

Modified-atmosphere storage under subatmospheric pressure and beef quality: I. Microbiological effects¹

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ABSTRACT: The microflora was studied in beef stored in stainless steel containers kept under reduced pressure (20 to 30 kPa) in a modified atmosphere (70% N₂ + 30% CO₂ or pure CO₂) at 3 to 4°C and 0 to 1°C at a headspace:meat volume ratio of 2:1. Samples were obtained at weekly intervals, 1 to 3 times. Total colony counts (TCC) for *Pseudomonas* spp. and *Brochothrix thermosphacta* were generally 1 to 2 log₁₀ cfu greater than in the control group of vacuum-packaged beef cuts stored at the same temperatures. In containers with the 70% N₂ + 30% CO₂ atmosphere at 20 to 30 kPa and 3 to 4°C, substantial growth of *Pseudomonas* sp. was observed (median of 6 log₁₀ cfu/cm² at d 21 of storage compared with 3 log₁₀ cfu/cm² for vacuum-packaged beef). *Pseudomonas* counts were lower when the container system was held at 0 to 1°C, especially when

combined with the pure CO₂ atmosphere. As expected for CO₂-enriched atmospheres, *B. thermosphacta* was the dominant spoilage bacterium, in the same log₁₀ order as the TCC. Lowering the storage temperature and changing the atmosphere to pure CO₂ resulted in a reduction of 1 log₁₀ for TCC (median values after 2 wk of storage). Although pathogenic bacteria such as *Campylobacter*, *Salmonella*, and *Listeria monocytogenes* were not detected in any sample, further studies are necessary to evaluate potential growth risks. The results demonstrate that CO₂-enriched and O₂-depleted atmospheres under low pressure have a limited effect on reducing bacterial growth, probably because the antibacterial activity of CO₂ is proportional to the effective concentration of this gas in the headspace. At pressures of 20 to 30 kPa, a headspace with pure CO₂ would still contain only approximately 20 to 30% CO₂.

Key words: beef, carbon dioxide, low pressure, microbiology, storage

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INTRODUCTION

Microbial spoilage of meat has always been of great concern to the food industry. Meat can be a vehicle for foodborne pathogenic microorganisms, unless handled with the utmost care. Modified-atmosphere packaging of meat can extend its shelf life. Modified-atmosphere packaging usually involves high concentrations of CO₂ (selectively inhibiting the growth of gram-negative bacteria), in combination with N₂ (replacing O₂ to hinder oxidation of fats and other ingredients) to inhibit the characteristic off-odors, off-colors, and spoilage associated with the growth of gram-negative psychrotrophs, and to slow product deterioration (Van Laack et al., 1996).

Occasional reports have claimed beneficial effects of the commercial use in abattoirs of plastic (low tech) storage containers kept under super- or subatmospheric pressure. The aim is to improve sensory meat characteristics, notably color (stability) and tenderness, without jeopardizing the microbiological condition (Aemig, 1992). However, systematic scientific studies on these potential effects are scarce, with one study (Smulders and Van Laack, 1992) on sensory meat quality characteristics failing to substantiate the above claims.

The microbiological quality of meat during extended storage is of great concern, and environmental considerations (i.e., reducing food packaging waste) are also relevant. The global challenge to reduce packaging wastage is now emphasized in legislation (European Union, 1994).

The purpose of the current study was to investigate meat quality under various modified-atmosphere packaging conditions in rigid, stainless steel containers. The present paper reports the effects of vacuum and controlled-atmosphere storage with N₂/CO₂ gas mixtures

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on the microbiological condition of beef muscles; the companion article in this issue discusses the sensory effects.

MATERIALS AND METHODS

Principle

The microbiological condition of beef muscles stored in rigid reusable containers at fixed temperature, atmospheric conditions, and pressure was compared with that of beef muscles stored in conventional vacuum-film packages for up to 21 d.

Three identical containers were available and these were opened successively at weekly intervals to allow testing of the storage effects at distinct times during storage.

Material for Analysis, Sampling, and Storage Equipment

Our study was conducted in a single EU-approved abattoir. Experimental animals were exclusively obtained from their commercial supply, and all had passed veterinary antemortem inspection before being admitted to slaughter.

