ELSEVIER

International Journal of Food Microbiology 59 (2000) 211–220

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Comparison between the growth of *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and *Salmonella* spp. in ground beef packed by three commercially used packaging techniques

H. Nissen[a,*], O. Alvseike[b,c], S. Bredholt[a], A. Holck[a], T. Nesbakken[c]

[a]*MATFORSK, Norwegian Food Research Institute, Oslovn. 1, N-1430 Ås, Norway*
[b]*The Norwegian School of Veterinary Science, P.O. Box 8146 Dep., N-0033 Oslo, Norway*
[c]*Norwegian Meat Research Centre, P.O. Box 396 Økern, N-0513 Oslo, Norway*

Received 21 November 1999; received in revised form 25 March 2000; accepted 5 June 2000

Abstract

Growth of the pathogens *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli* O157:H7 and strains of *Salmonella* were compared in ground beef packed in modified atmospheres of 60% CO_2/40% N_2/0.4% CO (high CO_2/low CO mixture), 70% O_2/30% CO_2 (high O_2 mixture) and in chub packs (stuffed in plastic casings). The ground beef was inoculated with rifampicin-resistant or nalidixic acid/streptomycin-resistant strains of the pathogens (final concentration 10^2–10^3 bacteria/g) and stored at 4 and 10°C for up to 14 days. At 4°C the shelf life, based on colour stability and background flora development, was prolonged for the high CO_2/low CO mixture compared to the two other packaging methods, but at 10°C the shelf life was <8 days for all the packaging methods. Growth of *Y. enterocolitica* was nearly totally inhibited both at 4 and 10°C in the high CO_2/low CO mixture, while the bacterial numbers in the samples packed in the high O_2 mixture increased from about 5×10^2 bacteria/g at day 0 to about 10^4 at day 5 at 4°C and to 10^5 at 10°C. Growth in the chub packs was even higher. *L. monocytogenes* showed very little growth at 4°C in all treatments. At 10°C there was slow growth from about 5×10^3 bacteria/g to about 10^4 at day 5 in the high CO_2/low CO mixture, while the numbers in the high O_2 mixture and the chub packs were about 10 times higher. Growth of *E. coli* O157:H7 at 10°C in the ground beef was nearly totally inhibited in both the high CO_2/low CO mixture and the high O_2 mixture. Growth in the chub packs was higher, as the number of bacteria increased 3 log in 5 days. The *Salmonella* strains (*S. typhimurium, S. dublin, S. enteritidis* and *S. enterica* 61:k:1,5,(7)) in the ground beef stored at 10°C for 5 and 7 days grew to a higher number in the high CO_2/low CO mixture than in the high O_2 mixture. This study shows that the growth of *Y. enterocolitica* and *L. mononcytogenes* in ground beef stored in the high CO_2/low CO mixture was not increased as a result of prolonging the shelf life. However, the observed growth of strains of *Salmonella* at 10°C in this mixture and in chub packs does emphasise the importance of temperature control during storage.

Keywords: Ground beef; Modified atmosphere packaging; High CO_2; Carbon monoxide; *Yersinia enterocolitica*; *Listeria monocytogenes*; *Escherichia coli* O157:H7; *Salmonella* spp.

*Corresponding author. Tel.: +47-64-970-100; fax: +47-64-970-333.
E-mail address: hilde.nissen@matforsk.no (H. Nissen).

0168-1605/00/$ – see front matter
PII: S0168-1605(00)00373-1

1. Introduction

Ground beef for retail sale is often ready-packed in modified atmospheres (MA) or in chub packs (stuffed in plastic casings). MA-packed (MAP) ground beef has a long microbiological shelf life and also maintains an attractive red colour. For the past decade the Norwegian meat industry has been using a gas mixture of 60–70% CO_2, 30–40% N_2, 0.3–0.5% CO (CO comes ready mixed in the N_2 from the supplier). The reason for adding CO to the gas mixture is that it will produce a long-lasting cherry-red colour of the meat (Sørheim et al., 1999), but the low concentration of CO has little effect on the microflora of the meat (Clark et al., 1976; Gee and Brown, 1978; Luno et al., 1998).

The use of CO at such low concentrations does not present any toxic threat to the consumers (Sørheim et al., 1997). The most commonly used gas mixture for retail-ready meat in other European countries is 70% O_2/30% CO_2 (Gill, 1996). The high oxygen concentration is necessary to keep the red colour of the meat (Lambert et al., 1991). It is therefore only possible to obtain half the CO_2 concentration used in the high CO_2/low CO mixture. The microbiological shelf life of the high O_2 mixture will be longer than in air, but less than in the high CO_2/low CO gas mixture (Sørheim et al., 1999).

The inclusion of CO is controversial because the stable cherry-red colour can last beyond the microbiological shelf life of the meat and thus mask spoilage (Kropf, 1980). The extended shelf life obtained by MAP may under some conditions imply increased risk of growth of pathogens (Silliker and Wolfe, 1980; Hintlian and Hotchkiss, 1986; Farber, 1991; Lambert et al., 1991). This issue has also been discussed by the European Commission (1997).

