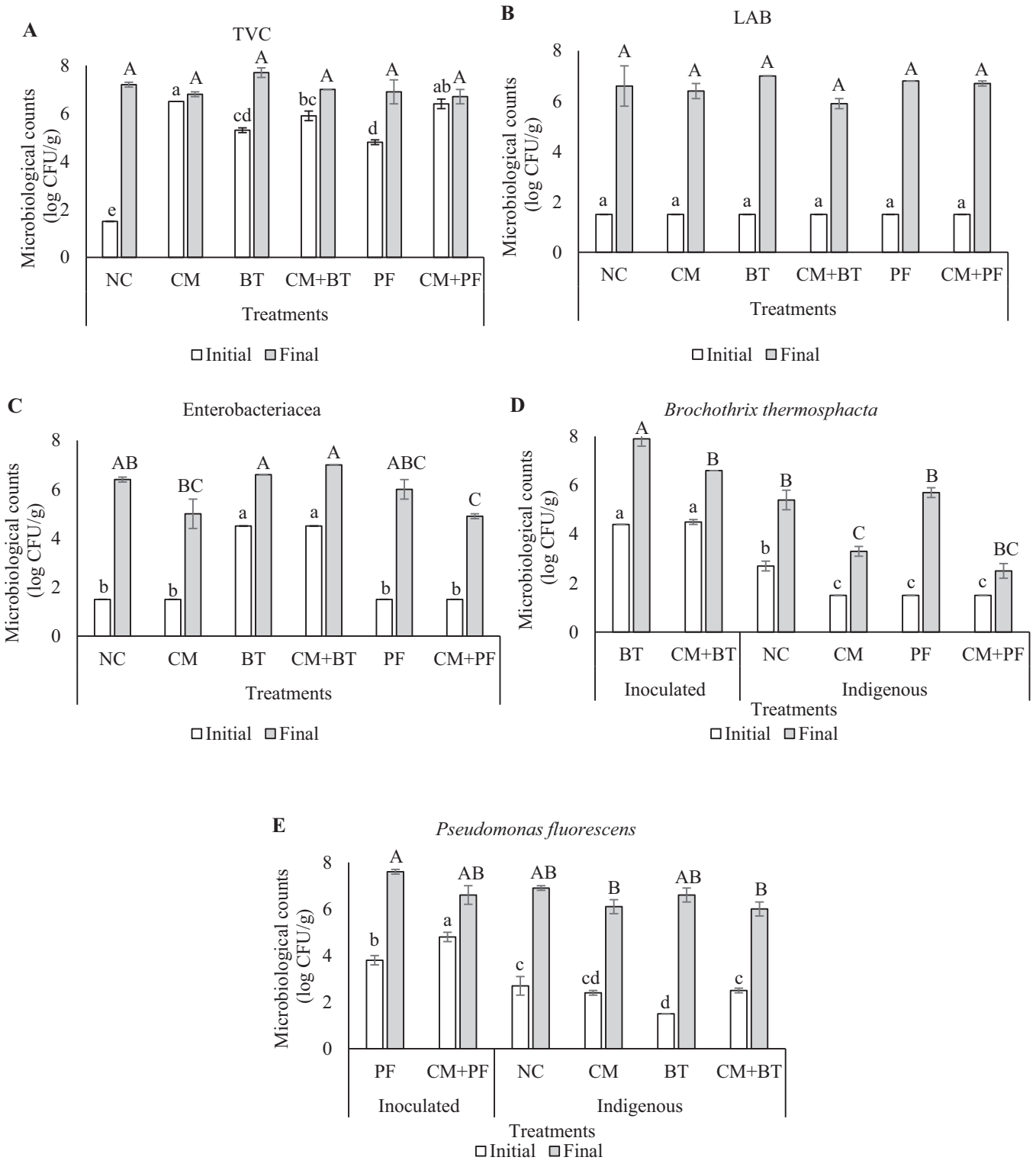

Fig. 2. Effect of *C. maltaromaticum* on the microbiological count in sliced cooked ham stored in MAP (70/30% N_2/CO_2) for 28 days with temperature abuse (10 days at 4 °C, followed by 18 days at 8 °C).
Treatments: NC = negative control; CM = pool of C. maltaromaticum; BT = pool of B. thermosphacta; PF = P. fluorescens; CM+BT = pool of C. maltaromaticum + pool of B. thermosphacta; CM+PF = pool of C. maltaromaticum + P. fluorescens. A = Total viable counts; B = Lactic acid bacteria counts; C = Enterobacteriaceae counts, D = inoculated B. thermosphacta counts are shown in BT and CM+BT and indigenous B. thermosphacta counts are shown in NC, CM, PF, and CM+PF; E = inoculated P. fluorescens counts are shown in PF and CM+PF and indigenous P. fluorescens counts are shown in NC, CM, BT, and CM+BT.Storage time: initial = 0 days; final = 28 days. Values are expressed as mean ± SE. Different lowercase letters show significant differences among microbial counts in samples on the first day of storage ($P < 0.05$). Different uppercase letters show significant differences among microbial counts in samples on the final day of storage ($P < 0.05$).

which was not affected by *P. fluorescens*, regardless of the inoculation level or storage time.

Inoculated *B. thermosphacta* population was also inhibited by *Carnobacterium* both in ground beef and in cooked ham. CM + BT showed lower *B. thermosphacta* counts (4.9 CFU/g) than BT (7.2 log CFU/g) at the end of storage (7 days) in ground beef ($P < 0.05$) and the rise in population during storage for *B. thermosphacta* was 3.5 log CFU/g in BT, whereas in CM + BT it was only 1.1 log CFU/g (Fig. 1). In ham, *B. thermosphacta* counts increased by 3.5 in BT and only 2.1 log CFU/g in CM + BT (Fig. 2).

Regarding the indigenous *B. thermosphacta*, in ground beef, *Carnobacterium* inhibited growth of the spoilage bacteria (at 7 days, *B. thermosphacta* counts were 5.5 log CFU/g in CM and CM + PF, while in NC, it was 6.7 log CFU/g) ($P < 0.05$). Moreover, the rise of indigenous *B. thermosphacta* population during storage was 4.7 log CFU/g in NC, and 3.2 and 3.5 log CFU/g in CM + PF and CM, respectively (Fig. 1). Likewise, in ham at 28 days, CM + PF and CM showed a lower count of indigenous *B. thermosphacta* than NC, with counts of 2.5, 3.3 and 5.4 log CFU/g ($P < 0.05$), respectively. Moreover, the rise in *B. thermosphacta* count over time was 2.7 CFU/g in NC, whereas, in CM + PF and CM, it was 1.0 and 1.8 log CFU/g (Fig. 2).