Sample Material. In 3 trials, thirty-six 18-mo-old Fleckvieh bulls were stunned with a captive bolt, suspended from the left hindleg, and subsequently exsanguinated. Electrical stimulation was not applied. After dressing, their prechill hot carcass weights ranged from 370 to 420 kg. All animals were classified according to the EUROP system (Allen and Oka, 2004) as U or R and fat classes 2 or 3.

The animals used in this study were consistently slaughtered on a Monday, and refrigerated for 48 h in air at $2 \pm 2^\circ\text{C}$ and an air velocity of 6 m/s. The carcasses were always hung at the same location in the same chill room before they were transported to the boning room (at 12°C), where both their left and right side LM were removed. These strip loin samples (approximately 40 to 50 cm long) were subsequently transported in refrigerated boxes to the nearby laboratory, where they were allocated to 1 of 2 storage treatments. Loins from one side of each animal were vacuum-packaged in plastic film, and from the other in the container (see next section).

In the first trial (A), the left-side muscles were always stored in the container, and the right side was vacuum-packaged in film. In the second (B) and third (C) trials, the right-side muscles were randomly assigned to container or film storage and their counterparts to the other treatment.

Description of the Container Storage System. Three 200-L, high-grade stainless steel barrels were equipped with a control unit for the introduction of gases (Figure 1). The atmosphere was modified to ultimately consist of 70 parts N_2 and 30 parts CO_2 at a given pressure between 20 and 30 kPa. In trial C only,

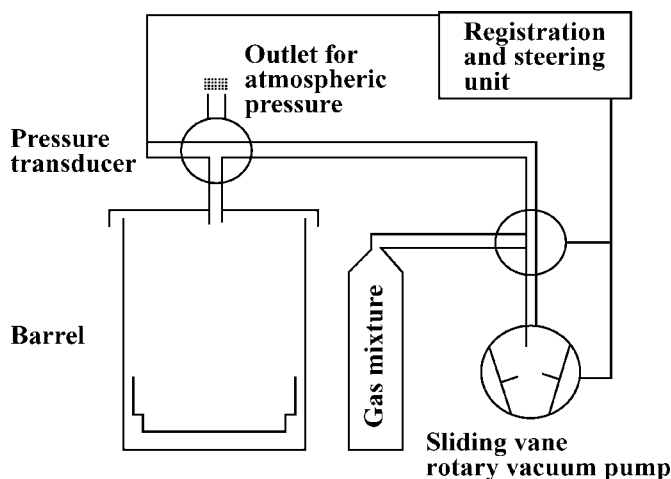


Figure 1. Design of the container storage unit. The barrel lid was equipped with a manually operated vent, to allow the introduction of air (atmospheric pressure) or, as shown in the figure, to connect the barrel to the vacuum pump-gas unit. The steering unit operated a 3-way valve to switch between gas flushing and vacuum drawing.

the barrel was filled with pure CO_2 under the aforementioned pressure. Muscle samples were placed in cleaned and disinfected trays, with the same circumference as the barrel's inner wall. A removable sterile perforated plate inset was situated 2 cm above the bottom of the tray to prevent the muscle sample surfaces from coming into contact with the drip accumulating in the tray. Trays (1 in each container) were covered with a sterile lid that was in contact with the meat.

All surfaces that could come in contact with the meat samples were made from V2A steel suitable for food-stuffs. The seam area where the lid was kept pressed to the barrel, as well as the inlets for temperature and pressure sensors and gas supply, were sealed using food-grade silicone (Rehau AG, Rehau, Germany). Before each trial, the barrel and all other stainless steel surfaces were meticulously cleansed and disinfected with 70% ethanol. During the study, the barrels and the control samples vacuum-packaged in film were kept in the chill room at temperatures of 3 to 4°C (trial A) or 0 to 1°C (trials B and C).

Filling of the Container and Storage. The trays were filled as densely as possible with the meat samples, taking particular care that environmental contamination could not occur. The individual packing the samples into trays wore sterile surgical gloves. Before covering the samples with the stainless steel lid, empty spaces between samples on each tray were filled with sterile polyamide-polyethylene bags filled with water. After this procedure, the ratio of gaseous headspace to meat volume was adjusted to be 2:1.