However, even if meat packed in high CO_2/low CO mixture acquires a stable colour, the shelf life based on odour is significantly longer in the high CO_2/low CO mixture only at 4°C (Sørheim et al., 1999). At this temperature *Yersinia enterocolitica* and *Listeria monocytogenes* are considered to be the most serious pathogens in meat. At abuse temperatures (>8°C) *Escherichia coli* O157:H7 and *Salmonella* spp. also may grow and increase the health risk to the consumers.

The objective of the present work was to evaluate the microbiological safety of ground beef when packed by three commercially used packaging techniques when challenged with pathogens at refrigerated temperatures (4 and 10°C).

2. Materials and methods

2.1. Preparation and packaging of the ground beef

The beef carcasses were de-boned, and trimmings with 14% fat were ground through a 4 mm plate. The beef was packed at a commercial meat plant within 1 h of grinding as described by Sørheim et al. (1999). The batch of ground beef was divided into 500 g portions which were packaged in 0.4% CO/60% CO_2/40% N_2 (high CO_2/low CO mixture), 70% O_2/30% CO_2 (high O_2) in an Ilapak Delta 2000 flowpacking machine (Ilapack Machine Auto S.A., Grancia, Switzerland). The packs consisted of polyethylene trays (Færch Plast, Holstebro, Denmark) in Cryovac BDF 550 shrinking film (Cryovac, Milan, Italy) with an O_2 transmission rate of 19 cm^3/m^2 per 24 h/atm at 23°C and 0% relative humidity (RH). Portions of 500 g ground beef were also packed in chub packs in a clipping machine (Poly-Clip, Frankfurt, Germany) using a red fishingnet-patterned, polyethylene film (SFK, Vidovre, Denmark) with an O_2 transmission rate of 500 cm^3/m^2 per 24 h/atm at 23°C and 0% RH.

2.2. Bacterial cultures and growth conditions

Strains of the following pathogens were inoculated in the ground beef. Of *Y. enterocolitica*, a mixture of three strains, Y 2A, Y 89 and Y 310 all isolated from pig tonsils, was used (Kapperud et al., 1990). Of *L. monocytogenes*, a mixture of three strains: 2230/92 (serovar 1) isolated from a refrigerated sample of cooked sausage implicated in a listeriosis outbreak in Norway, 187 (serovar 4b) isolated from a different type of cooked sausage and 167 (serovar 4b) isolated from a meat production plant was used (Blom et al., 1997, Nissen and Holck, 1998, Bredholt et al., 1999). The *Listeria* and *Yersinia* strains were made resistant to rifampicin by spreading 0.1 ml of overnight cultures onto agar plates of TSB medium (Oxoid, CM 129) containing 50 μg/ml rifampicin (Sigma, St. Louis, MO, USA). The growth rates of the resistant strains were essentially equal to those of

the parent strains when tested in TSB medium in a Bioscreen instrument (Labsystem Co., Helsinki, Finland) at the same temperature, pH and a_w (NaCl) concentrations. One strain of *E. coli* O157:H7 was used (NCTC 1200, National Collection of Type Cultures, Colindale, London). This non-toxic strain was resistant to 100 μg/ml nalidixic acid and 1000 μg/ml streptomycin. Of *S. diarizonae* serovar 61:k:1,5,(7) (*Salmonella* 61:k:1,5,(7), a mixture of three strains (492/91, 2431/93 and 2748/93 supplied from National Institute of Public Health, Oslo) was used.

In a second experiment, four rifampicin-resistant salmonella strains, *S. typhimurium* (E 1525/96 serovar 4,5,12:i:,2), *S. dublin* (E 1146/95 serovar 9,12:g,m:–)), *S. enteritidis* (E 457/96 serovar 9,12:g,m:–) and *Salmonella* 61:k:1,5,(7), were used to inoculate the MAP- packed ground beef. The three first strains were isolated from cattle, the last from sheep, all obtained from National Institute of Public Health, Norway. The growth rates (measured as above) of the resistant strains of *S. enteritidis* and *Salmonella* 61:k:1,5,(7) were essentially the same as the parent strains while the growth rates of *S. dublin* and *S. typhimurium* were slightly lower.

2.3. Inoculation and storage

After packaging, the ground beef was inoculated with stationary cultures (the bacteria were cultivated overnight at 30°C and kept in the refrigerator for 1 day before use to ensure stationary cells) of the different pathogenic bacteria. The stock cultures were diluted in peptone water (PW) (Bacto peptone, Difco, 1 g/l; NaCl, Merck, 8.5 g/l) and the strains belonging to the same species were mixed. A 50 μl suspension of each pathogen (about 10^5 bacteria/ml) was inoculated with a syringe through a gas probe self-sealing tape (Toray Engineering Co. Ltd, England) onto the surface of the ground beef in one of the corners of the MA packages. The rectangular packages thus had one pathogen inoculated in each corner. In the chub packs the pathogens were inoculated at least 3 cm apart. Analysis of control samples showed that no pathogens were found outside the 25 g ground beef immediately around the inoculated area. Packages inoculated only with *Y. enterocolitica* and *L. monocytogenes* (one corner each) were stored at 4°C and analysed after 0, 2, 5, 8 and 14 days while packages inoculated with all four pathogens were stored at 10°C and analysed after 0, 2, 5 and 8 days.

In the second experiment four serovars of *Salmonella* were inoculated in one corner each of the package of ground beef which was stored at 10°C and analysed after 0, 2, 5 and 7 days. Non-inoculated packages used as controls were also stored at 10°C.