The presence of *Carnobacterium* also significantly inhibited the growth of both inoculated and indigenous *P. fluorescens*. In ground beef, CM + PF had lower counts than PF (4.8 and 5.5 log CFU/g at 7 days, respectively) ($P < 0.05$). Additionally, the increase of inoculated *P. fluorescens* population during storage was 1.4 CFU/g in PF and only 0.7 CFU/g in CM + PF. Indigenous *P. fluorescens* population increased by 3.5 log CFU/g in NC, and only 0.7 and 0.9 log CFU/g in treatments with *Carnobacterium* (CM + BT and CM, respectively) (Fig. 1). Considering the inoculated *P. fluorescens* in ham, the rise of the population during storage in CM + PF was only 1.8 CFU/g, whereas in PF, it was 3.8 log CFU/g. Indigenous *P. fluorescens* count in ham increased by 4.2 log CFU/

Table 1
pH and dynamic behavior of headspace gases of ground beef stored in MAP (66/30/4% $O_2/CO_2/N_2$) for 3 days at 4 °C and 4 days at 8 °C.

Treatment	Storage days				*P*
	0	3	7	Mean	
	Physicochemical parameter				
	pH				
NC	5.69 ± 0.00^{bB}	5.71 ± 0.01^{bB}	5.85 ± 0.01^{aA}	5.75^{A}	0.0000
CM	5.70 ± 0.01^{abB}	5.70 ± 0.00^{bAB}	5.68 ± 0.01^{bcA}	5.69^{cd}	0.0260
BT	5.70 ± 0.01^{abB}	5.72 ± 0.01^{abB}	5.81 ± 0.00^{aA}	5.74^{ab}	0.0000
CM + BT	5.72 ± 0.00^{aA}	5.74 ± 0.00^{aA}	5.67 ± 0.02^{cB}	5.71^{bc}	0.0050
PF	5.72 ± 0.00^{aB}	5.74 ± 0.00^{aB}	5.79 ± 0.02^{abA}	5.75^{A}	0.0035
CM + PF	5.72 ± 0.00^{aA}	5.70 ± 0.00^{bA}	5.56 ± 0.05^{dB}	5.66^{d}	0.0160
Mean	5.71^{A}	5.72^{A}	5.73^{A}		0.1557
P	0.0008	0.0004	0.0000	0.0000	
	O_2 (%)			Mean	*P*
NC	65.83 ± 0.03^{cC}	69.53 ± 0.03^{aA}	68.53 ± 0.03^{aB}	67.97^{a}	0.0000
CM	66.03 ± 0.03^{bcB}	69.33 ± 0.09^{aA}	65.17 ± 0.09^{bC}	66.84^{b}	0.0000
BT	66.30 ± 0.00^{aC}	70.00 ± 0.40^{aA}	68.47 ± 0.03^{aB}	68.26^{a}	0.0001
CM + BT	66.07 ± 0.03^{bB}	69.40 ± 0.10^{aA}	65.17 ± 0.33^{bC}	66.88^{b}	0.0000
PF	66.23 ± 0.07^{abC}	69.73 ± 0.07^{aA}	68.37 ± 0.09^{aB}	68.11^{a}	0.0000
CM + PF	66.20 ± 0.06^{abB}	69.40 ± 0.06^{aA}	65.07 ± 0.23^{bB}	66.89^{b}	0.0000
Mean	66.11^{C}	69.57^{A}	66.79^{B}		0.0000
P	0.0001	0.1411	0.0000	0.0000	
	CO_2 (%)			Mean	*P*
NC	29.67 ± 0.44^{aA}	26.57 ± 0.19^{aB}	27.33 ± 0.17^{bB}	27.86^{b}	0.0007
CM	30.07 ± 0.03^{aB}	26.63 ± 0.03^{aC}	31.20 ± 0.06^{aA}	29.30^{a}	0.0000
BT	29.97 ± 0.13^{aA}	26.53 ± 0.12^{aC}	27.67 ± 0.09^{bB}	28.06^{b}	0.0000
CM + BT	29.93 ± 0.03^{aB}	26.70 ± 0.06^{aC}	31.40 ± 0.40^{aA}	29.34^{A}	0.0000
PF	29.67 ± 0.03^{aA}	26.57 ± 0.09^{aC}	27.90 ± 0.10^{bB}	28.04^{b}	0.0000
CM + PF	29.97 ± 0.09^{aB}	26.70 ± 0.00^{aC}	31.07 ± 0.13^{aA}	29.24^{a}	0.0000
Mean	29.88^{A}	26.62^{C}	29.43^{B}		0.0000
P	0.5805	0.7619	0.0000	0.0000	
	N_2 (%)			Mean	*P*
NC	4.50 ± 0.40^{aA}	3.90 ± 0.15^{aA}	4.13 ± 0.19^{aA}	4.18^{a}	0.3539
CM	3.90 ± 0.00^{aA}	4.03 ± 0.09^{aA}	3.63 ± 0.13^{abA}	3.86^{ab}	0.0554
BT	3.73 ± 0.13^{aA}	3.47 ± 0.28^{aA}	3.87 ± 0.10^{abA}	3.69^{b}	0.3923
CM + BT	4.00 ± 0.06^{aA}	3.90 ± 0.06^{aA}	3.43 ± 0.07^{bB}	3.78^{b}	0.0066
PF	4.10 ± 0.10^{aA}	3.70 ± 0.15^{aA}	3.73 ± 0.12^{abA}	3.84^{ab}	0.1194
CM + PF	3.83 ± 0.03^{aA}	3.90 ± 0.06^{aA}	3.87 ± 0.12^{abA}	3.87^{ab}	0.8424
Mean	4.01^{A}	3.82^{AB}	3.78^{B}		0.0326
P	0.1149	0.2317	0.0381	0.0148	

Treatments: NC = negative control; C-M = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.
Values are expressed as Mean ± SE. Different lowercase letters in the same column show significant differences among treatments ($P < 0.05$). Different uppercase letters in the same row show significant differences among storage time ($P < 0.05$).

g in NC, and 3.5 and 3.7 CFU/g in CM + BT and CM, respectively (Fig. 2).