Temperature was monitored using a multichannel recorder (DR 240, Yokogawa, Japan) connected with 2 Fe-Cu-Ni thermocouple probes (Conatex, St. Wendel, Germany) inserted in 2 gelatin blocks (FRPERC, Bris-

Table 1. Overview of storage conditions and sampling intervals

Trial	Sample size (n)	Storage temperature, °C	Pressure, kPa	Atmosphere	Sampling day (after slaughter)
A	18	3 to 4	20 to 30	70% N ₂ + 30% CO ₂	2, 9, ¹ 16, 23 ¹
B	12	0 to 1	20 to 30	70% N ₂ + 30% CO ₂	2, 9, 16 ²
C	6	0 to 1	20 to 30	100% CO ₂	2, 9, 16

¹Sample size = 12.²Sample = 6.

tol, UK). One of these was located inside the container (representing the meat temperature in the container) and the other outside the barrel (representing the meat temperature in the chill room). In addition, chill room temperature was recorded on a display outside the chill room. Table 1 includes the storage conditions and sampling intervals in trials A, B, and C.

Atmospheric Conditions in the Container. For drawing vacuum in the container, a vacuum pump (WB 80 B2 STP, Becker, Wuppertal, Germany) was used. Table 1 includes the various gas mixtures introduced. Beginning with trial B, the container was first “rinsed” with the gas mixture immediately after filling, to effectively and rapidly expel any residual O₂. The barrel was exhausted until the pressure fell below 5 kPa, and was then flushed with the gas mixture until a pressure of 90 kPa was reached. This procedure was repeated and then the pressure was reduced to the operating pressure of 20 to 30 kPa, which was maintained throughout the storage period. To restore the gas mixture throughout storage, a short withdrawal of gas from the barrels was conducted at 55-min intervals, followed by the injection of fresh modified atmosphere until the working pressure was reached.

Vacuum-Packaging Procedure. Counterpart muscle samples were packaged separately in vacuum bags [Combivac, foil type 20/70, Felzmann, Linz, Austria;

i.e., a polyamide-polyethylene vacuum film with an O₂ permeability of 50 cm³/(m²·24 h·bar), CO₂ permeability of 150 cm³/(m²·24 h·bar), N₂ permeability of 10 cm³/(m²·24 h·bar), and steam permeability of 2.6g/(m²·24 h·bar)] using a packaging machine (SuperVac, Laska, Traun, Austria) equipped with a rotary-vane vacuum pump (Type 021-336, Busch AG, Magden, Switzerland) with a nominal pumping speed of 20 m³/h and a final pressure of 0.2 kPa. The vacuum-packaged samples were stored in the chill room on trays located next to the containers, at temperatures given in Table 1.

Microbiological Examination

On d 2 (i.e., before the storage treatments), two 3- to 4-mm-thick slices of 4 × 5 cm each were excised from the surface of all muscles using a stainless steel, sterile, rectangular template. A similar procedure was followed upon opening the containers or vacuum packs after the various storage intervals. Subsequently, these slices were put in sterile plastic bags and macerated at high speed for 2 min in a Stomacher (Stomacher 400, Seward, UK). Beginning from this primary dilution, a 10-fold dilution series was prepared (Maximum recovery diluent, CM 733, Oxoid, Basingstoke, UK). Microbiological criteria were according to Van Laack et al. (1996) and Upmann et al. (2000); see Table 2. Media were

Table 2. Microbiological criteria assessed and media used in the trials

Item	Method reference	Detection limit
Psychrotrophic pathogens		
<i>Listeria</i> sp.	DIN EN ISO 11290-1 (1997)	P/A ¹ in 25 g
Pathogens capable of growing between 5 and 12°C		
<i>Salmonella</i> sp.	Pless et al. (1993)	P/A in 25 g
<i>Clostridium perfringens</i>	DIN EN 13410 (1999)	10 cfu/cm ²
Pathogens capable of growing between 31 and 45°C		
<i>Campylobacter jejuni</i>	DEN EN ISO 10272 (1995)	P/A in 25 g
General		
Total colony count	DIN 10161, Part 1 (1984)	100 cfu/cm ²
<i>Pseudomonas</i>	Kielwein (1969)	100 cfu/cm ²
<i>Brochothrix thermosphacta</i>	Baumgart (1990)	100 cfu/cm ²
Lactobacillaceae	DIN 10109 (1991)	100 cfu/cm ²
Indicator bacteria		
Enterobacteriaceae	DIN 10164, Part 1 (1986)	100 cfu/cm ²
<i>Escherichia coli</i>	Coli-ID kit ²	100 cfu/cm ²
Coliforms	Mallmann and Darby (1941)	3 (most probable number) cfu/cm ²