2.4. Microbial analyses

Samples of 25 g ground beef containing the inoculated pathogens were transferred to a stomacher bag and mixed with 150 ml PW (8.5 g NaCl, 1.0 g peptone/1000 ml water). Subsequently, 100 μl of a 10-fold dilution series were plated on blood agar (Bacto Blood Agar Base, Difco) with 5% sterile defibrinated horse blood, containing 50 μg/ml rifampicin for *L. monocytogenes* and *Y. enterocolitica* or 100 μg/ml nalidixic acid and 1000 μg/ml streptomycin sulphate for *E. coli* O157:H7. From the undiluted mixture an aliquot of 1 ml was also plated out. For enumeration of *Salmonella* spp. the selective medium Brilliant Green Agar (modified) (BGA; Oxoid, Basingstoke, Hampshire, England) was used. The colonies were confirmed on Triple Sugar Iron Agar (TSI; Difco, Detroit, MI, USA) and Urea agar (Urea Agar Base, Oxoid CM53 and Urea Solution, Oxoid SR20) followed by agglutination by monovalent antisera (provided by the National Institute of Public Health). In the second experiment, samples for detection of the four *Salmonella* strains were plated on blood agar containing 50 μg/ml rifampicin. Samples from non-inoculated packages were treated the same way and plated on MRS plates (CM359, Oxoid), pH 5.7, for determination of lactic acid bacteria and PCA (Difco, Detroit, MI, USA) plates for aerobic mesophilic counts of bacteria. The plates were incubated at 30°C for up to 2 days, all aerobically. Samples from two replicate packages were used for all analyses, except after 7 days storage in experiment 2 where three replicate packages were analysed.

2.5. Gas analyses, pH and odour

The concentrations of CO_2 and O_2 were determined in the MAP ground beef during storage at 4°C. CO_2 was determined using a Torrey PG-100 gas analyser and O_2 using a Torray LC 700-F gas

analyser (Torray Engineering, Osaka, Japan). The threshold levels for the CO_2 and O_2 analyses were 1% and 0.05%, respectively. Gas samples of 10 cm^3 were removed with a syringe through selfsealing patches on the packs. O_2 was measured in all packages before they were opened for microbial analysis. pH for all samples was measured in the stomacher solution (Knick pH meter, Portamess 751/752, Elektronische Messgeräte, GmbH and Co, Berlin, Germany). At sampling, the odour of the meat was evaluated as acceptable or not by the person who performed the microbial analysis.

2.6. Statistical analyses

Microbial data were subjected to analysis of variance (ANOVA) and Tukey's pairvise comparisons. It was deemed appropriate to perform ANOVA on these data after a $\log_{10}$ transformation, thereby obtaining a distribution more akin to the normal distribution on which ANOVA is based.

3. Results

The concentrations of CO_2 and O_2 were determined during storage at 4°C. In the high O_2 mixture the oxygen concentration remained constant at around 70% the first week and decreased to 43% after 2 weeks (results not shown). The CO_2 concentration remained constant at around 22% the first week and increased to 46% after 2 weeks and in the high CO_2/low CO gas mixture the concentration of O_2 was less than 0.2% during the whole storage period while the CO_2 concentration was constant around 47% (results not shown). The CO_2 concentration measured was lower than in the gas composition due to the absorption in the meat. The increasing CO_2 concentrations at the end of the storage period indicated that the CO_2 film barrier is effective. To ensure that no leakage had occurred, the O_2 concentration was measured in all the packages in all experiments before opening.

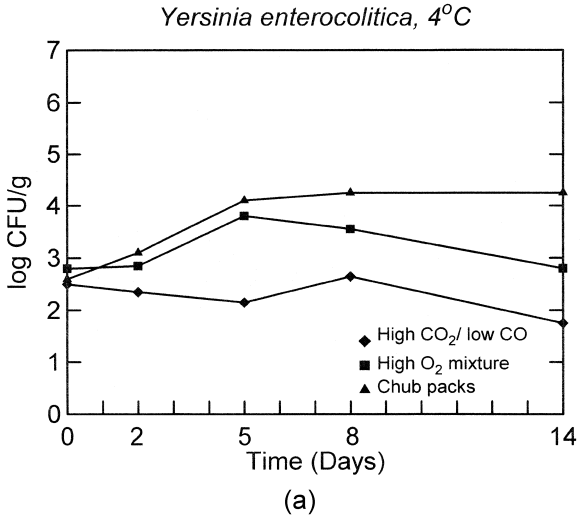
As expected the shelf life of the ground beef stored at 4°C was prolonged in the high CO_2/low CO mixture compared with the other packaging methods studied. This was due to the stable colour and reduced background flora resulting in little off-odour. Thus the ground beef packed in the high CO_2/low CO mixture still had an acceptable odour after 14 days of storage at 4°C, while the beef packed in high O_2 mixture and in the chub packs had some off-odours. The difference in shelf life was less at 10°C. After 5 days storage the ground beef packed in the high CO_2/low CO mixture had an acceptable smell (except for the packages inoculated with salmonella), while beef packed in the high O_2 mixture and the chub packs had a slight off-odour.