The inhibitory effect of *C. maltaromaticum* toward spoilage bacteria was previously assessed in vitro by Zhang et al. (2015), who showed that *C. maltaromaticum* could inhibit *B. thermosphacta* and *Pseudomonas* sp. in spot-lawn (cell-to-cell contact) assays and medium broth (cell-free supernatant). The effect was also verified in ricotta (Spanu et al., 2018) and shrimp (Laursen, Leisner, & Dalgaard, 2006), but not in meat. Thus, our study brings novel findings of the biopreservative effect of *C. maltaromaticum* in meat and meat products.

The inhibitory effect of *C. maltaromaticum* in food matrices may occur mainly due to the following mechanisms: (i) competition for nutrients, which leads to nutrient depletion; (ii) production of antagonist compounds such as diacetyl, and CO_2 (Kasra-Kermanshahi & Mobarak-Qamsari, 2015; Said, Gaudreau, Dallaire, Tessier, & Fliss, 2019); (iii) faster growth in the matrix and bacteriocin production, leading to bactericidal or bacteriostatic effects on spoilage bacteria (Brillet-Viel, Pilet, Courcoux, Prévost, & Leroi, 2016) and (iv) production of organic acids such as lactic, formic and acetic (Zhang et al., 2019).

The effects of *Carnobacterium* against the indigenous microbiota of the products represent well the case of using it as bioprotective culture in meat. Considering the microorganism investigated, *C. maltaromaticum* reduced the growth of both inoculated and indigenous *Pseudomonas* and *Brochothrix* in ground beef and cooked ham.

The population of *C. maltaromaticum* in CM, CM + BT, and CM + PF reached high counts at the end of the storage in ground beef: 7.3 (CM), 7.3 (CM + BT), 6.1 log CFU/g (CM + PF) and in ham: 6.6 (CM), 6.6 (CM + BT) and 6.1 log CFU/g (CM + PF) (Supplementary Material, Tables 1 and 2). As noticed, *C. maltaromaticum* growth remained practically constant during storage and was barely affected by the inoculated or indigenous spoilage microbiota. The high population of *Carnobacterium* in these meat matrices is sustained by a previous study by Imazaki et al. (2023), who isolated the *C. maltaromaticum* strains used in the present study from a long-term vacuum-packaged Australian beef, of which 98% of the final microbial composition was predominantly *Carnobacterium*. Other studies show that *C. maltaromaticum* can predominate over the spoilage population (Laursen et al., 2005) and persist in chilled meat until the end of the shelf-life (Holck, Pettersen, Moen, & Sørheim, 2014; Zhang, Badoni, Gänzle, & Yang, 2018).

3.2. Physicochemical analyses

There was an interaction between storage time and treatment for pH values in both products. Although the storage time alone did not influence the pH of ground beef, treatments did. The pH was slightly lower in treatments inoculated with carnobacteria (CM, CM + BT, and CM + PF) than in other treatments ($P < 0.05$). However, numerical differences in pH values between these treatments and NC were <0.3 decimals. In beef at 7 d of storage (Table 1), pH values were: 5.68 (CM), 5.67 (CM + BT), and 5.56 (CM + PF); and in ham at 28 d (Table 3): 5.66 (CM), 5.66 (CM + BT), 5.74 (CM + PF). Remarkably, these values remained within the normal pH range for beef 5.4–5.8 (Macdougall & Taylor, 2007) and cooked ham 5.6–6.2 (Arnau, Guerrero, Casademont, & Gou, 1995). This observation confirms that *Carnobacterium* is not a strong acid producer and did not affect the meat pH at a noticeable value (Leisner et al., 2007).

There was an interaction between treatments and storage time for the headspace gas composition for both products, except for nitrogen (N_2), in ground beef, for which, each effect was independent ($P < 0.05$). In our study conditions, *C. maltaromaticum* influenced the gaseous composition of products stored in MAP. Notably in beef (66/30/4% O_2/CO_2/N_2), treatments inoculated with carnobacteria displayed lower O_2 and elevated CO_2 levels when compared to the other treatments. Over 7 days of storage, O_2 concentrations decreased in CM (−0.9%), CM + BT (−0.9%), and CM + PF (−1.1%). In contrast, CO_2 levels exhibited an increase in CM (1.1%), CM + BT (1.5%), and CM + PF (1.1%) during the same timeframe (Table 1). These findings corroborate the results of the study of Laursen et al. (2006) with *Carnobacterium* in shrimp (MAP – 50/30/20% CO_2/N_2/O_2). In fact, *Carnobacterium* spp. uses a significant amount of oxygen for exponential growth, when present in abundance (aerobic conditions), increasing the growth rate (Kolbeck, Kienberger, Kleigrewe, Hilgarth, & Vogel, 2021). Moreover, the production of CO_2 may be a metabolic result (Leisner et al., 2007). The CO_2 can inhibit Enterobacteriaceae, although *B. thermosphacta* growth is unaffected

Table 2
Effect of the addition of a pool of *C. maltaromaticum* and spoilage bacteria on the instrumental color (L*, a*, b* and ΔE*) of ground beef during storage in MAP (66/30/4% O_2/CO_2/N_2) for 3 days at 4 °C and 4 days at 8 °C.