¹P/A = presence/absence.²Colony counting on chromogenic selective agar (Coli-ID, bioMerieux, Marcy l’Etoile, France) after incubation at 42°C for 24 h, according to the manufacturer’s instructions.

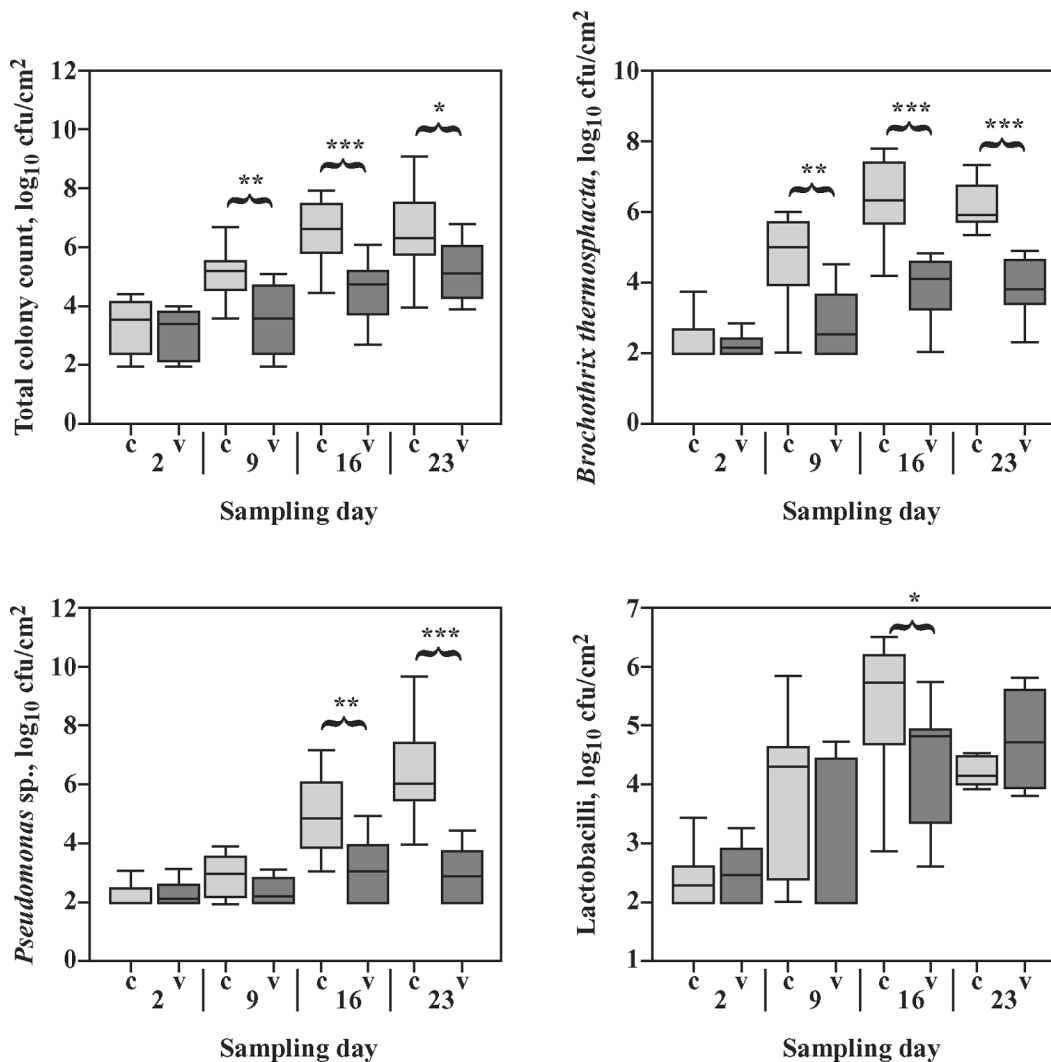


Figure 2. The effects of 1- to 3-wk of storage in modified atmosphere (N₂/CO₂) in containers (C, left-side samples) vs. vacuum-storage in packaging films (V, right-side counterparts) on the surface microflora of beef LM expressed as log₁₀ cfu/cm² in trial A (n = 18 for d 2 and 16, and n = 12 for d 9 and 23). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