After 8 days storage there was a strong off-odour for all treatments, but the ground beef in the high CO_2/low CO mixture still looked bright red. Similar observations were made by Sørheim et al. (1999). The O_2 content in the high CO_2/low CO mixture was virtually zero throughout storage at both temperatures. At 10°C the O_2 content in the high O_2 gas mixture decreased from 70% to about 35% after 8 days storage, probably due to aerobic bacterial metabolism. The chub packs had an O_2-permeable casing which probably was the cause of the high bacterial growth in these packs at both temperatures.

Growth of *Y. enterocolitica* was inhibited both at 4 and 10°C in the high CO_2/low CO mixture (Fig. 1a and b). The number in the samples packed in the high O_2 mixture, however, increased from about 5×10^2 cfu/g at day 0 to about 10^4 cfu/g at day 5 at 4°C, and increased to 10^5 cfu/g at 10°C. Growth in the chub packs at 4°C was higher than for the other treatments. Growth in chub packs was also higher than in high O_2 at 10°C ($P = 0.007$).

L. monocytogenes (Fig. 2a) was inhibited at 4°C for all treatments. At 10°C (Fig. 2b) there was slow growth, from about 5×10^3 bacteria/g to about 10^4 at day 5, in the high CO_2/low CO mixture and in the chub packs. This was approximately 10-fold lower cfu/g at day 5 than in the high O_2 mixture ($P = 0.040$).

Ground beef inoculated with *E. coli* O157:H7 and strains of *Salmonella* was stored at 10°C. Growth of *E. coli* O157:H7 was slow both in the high CO_2/low CO mixture and the high O_2 mixture (Fig. 3) giving less than 10^4 cfu/g at day 5. Growth in the chub packs was greater than in the high CO_2/low CO-mixture ($P = 0.011$) and in the high O_2 mixture ($P = 0.019$), reaching 10^5 cfu/g. The number of lactic acid bacteria in the non-inoculated packages were lower in the high CO_2/low CO mixture at 4°C (Fig. 4). At 10°C the growth was significantly higher in the chub packs. At start of the experiment the pH



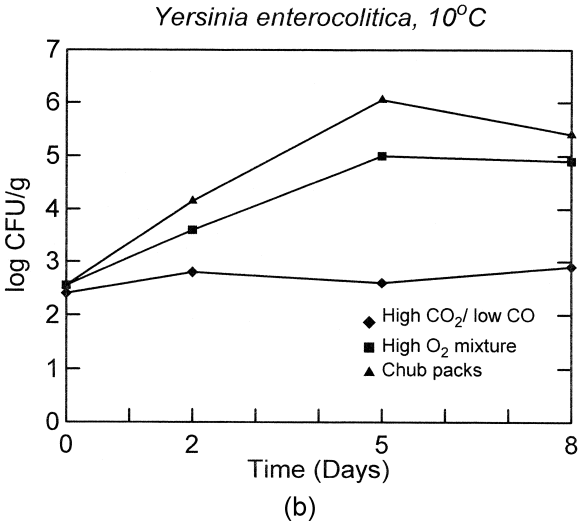
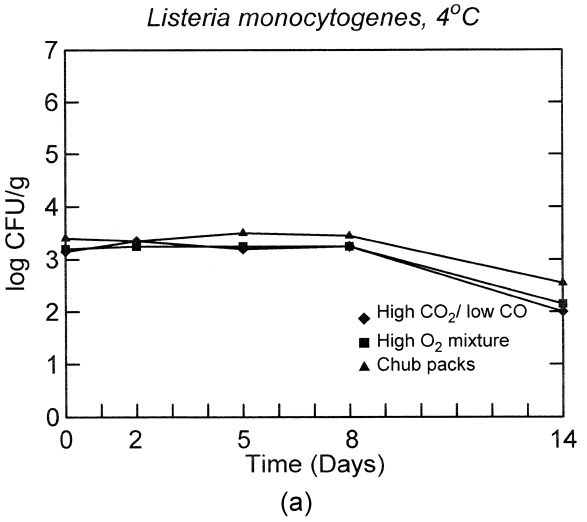


Fig. 1. Growth of *Yersinia enterocolitica* inoculated in ground beef packed in high CO_2/low CO mixture (0.4% CO/60% CO_2/40% N_2), high O_2 (70% O_2/30% CO_2) or in chub packs. The ground beef was stored at (a) 4°C or (b) 10°C.



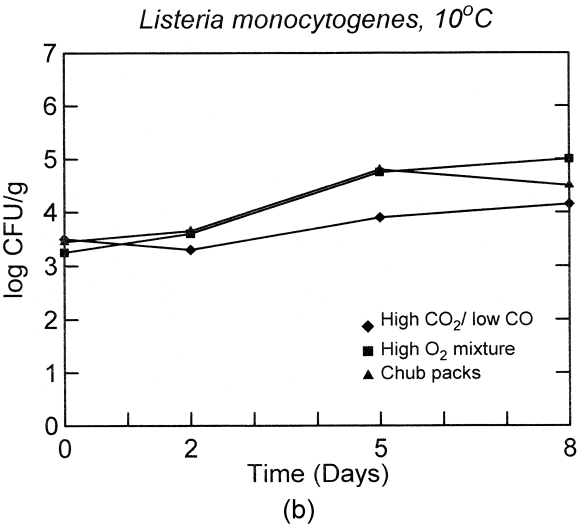


Fig. 2. Growth of *Listeria monocytogenes* inoculated in ground beef packed in high CO_2/low CO mixture (0.4% CO/60% CO_2/40% N_2), high O_2 (70% O_2/30% CO_2) or in chub packs. The ground beef was stored at (a) 4°C or (b) 10°C.

in the ground beef was about 5.8 in all packages. After 5 days storage the pH was about 5.7 in the high CO_2/low CO mixture, 5.5 in the high O_2 mixture and 5.3 in the chub packs.