	Storage days				
Treatment	0	3	7		
		L*		Mean	*P*
NC	37.22 ± 0.38^{bcB}	40.52 ± 0.53^{aA}	42.26 ± 0.60^{abA}	40.00^{b}	0.0000
CM	37.14 ± 0.47^{bcB}	40.40 ± 0.61^{aA}	41.26 ± 0.43^{abA}	39.60^{bc}	0.0000
BT	37.97 ± 0.49^{abB}	41.00 ± 0.62^{aA}	40.53 ± 0.51^{bA}	39.84^{b}	0.0006
CM + BT	36.09 ± 0.36^{cdC}	39.84 ± 0.36^{aB}	41.66 ± 0.62^{abA}	39.20^{bc}	0.0000
PF	39.33 ± 0.48^{aC}	41.51 ± 0.26^{aB}	43.40 ± 0.44^{aA}	41.41 ^{A}	0.0000
CM + PF	34.89 ± 0.36^{dB}	39.67 ± 0.31^{aA}	40.99 ± 0.60^{bA}	38.52^{c}	0.0000
Mean	37.11^{C}	40.49^{B}	41.68 ^{A}		0.0000
P	0.0000	0.0639	0.0050	0.0000	
		a*		Mean	*P*
NC	20.31 ± 0.56^{aA}	19.95 ± 0.39^{aA}	14.09 ± 0.51^{aB}	18.12 ^{A}	0.0000
CM	18.98 ± 0.54^{abA}	18.57 ± 0.50^{aA}	14.70 ± 0.48^{aB}	17.42^{ab}	0.0000
BT	19.33 ± 0.81^{abA}	18.82 ± 0.50^{aA}	14.89 ± 0.63^{aB}	17.68^{ab}	0.0000
CM + BT	18.59 ± 0.46^{abA}	18.96 ± 0.35^{aA}	13.69 ± 0.33^{aB}	17.08^{ab}	0.0000
PF	17.89 ± 0.44^{bB}	19.46 ± 0.26^{aA}	13.42 ± 0.36^{aC}	16.92^{b}	0.0000
CM + PF	19.12 ± 0.53^{abA}	18.71 ± 0.32^{aA}	13.43 ± 0.47^{aB}	17.09^{ab}	0.0000
Mean	19.04 ^{A}	19.08 ^{A}	14.04^{B}		0.0000
P	0.0890	0.1300	0.1186	0.0258	
		b*		Mean	*P*
NC	27.76 ± 0.36^{aA}	20.59 ± 0.25^{aB}	11.44 ± 0.15^{abcC}	19.93^{a}	0.0000
CM	26.71 ± 0.36^{aA}	18.92 ± 0.42^{bB}	10.68 ± 0.35^{bcC}	18.77^{bc}	0.0000
BT	26.70 ± 0.63^{aA}	19.60 ± 0.28^{abB}	10.64 ± 0.37^{bcC}	18.98^{bc}	0.005
CM + BT	26.05 ± 0.49^{aA}	19.45 ± 0.21^{abB}	10.29 ± 0.30^{cC}	18.59^{c}	0.0000
PF	26.70 ± 0.36^{aA}	19.92 ± 0.24^{abB}	11.72 ± 0.25^{abC}	19.45^{ab}	0.0000
CM + PF	26.59 ± 0.41^{aA}	18.74 ± 0.31^{bB}	11.96 ± 0.37^{aC}	19.10^{abc}	0.0000
Mean	26.75^{A}	19.54^{B}	11.12^{C}		0.0000
P	0.1832	0.0003	0.0007	0.0001	
		ΔE			
NC		18.18			
CM		17.10			
BT		16.86			
CM + BT		17.42			
PF		16.15			
CM + PF		16.85			

Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*. Values are expressed as Mean ± SE. Different lowercase letters in the same column show significant differences among treatments ($P < 0.05$). Different uppercase letters in the same row show significant differences among storage time ($P < 0.05$).

Table 3
pH and dynamic behavior of headspace gases of sliced cooked ham stored in MAP (70/30% N_2/CO_2) for 28 days with temperature abuse (10 days at 4 °C, followed by 18 days at 8 °C).

	0	5	10	19	28	Mean	*P*
	Physicochemical parameter						
	pH						
NC	6.07 ± 0.02^{bAB}	6.09 ± 0.01^{aA}	6.07 ± 0.02^{bAB}	6.10 ± 0.03^{aA}	5.94 ± 0.06^{aB}	6.05^{A}	0.0284
CM	5.93 ± 0.01^{cB}	6.13 ± 0.05^{aA}	5.93 ± 0.01^{cB}	5.88 ± 0.01^{bB}	5.66 ± 0.01^{cC}	5.91^{bc}	0.0000
BT	6.01 ± 0.01^{bA}	6.02 ± 0.03^{aA}	6.01 ± 0.01^{bA}	5.86 ± 0.04^{bAB}	5.77 ± 0.06^{abcB}	5.93^{b}	0.0012
CM + BT	6.07 ± 0.03^{bA}	5.87 ± 0.02^{bB}	6.07 ± 0.03^{bA}	5.70 ± 0.02^{cdC}	5.66 ± 0.00^{cC}	5.87^{c}	0.0000
PF	6.18 ± 0.00^{aA}	6.05 ± 0.00^{aB}	6.18 ± 0.00^{aA}	5.81 ± 0.03^{bcC}	5.91 ± 0.04^{abC}	6.03^{a}	0.0000
CM + PF	6.05 ± 0.01^{bA}	5.87 ± 0.01^{bB}	6.05 ± 0.01^{bA}	5.64 ± 0.04^{dD}	$5.74 \pm 0.01b^{bcC}$	5.87^{c}	0.0000
Mean	6.05^{A}	6.00^{B}	6.05^{A}	5.83^{C}	5.78^{C}		0.0000
P	0.0000	0.0000	0.0000	0.0000	0.0006	0.0000	
			O_2 residue (%)			Mean	*P*
NC	0.68 ± 0.00^{aB}	0.86 ± 0.00^{aAB}	0.94 ± 0.01^{aA}	0.29 ± 0.11^{aC}	0.00 ± 0.00^{aD}	0.55^{a}	0.0000
CM	0.66 ± 0.00^{abA}	0.62 ± 0.02^{cA}	0.10 ± 0.01^{bC}	0.21 ± 0.01^{abBC}	0.00 ± 0.00^{aD}	0.32^{c}	0.0000
BT	0.68 ± 0.01^{aC}	0.84 ± 0.00^{aB}	0.96 ± 0.01^{aA}	0.11 ± 0.03^{abD}	0.00 ± 0.00^{aE}	0.52^{ab}	0.0000
CM + BT	0.66 ± 0.01^{aA}	0.67 ± 0.01^{bA}	0.10 ± 0.00^{bB}	0.16 ± 0.01^{abB}	0.10 ± 0.10^{aB}	0.34^{c}	0.0000
PF	0.63 ± 0.00^{cC}	0.84 ± 0.00^{aB}	0.93 ± 0.03^{aA}	0.02 ± 0.02^{bD}	0.00 ± 0.00^{aD}	0.48^{b}	0.0000
CM + PF	0.64 ± 0.00^{bcA}	0.56 ± 0.01^{dB}	0.09 ± 0.00^{bD}	0.14 ± 0.00^{abC}	0.00 ± 0.00^{aE}	0.29^{c}	0.0000
Mean	0.66^{B}	0.73^{A}	0.52^{C}	0.15^{D}	0.02^{E}		0.0000
P	0.0000	0.0000	0.0000	0.0269	0.4175	0.0000	
			CO_2 (%)			Mean	*P*
NC	28.47 ± 0.07^{bA}	26.03 ± 0.03^{aB}	26.03 ± 0.20^{bB}	25.20 ± 0.38^{cB}	27.97 ± 0.42^{aA}	26.74^{ab}	0.0000
CM	28.83 ± 0.29^{abA}	26.40 ± 0.17^{aC}	27.27 ± 0.09^{aB}	25.87 ± 0.09^{bcCD}	25.30 ± 0.25^{bD}	26.73^{ab}	0.0000
BT	28.33 ± 0.09^{bA}	26.30 ± 0.06^{aBC}	25.57 ± 0.24^{bC}	26.43 ± 0.12^{abB}	26.97 ± 0.20^{aB}	26.72^{ab}	0.0000
CM + BT	29.03 ± 0.12^{abA}	26.57 ± 0.26^{aC}	27.20 ± 0.10^{aB}	26.90 ± 0.06^{aBC}	25.53 ± 0.23^{bD}	27.05^{a}	0.0000
PF	29.53 ± 0.35^{aA}	25.97 ± 0.09^{aB}	25.37 ± 0.18^{bB}	26.13 ± 0.30^{abcB}	25.53 ± 0.23^{bB}	26.51^{b}	0.0000
CM + PF	29.47 ± 0.03^{aA}	26.30 ± 0.06^{aC}	27.30 ± 0.15^{aB}	26.23 ± 0.15^{abC}	25.13 ± 0.22^{bD}	26.89^{a}	0.0000
Mean	28.94^{A}	26.26^{BC}	26.46^{B}	26.13^{BC}	26.07^{C}		0.0000
P	0.0039	0.0742	0.0000	0.0027	0.0000	0.0033	
			N_2 (%)			Mean	*P*
NC	70.86 ± 0.07^{aC}	73.11 ± 0.03^{aB}	73.02 ± 0.21^{abcB}	74.51 ± 0.27^{aA}	72.03 ± 0.42^{cB}	72.71^{ab}	0.0000
CM	70.51 ± 0.28^{abC}	72.98 ± 0.19^{aB}	72.64 ± 0.09^{cB}	73.93 ± 0.08^{abA}	74.70 ± 0.25^{aA}	72.95^{ab}	0.0000
BT	70.99 ± 0.08^{aB}	72.86 ± 0.06^{aA}	73.48 ± 0.23^{abA}	73.46 ± 0.11^{bcA}	73.03 ± 0.20^{bA}	72.76^{ab}	0.0000
CM + BT	70.30 ± 0.13^{abC}	72.76 ± 0.26^{aB}	72.70 ± 0.10^{bcB}	72.94 ± 0.05^{cB}	74.36 ± 0.15^{aA}	72.61^{b}	0.0000
PF	69.84 ± 0.35^{bC}	73.20 ± 0.09^{aB}	73.70 ± 0.20^{aAB}	73.85 ± 0.28^{abAB}	74.47 ± 0.23^{aA}	73.01^{a}	0.0000
CM + PF	69.90 ± 0.03^{bD}	73.14 ± 0.06^{aBC}	72.61 ± 0.15^{cC}	73.63 ± 0.15^{bcB}	74.87 ± 0.22^{aA}	73.83^{ab}	0.0000
Mean	70.40^{C}	73.01^{B}	73.02^{B}	73.72^{A}	73.91^{A}		0.0000
P	0.0051	0.2642	0.0025	0.0013	0.0000	0.0184	

Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.
Values are expressed as Mean ± SE. Different lowercase letters in the same column show significant differences among treatments ($P < 0.05$). Different uppercase letters in the same row show significant differences among storage time ($P < 0.05$).

(Djenane & Roncalés, 2018). Also, *P. fluorescens* can show slight signs of growth regardless of high concentrations of CO_2; in fact, residual O_2 levels as low as 0.1% are enough to allow *Pseudomonas* sp. growth even in products stored in low-oxygen MAP. Indeed, package permeability also allows growth despite the absence of oxygen in the packaging (Stoops, Maes, Claes, & Van Campenhout, 2012). The N_2 exhibited a slight decline over the storage period. Treatments had minimal impact on N_2 concentration, with the only noticeable difference detected between NC and CM + BT.

In ham (70/30% N_2/CO_2), the concentration of CO_2 decreased in all treatments. This decrease in the CO_2 concentration in ham may be related to the gas absorption rate by the different meat matrices. Devlieghere and Debevere (2000) reported that sliced cooked ham seems to reach a faster absorption equilibrium of CO_2 dissolved in the matrix (60 min) than ground beef (12*h*). By the end of storage, CM, CM + BT, PF, and CM + PF showed the most significant decrease in CO_2 levels (−3.5, −3.5, −4 and − 4.3%, respectively), whereas N_2 increased the most (4.2, 4.1, 4.6 and 5.0%, in that order, respectively) (Table 3).

A crucial quality parameter of meat is its color, which is the first attribute consumers evaluate to predict meat quality and freshness when purchasing the product. The acceptance of raw and cooked meat relies intensely on color and color uniformity (Hunt et al., 2012). Color disparities are calculated as ΔE, and when ΔE > 3, color differences are visible to the human eye, leading to product rejection (Olivera, Bambicha, Laporte, Cárdenas, & Mestorino, 2013).

In ground beef, the storage time influenced color coordinates in all treatments, increasing luminosity and decreasing redness and yellowness. No consistent effect of treatments on color coordinates was observed. When considering the mean values, it is noteworthy that, in general, PF exhibited greater luminosity and yellowness, but lower redness. Nevertheless, all treatments showed perceptible color alterations (ΔE > 3) at the end of storage, which is likely related to the high O_2 content (66%) in MAP (CM = 17.1; CM + PF = 16.9; CM + BT = 17.4; BT = 16.9; PF = 16.2; NC = 18.2) (Table 2). Thus, these values exceed the theoretical customer decline threshold (Olivera et al., 2013). Jakobsen and Bertelsen (2000) showed that beef stored in MAP with O_2 content higher than 55% had issues with color stabilization throughout the shelf-life. When beef is stored in an aerobic atmosphere, the high concentrations of O_2 can cause myoglobin oxidation, leading to meat discoloration (Zhang, Xiao, & Ahn, 2013).

In ham, treatments and storage time affected only yellowness. The individual effect of the storage time was not observed for the luminosity and redness. These coordinates were also not affected by treatments at 28 days. There were differences between treatments and time for yellowness. However, the numerical values were close and would not interfere with the product's visual quality since ΔE* values were below

3.0 in all treatments. Treatments inoculated with *C. maltaromaticum* showed lower color alterations (CM = 0.70, CM + BT = 0.68, and CM + PF = 1.56) compared to other treatments (NC = 1.33, BT = 1.11, and PF = 2.74). The effect of carnobacteria on color would be unnoticeable in the ham samples as ΔE* values were lower than 1. ΔE* values <1 would theoretically be imperceptible to the consumer's eye and values between 1 and 3 would not be rejected (Hunt et al., 2012). This result is important considering the capability of *Pseudomonas* to alter the color of meat (Zagorec & Champomier-Vergès, 2017). Thus, the joint incorporation of carnobacteria helped preserve the color stability of the ham (Table 4). The negligible influence of the *C. maltaromaticum* strains on meat color was previously reported in raw and cooked beef patties (Casaburi et al., 2011; Imazaki et al., 2023; Said et al., 2019) and in ham (Danielski et al., 2020).