obtained from BioMerieux (Marcy l'Etoile, France), Merck (Darmstadt, Germany), and Oxoid.

Statistical Analysis

Data were analyzed using the SPSS 11.5 for Windows software package (SPSS Inc., Chicago, IL). For hypothesis testing, the U-test (Wilcoxon-Mann-Whitney) was applied. Significant differences were displayed at the error levels of *P* < 0.05, *P* < 0.01, and *P* < 0.001. Results are displayed as vertical box-and-whisker plots, wherein the box comprises the middle 50% of the data, with the median as a horizontal line in the box. The whiskers extending from the boxes represent the minimum and maximum values.

RESULTS

Total Colony Counts, Lactobacillaceae, and Spoilage Bacteria

The data presented in Figures 2, 3 and 4 demonstrate the combined effects of chill storage and modified gas

atmosphere on the development of the microflora on beef cuts.

At 3 to 4°C and at the common 70:30 N₂/CO₂ gas mix, total colony counts (TCC) and counts of *Pseudomonas* sp. and *B. thermosphacta* were significantly (e.g., for d 16: *P* = 0.000, 0.005, and 0.000, respectively) greater (generally 1 to 2 log₁₀ units) on container-stored than on vacuum-packaged beef strip loins (trial A). As expected, lowering the storage temperature to 1 to 2°C (trial B) resulted in microbial numbers approximately 1 log₁₀ unit lower than in trial A.

After the containers had been flushed with 100% CO₂ (trial C) at both storage intervals tested, TCC were greater compared with the vacuum-packaged samples, in spite of the significantly greater (*P* = 0.041) initial microbial surface load on the latter samples. However, in trial C, the microbial concentrations of *Pseudomonas* sp. and *B. thermosphacta* were low.

The growth of Lactobacillaceae was generally slower at 0 to 1°C (Figures 3 and 4) than at 3 to 4°C (Figure 2). Under both container and vacuum-packaged storage, *B.*