Due to growth of other bacteria on the selective plates, only approximate numbers of *Salmonella* 61:k:1,5,(7) were obtained, but growth of about 1.5 log units was observed both in the high CO_2/low CO mixture and the chub packs (results not shown). This increase was not seen in the high O_2 mixture. To verify these results and investigate whether they were valid for other serovars more virulent to humans, such as *S. typhimurium, S. dublin* and *S. enteritidis*, a second experiment was performed. The results (Fig. 5a, b, c and d) show that after 2 days of storage at 10°C there was essentially no growth of the *Salmonella* strains in ground beef packed in the high CO_2/low CO mixture or the high O_2 mixture, while the numbers of *Salmonella* in the chub packs

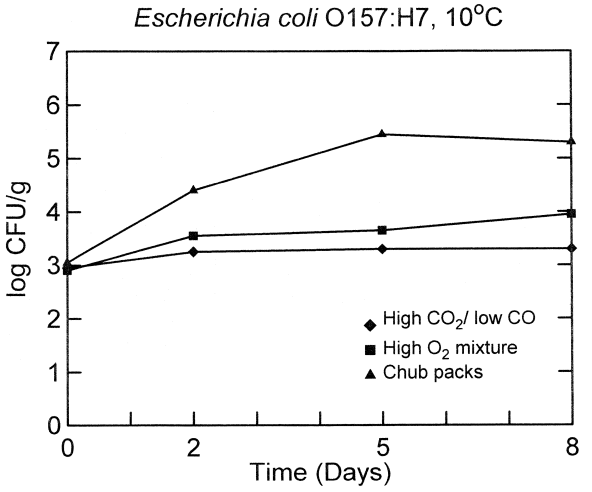


Fig. 3. Growth of *Escherichia coli* O157: H7 inoculated in ground beef packed in high CO_2/low CO mixture (0.4% CO/60% CO_2/40% N_2), high O_2 (70% O_2/30% CO_2) or in chub packs, stored at 10°C.

were about 10-fold higher. After 5 days there was a slight off-odour in all the packages except for one package with high CO_2/low CO mixture which smelled strongly of H_2S. In this package the numbers of all the *Salmonella* strains were higher than in the replicate package and were of the same magnitude as the numbers in the chub packs. In the O_2 mixture there was no growth of *S. dublin* and *S. enteritidis* and only a low growth of *Salmonella* 61:k:1,5,(7) and *S. typhimurium*.

The growth of the *Salmonella* strains was still greatly inhibited in the high O_2 mixture, while growth in the high CO_2/low CO mixture was just as high or even higher than in the chub packs.

In the non-inoculated packages the lactic acid bacteria rapidly constituted most of the background flora (not shown). After 5 days storage the numbers were higher in the chub-packed samples, but after 8 days there were no obvious differences (Fig. 6). The pH in the non-inoculated ground beef followed the same pattern as in experiment 1 (not shown).

4. Discussion and conclusions

Ground beef is a high-risk product because pathogens may be mixed into the ground product which may not be sufficiently heated before consumption.