3.3. Shelf-life prediction

A logistic regression model was employed to predict the shelf-life, measured in days, of the products. The use of *Carnobacterium* to increase a product's shelf-life has not been reported before according to the bacterial growth prediction model and the spoilage threshold for each bacterium. This prediction was based on the time required for the microbial population to reach the threshold at which the products become unfit for consumption due to the emergence of detectable off-odors. The spoilage thresholds stood at 7 log CFU/g for *Pseudomonas* and 6 log CFU/g for *Brochothrix*, as these levels correlate with noticeable deterioration-induced odors (Mills et al., 2014; Pellissery et al., 2020).

According to the model, ground beef inoculated with BT would show spoilage (6 CFU/g) within 5 days of storage, while in samples inoculated with CM + BT, the *Brochothrix* population would take twice the time (10 days) to reach 5.1 log CFU/g (Fig. 3). In the case of ham, samples would reach spoilage earlier in CM + BT (9 days) than in BT (13 days). However, these treatments displayed a similar count of *B. thermosphacta* on day 16, but from that point forward, the count in CM + BT would stabilize, while the count in BT would continue to rise (Fig. 4).

Regarding indigenous *B. thermosphacta* in the beef, samples without bacterial inoculation (NC) would also reach spoilage after 5 days. Nonetheless, in the case of the CM treatment, bacterial growth would remain below the spoilage threshold, reaching a maximum of 5.6 log CFU/g by the 9th day (Fig. 3). Hence, the addition of CM could potentially extend the shelf-life of ground beef by a minimum of 80%. Whereas in ham, the NC samples would reach the estimated spoilage threshold in 29 days of storage. The CM would not reach the spoilage limit, with a maximum growth of 3.3 log CFU/g in 33 days, representing, at minimum, an extension of 13.8% in shelf-life when compared to NC (Fig. 4).

Ground beef inoculated with *P. fluorescens* (PF) would reach spoilage after 9 days of storage. In contrast, the spoilage threshold would not be reached in CM + PF, with the maximal expected population reaching 5 log CFU/g by the 9th day (Fig. 3). The ham samples inoculated with *P. fluorescens* in PF treatment would reach the estimated spoilage in 18 days of storage, whereas in CM + PF, it would be reached after 33 days (83.3% increase compared to PF) (Fig. 4).

In beef, the indigenous *P. fluorescens* growth was predicted to reach a

Table 4
Effect of the addition of a pool of *C. maltaromaticum* and spoilage bacteria on instrumental color (L*, a*, b* and ΔE*) of sliced cooked ham during storage in MAP (70/30% N_2/CO_2) for 28 days (10 days at 4 °C, followed by 18 days at 8 °C).

	Storage days						
Treatment	0	5	10	19	28	Mean	*P*
			L*				
NC	65.01 ± 0.67^{aA}	65.38 ± 0.54^{aA}	66.05 ± 0.89^{aA}	65.29 ± 1.33^{aA}	66.23 ± 0.97^{aA}	65.59^{a}	0.8623
CM	65.17 ± 0.74^{aA}	63.65 ± 1.14^{aA}	66.19 ± 0.82^{aA}	65.01 ± 0.78^{aA}	65.54 ± 0.80^{aA}	65.11^{ab}	0.3346
BT	65.48 ± 0.95^{aA}	67.02 ± 0.86^{aA}	66.77 ± 0.66^{aA}	63.70 ± 1.13^{aA}	64.58 ± 0.87^{aA}	65.51^{ab}	0.0553
CM + BT	64.17 ± 1.18^{aA}	64.72 ± 1.05^{aA}	64.40 ± 1.39^{aA}	64.14 ± 1.00^{aA}	64.49 ± 0.88^{aA}	64.39^{ab}	0.9958
PF	65.15 ± 1.49^{aA}	64.87 ± 1.12^{aA}	64.71 ± 0.80^{aA}	65.07 ± 1.15^{aA}	62.82 ± 1.37^{aA}	64.53^{ab}	0.6355
CM + PF	64.96 ± 0.80^{aA}	63.78 ± 1.26^{aA}	64.31 ± 0.91^{aA}	62.85 ± 1.03^{aA}	63.91 ± 0.69^{aA}	63.69^{b}	0.3118
Mean	64.99 ^{A}	64.90 ^{A}	65.41 ^{A}	64.34 ^{A}	64.60 ^{A}		0.2839
P	0.9690	0.2154	0.2847	0.5677	0.0490	0.0259	
			a*				*P*
NC	7.54 ± 0.30^{aA}	7.91 ± 0.30^{aA}	8.51 ± 0.61^{aA}	7.76 ± 0.72^{aA}	7.65 ± 0.47^{aA}	7.87^{a}	0.6903
CM	7.91 ± 0.35^{aA}	9.01 ± 0.65^{aA}	7.86 ± 0.39^{aA}	7.68 ± 0.50^{aA}	7.73 ± 0.30^{aA}	8.04^{a}	0.2225
BT	7.93 ± 0.55^{aA}	7.64 ± 0.43^{aA}	7.45 ± 0.42^{aA}	8.90 ± 0.67^{aA}	8.00 ± 0.35^{aA}	7.98^{a}	0.2902
CM + BT	7.87 ± 0.55^{aA}	8.45 ± 0.47^{aA}	8.05 ± 0.57^{aA}	8.15 ± 0.47^{aA}	8.43 ± 0.36^{aA}	8.19^{a}	0.9024
PF	7.23 ± 0.80^{aA}	8.19 ± 0.59^{aA}	7.87 ± 0.42^{aA}	7.68 ± 0.60^{aA}	8.64 ± 0.63^{aA}	7.92^{a}	0.5653
CM + PF	7.78 ± 0.56^{aA}	8.68 ± 0.55^{aA}	8.21 ± 0.41^{aA}	8.56 ± 0.57^{aA}	8.85 ± 0.51^{aA}	8.41^{a}	0.6178
Mean	7.71 ^{A}	8.31 ^{A}	7.99 ^{A}	8.12 ^{A}	8.22 ^{A}		0.2954
P	0.9329	0.4442	0.7211	0.5897	0.3112	0.5826	
			b*				*P*
NC	8.41 ± 0.14^{cBC}	8.11 ± 0.11^{aC}	8.76 ± 0.17^{aB}	10.04 ± 0.13^{aA}	8.93 ± 0.13^{aB}	8.85^{ab}	0.0000
CM	9.32 ± 0.15^{aB}	8.13 ± 0.21^{aC}	8.67 ± 0.10^{aC}	9.99 ± 0.15^{aA}	8.75 ± 0.16^{abBC}	8.97^{ab}	0.0000
BT	8.87 ± 0.18^{abcB}	8.28 ± 0.08^{aC}	8.53 ± 0.13^{aBC}	10.17 ± 0.18^{aA}	8.21 ± 0.12^{bC}	8.81^{ab}	0.0000
CM + BT	8.94 ± 0.23^{abcBC}	8.50 ± 0.18^{aBC}	8.24 ± 0.26^{aC}	10.49 ± 0.15^{aA}	9.15 ± 0.17^{aB}	9.06^{a}	0.0000
PF	8.44 ± 0.18^{bcB}	8.26 ± 0.23^{aB}	8.14 ± 0.15^{aB}	9.93 ± 0.19^{aA}	8.64 ± 0.21^{abB}	8.68^{b}	0.0000
CM + PF	9.17 ± 0.22^{abB}	8.42 ± 0.18^{aC}	8.46 ± 0.19^{aBC}	10.10 ± 0.18^{aA}	8.74 ± 0.16^{abBC}	8.98^{ab}	0.0000
Mean	8.86^{B}	8.28^{D}	8.47^{CD}	10.12 ^{A}	8.74^{BC}		0.0000
P	0.0023	0.5488	0.0980	0.2204	0.0038	0.0069	
			ΔE				
NC			1.33				
CM			0.70				
BT			1.11				
CM + BT			0.68				
PF			2.74				
CM + PF			1.56				

Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.
Values are expressed as Mean ± SE. Different lowercase letters in the same column show significant differences among treatments ($P < 0.05$). Different uppercase letters in the same row show significant differences among storage time ($P < 0.05$).

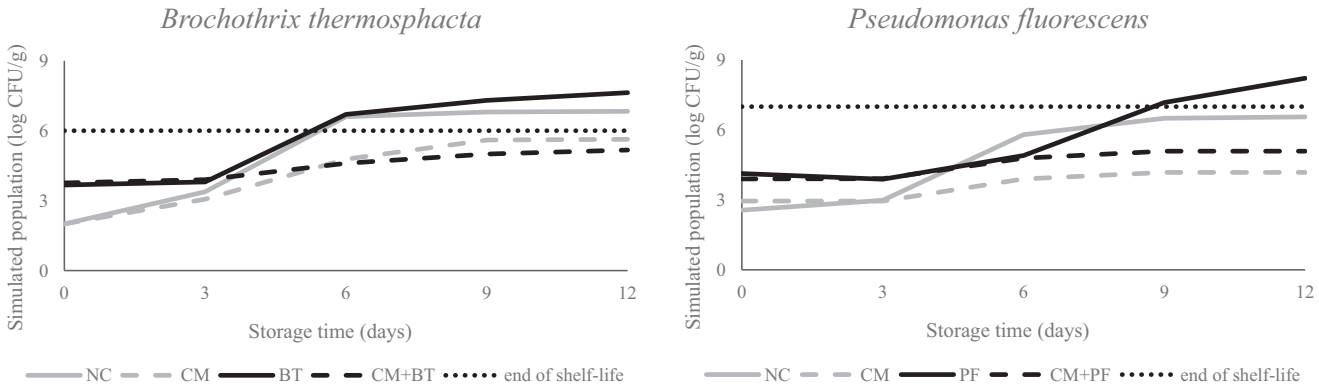

Fig. 3. Prediction of shelf-life based on the effect of *C. maltaromaticum* on the populations of *B. thermosphacta* and *P. fluorescens* in ground beef stored in MAP (66/30/4% $O_2/CO_2/N_2$) for 3 days at 4 °C and 4 days at 8 °C.

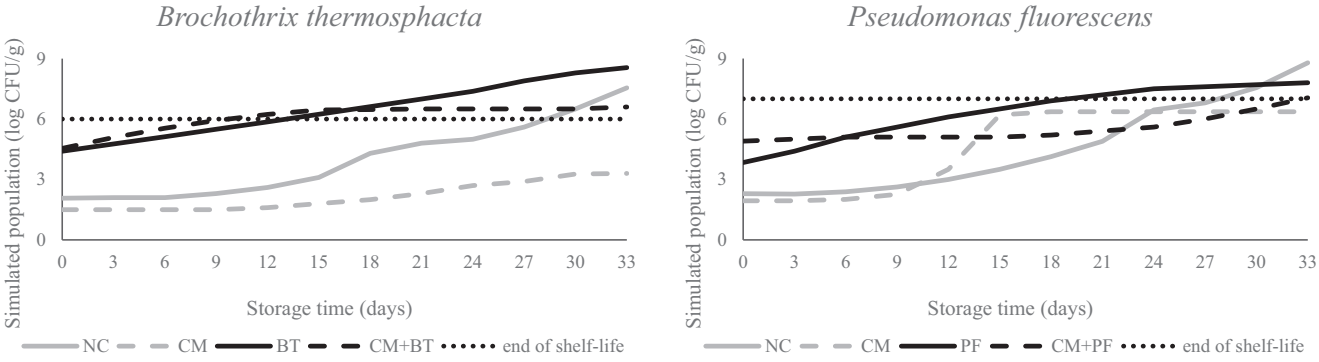

Fig. 4. Prediction of shelf-life based on the effect of *C. maltaromaticum* on the populations of *B. thermosphacta* and *P. fluorescens* in sliced cooked ham stored in MAP (70/30% N_2/CO_2) for 28 days (10 days at 4 °C, followed by 18 days.
Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.
The storage time (days) expresses the predicted shelf-life with a logistic regression model based on the estimated microbial population according to Y=a + b/(1 + (x/c)d). Where y is the microbial population count (Y=F(x)∞); x is the storage time (days) and a, b, c, and d are independent variables. The R^2 values for the simulated *B. thermosphacta* population are: 0.834974 (NC), 0.879554 (CM), 0.879554 (BT) and 0.961514 (CM + BT), and for *P. fluorescens*: 0.936228 (NC), 0.959526 (CM), 0.954111 (PF) and 0.653685 (CM + PF).
End of shelf-life was considered at the spoilage threshold: 7 log CFU/g for *Pseudomonas* spp. and 6 log CFU/g for *Brochothrix* spp.

maximum of 6.5 log CFU/g in NC after 9 days of storage, all while remaining under the spoilage threshold. A similar prediction was made for the CM treatment, although the maximum growth was estimated to be 4.2 log CFU/g by day 10, indicating an extension of >11.1% in shelf-life (Fig. 3). Ham samples in NC would reach the spoilage threshold in 29 days, whereas in CM, the limit would not be reached within the same period, and the growth would remain at 6.4 log CFU/g by the 15th day of storage (Fig. 4).