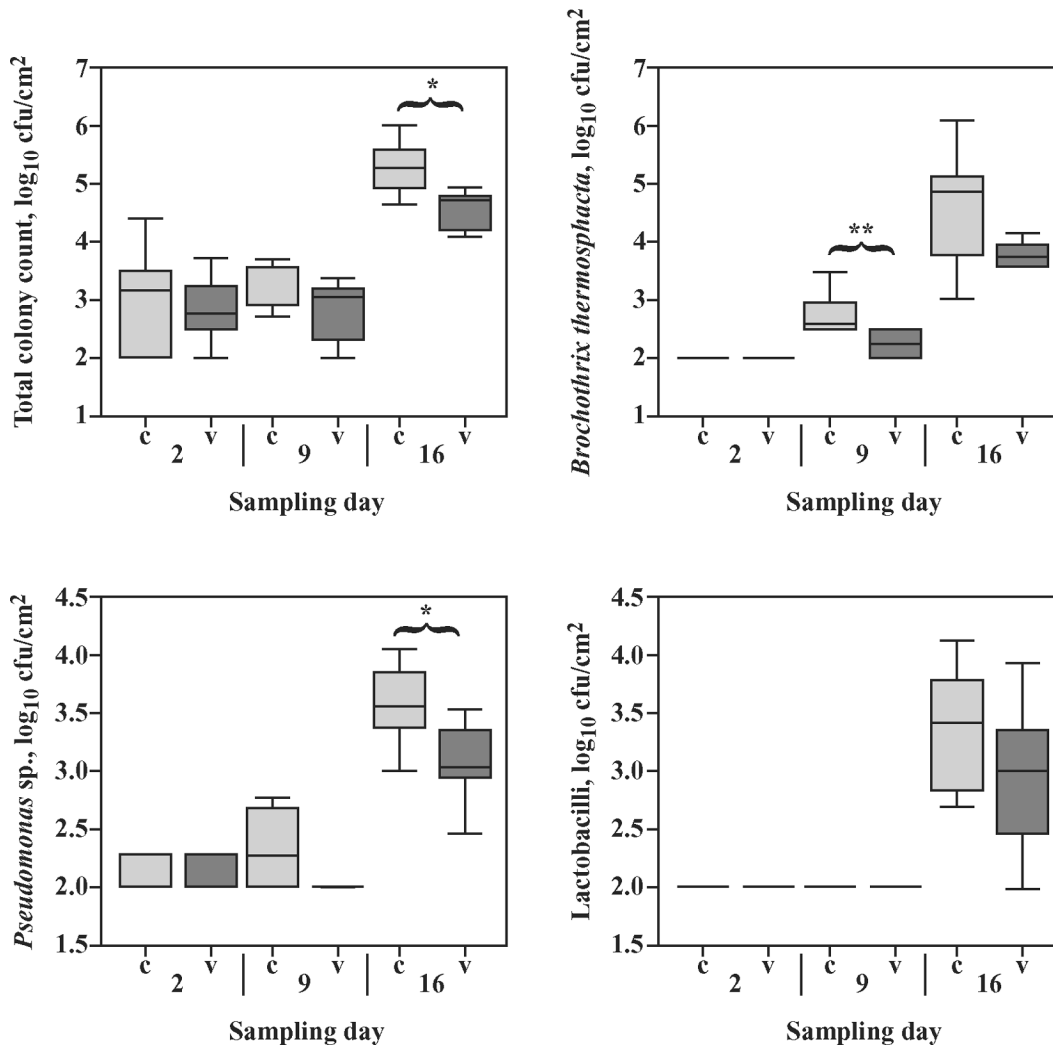


Figure 3. The effects of 1- to 2-wk storage in modified atmosphere (N₂/CO₂) in containers (C) vs. vacuum-storage in packaging films (V) on the surface microflora of beef LM expressed as log₁₀ cfu/cm² in trial B (randomized sample distribution; n = 12 for d 2 and 9, and n = 6 for d 16). **P* < 0.05; ***P* < 0.01; horizontal lines at 2 log₁₀ cfu/cm² indicate that all results were below the limit of detection.

thermosphacta counts were similar to, or exceeded, the Lactobacillaceae counts.

Indicator Bacteria and Pathogenic Bacteria

The number of coliforms (most probable number) was generally <30/cm² throughout all experiments, and those for *Escherichia coli* <10 cfu/cm². Enterobacteriaceae counts ranged from <100 to a maximum of 400 cfu/cm². None of the samples tested positive for the pathogenic bacteria *Listeria monocytogenes*, *Salmonella* sp., *Campylobacter* sp. (25-g sample size), or *Clostridium perfringens* (<10 cfu/cm²) (data not shown).

DISCUSSION

Antimicrobial Efficacy of CO₂ as Influenced by Temperature, Headspace, and Pressure in the Container System

Atmospheric CO₂ absorbed by the meat's surface layer is metabolized to carbonic acid, which is the com-

pound demonstrating antibacterial activity (Down, 1996; Blakistone, 1998). Hence, the amount of CO₂ in the container is critical. This amount will depend on the meat volume:headspace ratio (Devlieghere et al., 1998), the (subatmospheric) pressure, and on the composition of the gas mixture. For economic reasons, it would be desirable to operate the container storage system with all trays full with meat, at the lowest possible pressure, but this would inevitably jeopardize the protective effect of CO₂.

The more pronounced effect of CO₂ at lower storage temperatures in trials B and C is explained by the fact that the solubility of carbonic acid in meat increases with decreasing temperature (O'Connor-Shaw and Reyes, 1999). When the storage temperature is reduced, a more pronounced effect of CO₂ has been observed, as reported by Blickstad et al. (1981) and Greer et al. (1993).