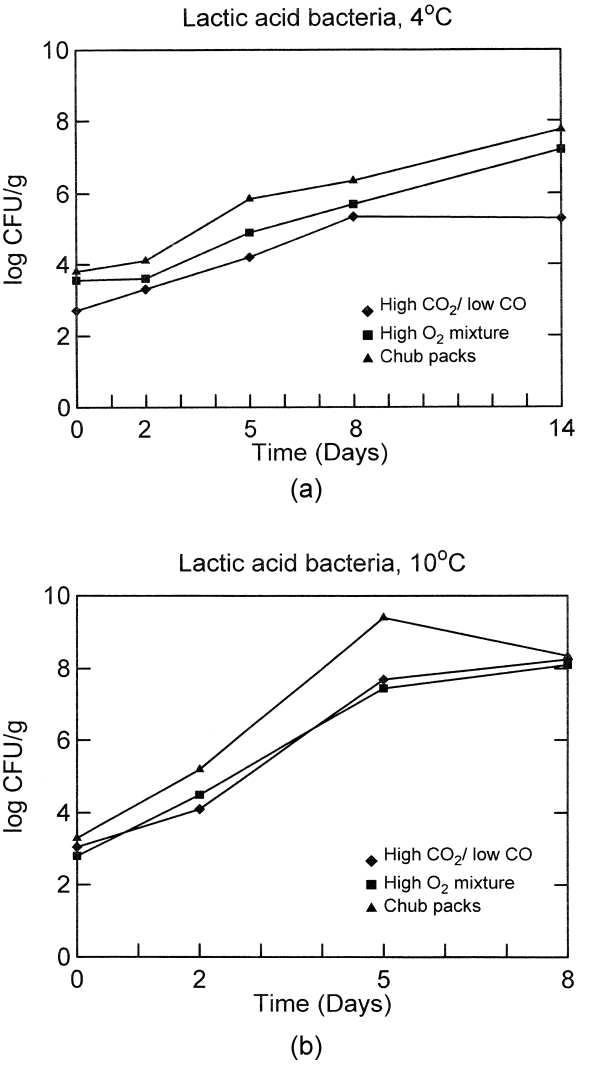


Fig. 4. Growth of lactic acid bacteria (cfu/g on MRS, pH 5.7) in non-inoculated ground beef packed in high CO_2/low CO mixture (0.4% CO/60% CO_2/40% N_2), high O_2 (70% O_2/30% CO_2) or in chub packs. The ground beef was stored at (a) 4°C or (b) 10°C.

To inhibit growth of spoilage bacteria and increase shelf life, MAP is often used by retailers. However, there is a possibility that some pathogenic bacteria may be less inhibited by MAP. The question 'Do modified atmospheres enhance risk to the consumers health, but delay signs of spoilage' raised by Hintlian and Hotchkiss (1986) is therefore relevant. When evaluating the safety of ground beef in the high CO_2/low CO mixture compared to other commercially available packaging methods, we have focused

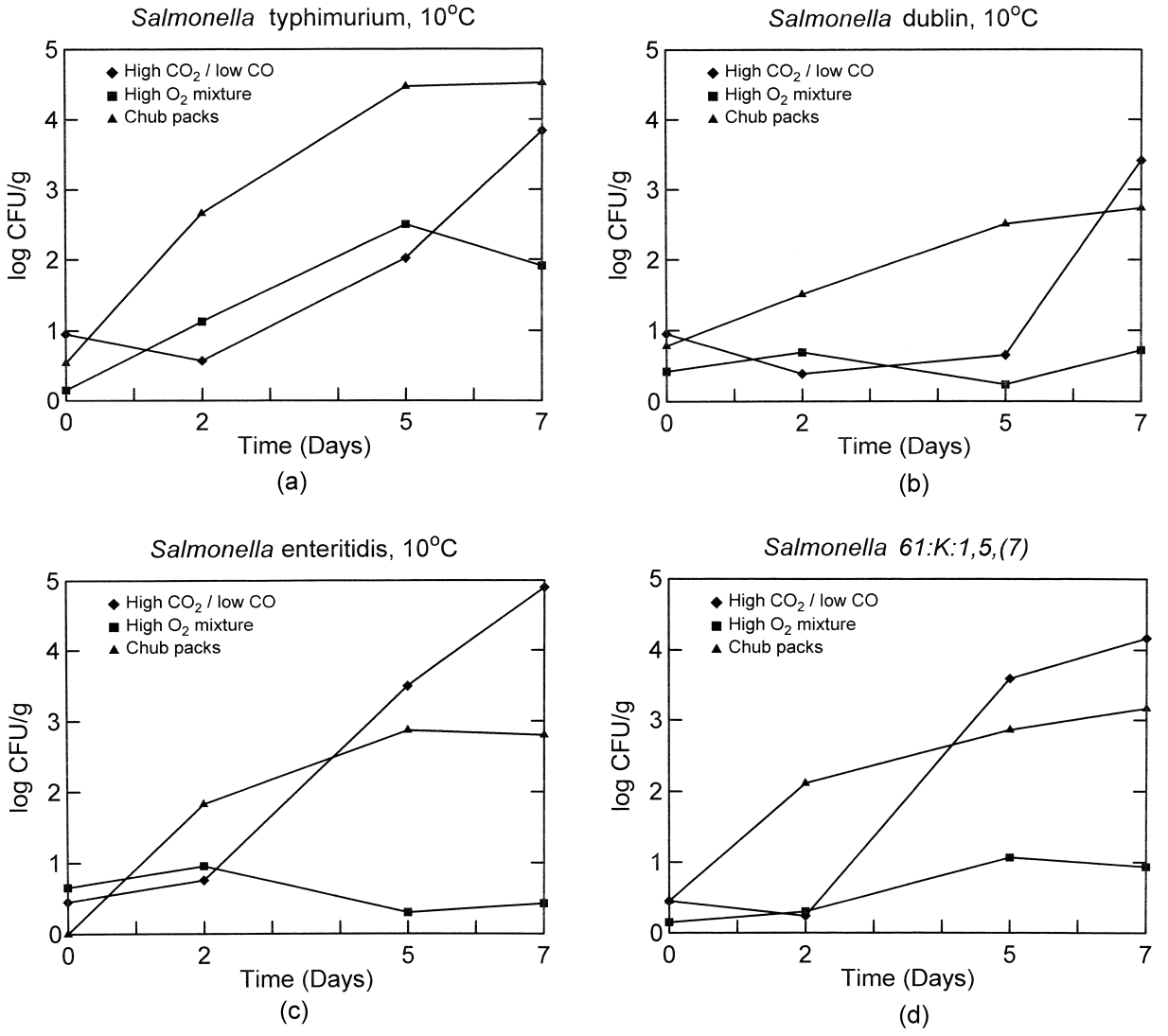


Fig. 5. Growth of strains of *Salmonellae* inoculated in ground beef packed in high CO_2/low CO mixture (0.4% CO/60% CO_2/40% N_2), high O_2 (70% O_2/30% CO_2) or in chub packs, stored at 10°C. (a) *S. typhimurium*, (b) *S. dublin*, (c) *S. enteritidis* and (d) *Salmonella* 61:k:1,5,(7).

on bacteria that show good growth at or below 10°C and are most relevant for meat products.