Other studies with LAB showed that *Lactobacillus curvatus* CRL705 (6.0 log CFU/g) could increase the shelf-life of vacuum-packaged raw beef (60 days at 2 °C) in 16.7% (10 days increase) based on appearance acceptance (Castellano, González, Carduza, & Vignolo, 2010). The increase in shelf-life is significant, showing that *C. maltaromaticum* can be competitive with other LABs in the shelf-life improvement of beef. For instance, Bredholt, Nesbakken, and Holck (2001) reported that a non-bacteriogenic *Lactobacillus sakei* strain increased the shelf-life of sliced cooked ham (28 days) by 17.9% (5 days increase) at concentrations of 5.0 to 6.0 log CFU/g.

The pool of *C. maltaromaticum* tested proved to be an effective potential bioprotective culture. As observed *C. maltaromaticum* can reduce spoilage population and remain mostly stable until the end of shelf-life, corroborating the fact that *Carnobacterium* tends to survive and thrive in contamination in abattoirs, processing, storage, and distribution of meat products (Chen et al., 2020). These strains can also grow in the presence of chemical preservatives such as nitrite, and acids such as acetic, lactic acid, ascorbic acid, benzoic, citric, and sorbic acids (Laursen et al., 2005). Moreover, the weak acid-producing potential and the fact that *C. maltaromaticum* can sustain a low pH variation (Edima et al., 2008) show an advantage over most LAB because the influence of a substantial pH decrease affects the quality parameters of these types of meat products (Singh, 2018). In addition, the neglected effect on the meat color benefits the product acceptance by consumers. Therefore, the use of bioprotective cultures along with good hygiene practices could help preserve the meat quality, stabilizing the color variation and reducing the spoilage population (Grispoldi, Karama, Sechi, Iulieto, & Cenci-Goga, 2020). In conclusion, *C. maltaromaticum* could be exploited as a natural preservative in meat to enhance quality and prolong the product shelf-life.

4. Conclusion

The pool of *C. maltaromaticum* strains (CM_824, CM_827, and

CM_289) reduced the counts of both inoculated and indigenous spoilage bacteria (*Brochothrix* sp., *B. thermosphacta*, *Pseudomonas* sp., *P. fluorescens* and Enterobacteriaceae). The inhibitory effect was observed in food matrices (sliced cooked ham and ground beef) and atmospheres tested with the potential increase in the shelf-life of the products.

This study supports the use of *C. maltaromaticum* as a bioprotective culture due to the following attributes: inhibition of spoilage microorganisms, stable growth under storage conditions until the end of shelf-life, minor alterations of the physicochemical quality parameters of the products and extension of products shelf-life.

Author statement

The authors state that the content of this manuscript was not published nor is it under consideration for publication elsewhere. The final version of the manuscript is approved by all authors. We confirm that we have read and have followed all the ethical standards for manuscripts submitted to this Journal. The authors have no conflict of interest to declare.

Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.

A = Total viable counts; B = Lactic acid bacteria counts; C = Enterobacteriaceae counts, D = inoculated *B. thermosphacta* counts are shown in BT and CM + BT and indigenous *B. thermosphacta* counts are shown in NC, CM, PF, and CM + PF; E = inoculated *P. fluorescens* counts are shown in PF and CM + PF and indigenous *P. fluorescens* counts are shown in NC, CM, BT, and CM + BT.

Storage time: initial = 0 days; final = 7 days.

Values are expressed as mean ± SE. Different lowercase letters show significant differences among microbial counts in samples on the first day of storage ($P < 0.05$). Different uppercase letters show significant differences among microbial counts in samples on the final day of storage ($P < 0.05$).

Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.

A = Total viable counts; B = Lactic acid bacteria counts; C = Enterobacteriaceae counts, D = inoculated *B. thermosphacta* counts are shown in BT and CM + BT and indigenous *B. thermosphacta* counts are shown in NC, CM, PF, and CM + PF; E = inoculated *P. fluorescens* counts are shown in PF and CM + PF and indigenous *P. fluorescens* counts are shown in NC, CM, BT, and CM + BT.

Storage time: initial = 0 days; final = 28 days.

Values are expressed as mean ± SE. Different lowercase letters show significant differences among microbial counts in samples on the first day of storage ($P < 0.05$). Different uppercase letters show significant differences among microbial counts in samples on the final day of storage ($P < 0.05$).

Treatments: NC = negative control; CM = pool of *C. maltaromaticum*; BT = pool of *B. thermosphacta*; PF = *P. fluorescens*; CM + BT = pool of *C. maltaromaticum* + pool of *B. thermosphacta*; CM + PF = pool of *C. maltaromaticum* + *P. fluorescens*.

The storage time (days) expresses the predicted shelf-life with a logistic regression model based on the estimated microbial population according to Y=a + b/(1 + (x/c)d). Where y is the microbial population count (Y=F(x)∞); x is the storage time (days) and a, b, c, and d are independent variables. The R^2 values for the simulated *B. thermosphacta* population are: 0.998823 (NC), 0.996798 (CM), 0.999527 (BT) and 0.963082 (CM + BT), and for *P. fluorescens:* 0.998891 (NC), 0.968915 (CM), 0.897622 (PF) and 0.963082 (CM + PF).

End of shelf-life was considered at the spoilage threshold: 7 log CFU/g for *Pseudomonas* spp. and 6 log CFU/g for *Brochothrix* spp.

CRediT authorship contribution statement

Caroline Maria de Andrade Cavalari: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Pedro Henrique Imazaki:** Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Barbara Pirard:** Investigation. **Sarah Lebrun:** Data curation, Investigation, Methodology. **Raphael Vanleyssem:** Investigation, Methodology. **Céline Gemmi:** Investigation. **Céline Antoine:** Investigation. **Sébastien Crevecoeur:** Methodology. **Georges Daube:** Methodology. **Antoine Clinquart:** Investigation, Methodology, Project administration, Writing – original draft. **Renata Ernlund Freitas de Macedo:** Conceptualization, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2024.109441.

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