Under normal pressure, atmospheres with 20% CO₂ were effective in suppressing the multiplication of com-

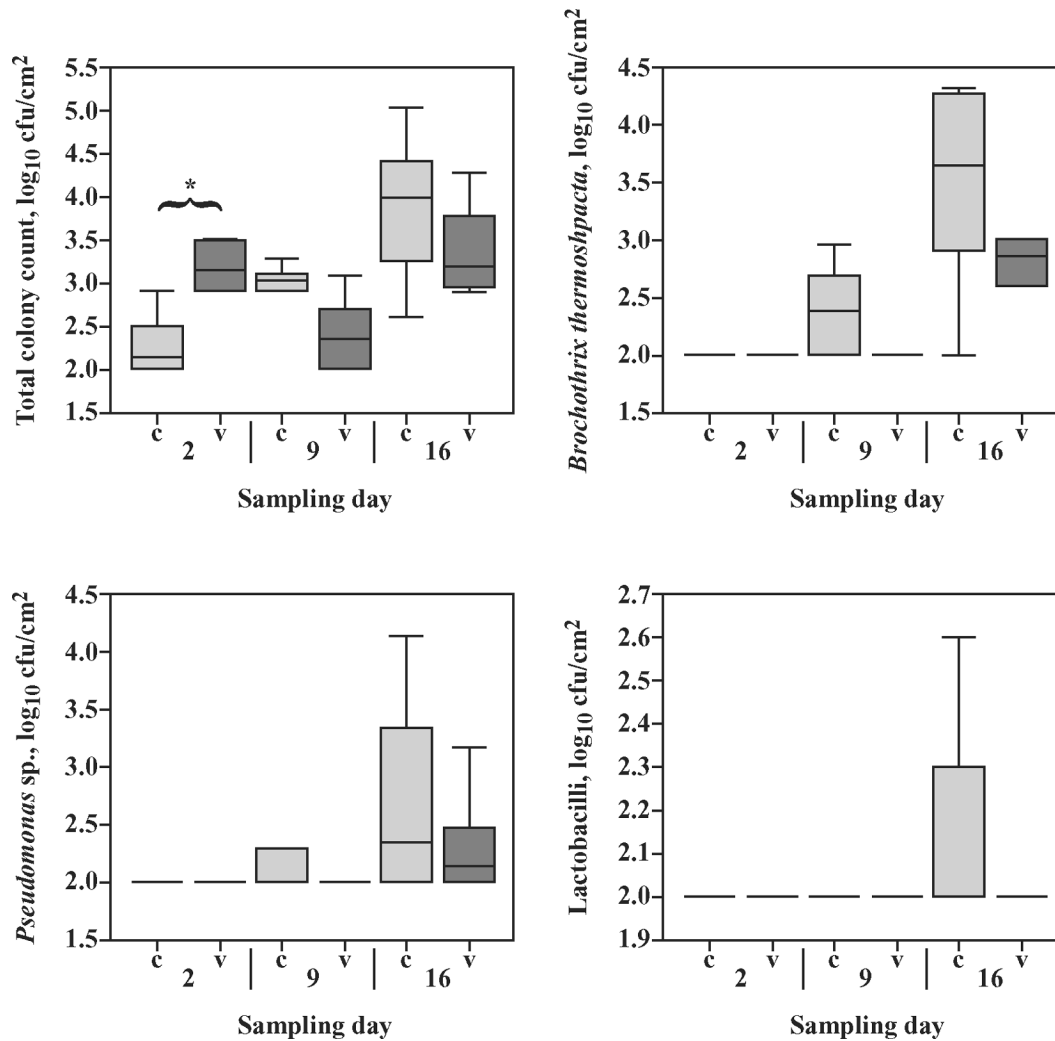


Figure 4. The effect of 1- to 2-wk storage in containers (C) filled with 100% CO₂ vs. vacuum-storage in packaging films (V) on the surface microflora of beef LM expressed as log₁₀ cfu/cm² in trial C (randomized sample distribution; n = 6). *P < 0.05; horizontal lines at 2 log₁₀ cfu/cm² indicate that all results were below the limit of detection.