The ability of *Y. enterocolitica* to multiply at low temperatures is of considerable concern to food producers, particularly in countries like Australia, Canada, Denmark, Germany, New Zealand, Norway and Sweden where *Y. enterocolitica* has surpassed *Shigella* and now rivals *Salmonella* and *Campylobacter* as a cause of acute bacterial gastroenteritis (Nesbakken, 2000). In our study, growth of *Y. enterocolitica* was totally inhibited in ground beef packed in the high CO_2/low CO mixture even at 10°C while it grew fairly well both in the high O_2 mixture and in the chub packs. Manu-Tawiah et al. (1993) found that pork chops packed in different MAs with 20 or 40% CO_2 with or without O_2 allowed growth of *Y. enterocolitica*, but the CO_2 concentration applied was lower than in the high CO_2/low CO mixture (60%) used in our study. Growth of *Y. enterocolitica* has also been observed

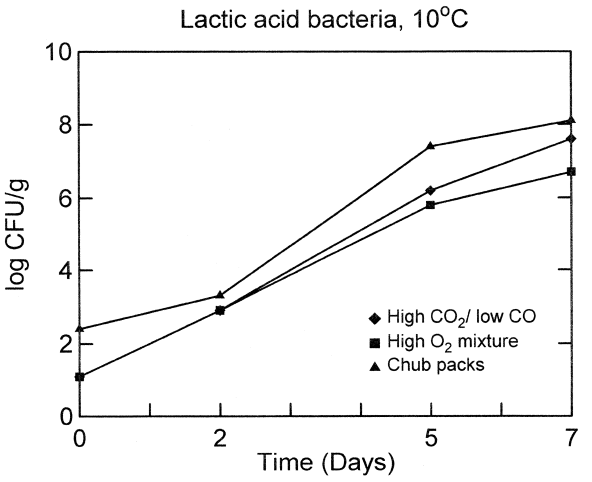


Fig. 6. Growth of lactic acid bacteria (cfu/g on MRS, pH 5.7) in non-inoculated ground beef packed in high CO_2/low CO mixture (0.4% CO/60% CO_2/40% N_2), high O_2 (70% O_2/30% CO_2) or in chub packs. The ground beef was stored at (a) 4°C or (b) 10°C.

in 100% CO_2 at low temperatures, but the pH of the meat was higher (Gill and Reichel, 1989; Hudson et al., 1994).

L. monocytogenes is also a pathogen that grows well at low temperatures, but in our study there was no growth of this bacterium in the ground beef in any of the packages at 4°C, and only slow growth at 10°C. This agrees with results of Farber and Daley (1994) and Manu-Tawiah et al. (1993) who found no growth or long lag times of *L. monocytogenes* in different meat products when stored at 4°C. Kaya and Schmidt (1989) found no growth in minced meat over 14 days at 4 or 8°C (low inoculum), while Hudson et al. (1994) found growth in vacuum-packed roast beef (pH 6.1).

At the abusive storage temperature of 10°C, *E. coli* O157:H7 in the chub packs multiplied quickly. However, growth was nearly totally inhibited in the high CO_2/low CO mixture and in the high O_2 mixture. This is in accordance with the predictive model of Sutherland et al. (1997). Their study showed that *E. coli* O157:H7 is relatively tolerant for CO_2, but growth could be inhibited at 10°C at high CO_2 concentrations and pH < 6.0.

In our study, growth of *Salmonella* spp. was not inhibited in ground beef packed in high CO_2/low CO mixture and stored at 10°C, contrary to what is found in many other studies (e.g. D'Aoust, 1991). Although *Salmonella* may grow well and out-compete the background flora on fresh meat stored at 10°C (Alford and Palumbo, 1969; Mackey and Kerridge, 1988), most reports claim that growth will be inhibited in MAP at this temperature (Silliker and Wolfe, 1980; D'Aoust, 1991; Gill and Delacy, 1991). The competitive flora may, however, also play a role (Garcia de Fernando et al., 1995), and in the *Salmonella* challenging experiment the number of lactic acid bacteria was initially very low (Fig. 6). Nychas and Tassou (1996) found that high CO_2 atmospheres were more inhibitory for growth of *S. enteritidis* on fresh poultry at 10°C than were high O_2 atmospheres, the opposite of what we found for ground beef. Luithen et al. (1982) found that numbers of *S. typhimurium* increased significantly on samples wrapped with oxygen-permeable film but remained low and fairly constant for vacuum or gas-treated steaks. However, in our study the O_2 concentration was $\leq 0.02\%$ in all the packages. Oxidative stress reactions in *Salmonella* have recently been reported (Stephens et al., 1999). This may explain the inhibition of growth (longer lag phase) in the high O_2 mixture in our study.