mon meat spoilage bacteria, such as *Pseudomonas* sp. and *Acinetobacter* and *Moraxella* spp. (Clark and Lentz, 1969). The container storage system under study relied on subatmospheric pressure conditions and consequently resulted in reduced CO₂ concentration in the headspace. Because the antibacterial activity of CO₂ dissolved in the aqueous phase of the meat is proportional to the effective concentration of this gas in the headspace (Devlieghere et al., 1998), a headspace with pure CO₂ would contain only about 20 to 30% CO₂ at pressures of 20 to 30 kPa, which explains the observed moderate effects on reducing bacterial growth. However, we assume that the periodical flushing (i.e., additional supply of CO₂) should cause a steady build-up of carbonic acid in the meat's surface layers. This, and meat respiration, should result in conditions comparable to vacuum packaging, in which both evacuation and meat respiration result in a CO₂/N₂ atmosphere (Van Laack et al., 1987; Zeuthen and Mead, 1996).

Growth of Spoilage Bacteria

During container storage in trial A, greater *Pseudomonas* counts were observed, which is not expected under CO₂/N₂ atmospheres (Clark and Lentz, 1969; Kakouri and Nychas, 1994) and indicates the presence of residual O₂. The results, however, demonstrate that the microbiological condition of beef stored in the container system was inferior to vacuum-packaged beef, even under strict hygiene procedures.

In the O₂-depleted, high-CO₂ atmosphere, growth of the obligate aerobic *Pseudomonas* sp. was generally slower than that of the facultative anaerobic *B. thermosphacta*. The relative insensitivity of the latter organism to atmospheres containing CO₂, which has been demonstrated for beef, chicken, and fish, (Nychas and Arkoudelos, 1990; Drosinos and Nychas, 1996; Tsigarida et al., 2000) accounts for its dominant role in the spoilage of food stored under protective atmospheres (Gill, 1998).

Indicator Bacteria and Microbial Pathogens

Counts of indicator bacteria were consistently low. Although these microorganisms are facultative anaerobes, and thus less susceptible to the atmospheric conditions created in the trials described above, the low temperature will reliably stop their multiplication (ICMSF, 1996, 1998).

Garcia de Fernando et al. (1995) concluded that CO₂-enriched atmospheres provide more protection against multiplication of the pathogenic psychrotrophic and psychrotolerant bacteria *Listeria* sp., *Salmonella* sp., *Yersinia*, and *Aeromonas* sp. However, the protective effect is a combination of low temperature, high CO₂ (preferably >40%), and low pH of meat.

With respect to growth inhibition of *L. monocytogenes*, Ingham et al. (1990) and Gill and Reichel (1989) demonstrated that CO₂ is effective only in combination with low temperatures; CO₂ concentrations of approximately 10% at 4 to 30°C are not effective (ICMSF, 1996). Tsigarida et al. (2000) observed an association between the growth of *Pseudomonas* and *L. monocytogenes* for beef packaged under various atmospheric conditions, implying that all container conditions resulting in considerable *Pseudomonas* growth constitute a potential meat safety hazard.

The results allow the conclusion, that, at least in the current experimental set-up, CO₂-enriched and O₂-depleted atmospheres at low pressure are of limited value in preventing microbial spoilage of beef stored in rigid, sealed containers. Keepability of beef and prevention of the growth of pathogenic bacteria can only be expected in combination with temperatures of 0 to 1°C. *Brochothrix thermosphacta* and *Pseudomonas* sp. represented the dominant spoilage flora. Because the distribution of microorganisms in meat is generally rather heterogeneous (Mossel et al., 1995; Upmann et al., 2000), which results in great deviations in microbial counts (see Figures 2 through 4), the growth and survival of these spoilage bacteria need to be further investigated.

IMPLICATIONS

The use of stainless steel containers for extended storage of large quantities of beef does not improve the shelf life of beef compared with vacuum-pack storage. In our experiments container storage was combined with the inclusion of a gas mixture (N₂/CO₂ in a 70:30 ratio) or (in 1 trial) pure CO₂. The microbiological condition (total colony counts and major spoilage organisms) of meat stored in containers was inferior compared with that stored in vacuum packs. Storage in pure CO₂ (trial C) at 0 to 1°C yielded a microbiological quality that was almost comparable with storage under vacuum.

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