The present study shows that the prolonged shelf life (due to stable colour and reduced background flora) at 4°C did not increase the risk of growth of *Y. enterocolitica* and *L. monocytogenes* in ground beef stored in the high CO_2/low CO gas mixture. This is probably due to the high CO_2 concentration that is inhibitory to most microorganisms (Dixon and Kell, 1989). Even at the abusive temperature of 10°C, the numbers of pathogens at the end of the shelf life (5 days) were less or the same as were found in the chub packs. The observed growth of *Salmonella* in the high CO_2/low CO mixture and chub packs does however emphasise the importance of temperature control during storage. There is a wide range of temperature criteria for chilled foods at retail in European countries. The values range from -1°C to 10°C, with most temperatures being between 4°C and 8°C (European Commission, 1996). These aspects should also be considered together with the conclusions of the EU report (European Commission, 1997) which states that MAP has proven to enhance the product quality by inhibiting the spoilage bacteria. MAP may also constitute a hurdle to the growth of some pathogens, and the safety of MAP products are mostly threatened by temperature abuse.

Acknowledgements

We thank Oddvin Sørheim, MATFORSK, Vestfold-Buskerud-Telemark Slakteri A/L, Sem and Åse Spangelo, Hydrogas AS Utviklingssenter, Porsgrunn for assistance in the production and packing of the ground beef. We also thank Janina Berg, Birgitta Baardsen and Ingvild Rosshaug for able technical assistance and Per Lea for statistics. The Agricultural Food Research Foundation and 'Omsetningsrådet' financially supported the work.

References

Alford, J.A., Palumbo, S.A., 1969. Interaction of salt, pH and temperature on the growth and survival of salmonellae in ground pork. Appl. Microbiol. 17, 528–532.

Blom, H., Nerbrink, E., Dainty, R.H., Hagtvedt, T.A., Borch, E., Nissen, H., Nesbakken, T., 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum packed sensory acceptable servelat sausage and cooked ham stored at 4°C. Int. J. Food Microbiol. 38, 71–76.

Bredholt, S., Nesbakken, T., Holck, A., 1999. Protective cultures inhibit growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in cooked, sliced, vacuum- and gas-packaged meat. Int. J. Food Microbiol. 53, 43–52.

Clark, D.C., Lentz, C.P., Roth, W.D., 1976. Use of carbon monoxide for extending shelf-life of prepackaged fresh beef. Can. Inst. Food Sci. Technol. J. 9, 114–117.

D'Aoust, J.Y., 1991. Psychrotrophy and foodborne *Salmonella*. Int. J. Food Microbiol. 13, 207–216.

Dixon, N.M., Kell, D.B., 1989. A review. The inhibition by CO_2 of the growth and metabolism of micro-organisms. J. Appl. Bacteriol. 67, 109–136.

European Commission, 1996. Scientific co-operation on questions relating to food. Task 2.2: Studies relating to temperature control. Directorate-General III, 10.51996, 31–33.

European Commission, 1997. Food science and techniques. Reports of the scientific committee for food (40th series) Directorate-General for Industry, pp. 51–62.

Farber, J.M., 1991. Microbiological aspects of modified-atmosphere packaging tecnology — a review. J. Food Protect. 54, 58–70.

Farber, J.M., Daley, E., 1994. Presence and growth of *Listeria monocytogenes* in naturally-contaminated meats. Int. J. Food Microbiol. 22, 33–42.

Garcia de Fernando, G.D., Nychas, G.J.E., Peck, M.W., Ordonez, J.A., 1995. Growth/survival of psychotrophic pathogens under modified atmospheres. Int. J. Food Microbiol. 28, 221–231.

Gee, D.L., Brown, W.D., 1978. Extension of shelf life in refrigerated ground beef under an atmosphere containing carbon dioxide and carbon monoxide. J. Agric. Food Chem. 25, 274–276.

Gill, C.O., Reichel, M.P., 1989. Growth of the cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. Food Microbiol. 6, 223–230.

Gill, C.O., Delacy, K.M., 1991. Growth of *Escherichia coli* and *Salmonella typhimurium* on high-pH beef packed under vacuum or carbon dioxide. Int. J. Food Microbiol. 13, 21–30.

Gill, C.O., 1996. Extending the storage life of meats. Meat Sci. 43 (Suppl.), S99–S109.

Hintlian, C.B., Hotchkiss, J.H., 1986. The safety of modified atmosphere packaging: a review. Food Technol. 40, 70–76.

Hudson, J.A., Mott, S.J., Penney, N., 1994. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and saturated carbon dioxide controlled atmosphere-packaged sliced roast beef. J. Food Protect. 57, 204–208.

Kapperud, G., Nesbakken, T., Aleksic, S., Mollaret, H.H., 1990. Comparison of restriction endonuclease analysis and phenotypic typing methods for differentiation of *Yersinia enterocolitica* isolates. J. Clin. Microbiol. 28, 1125–1131.

Kaya, M., Schmidt, U., 1989. Verhalten von *Listeria monocytogenes* im Hackfleisch bei Kühl- und Gefrierlagerung. Fleischwirtschaft 69, 617–620.

Kropf, D.H., 1980. Effects of retail display conditions on meat color. Proc. Reprocial Meat Conf. 33, 15–32.

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

Luithen, L.S., Marchello, J.A., Dryden, F.D., 1982. Growth of *Salmonella typhimurium* and mesophilic organisms on beef steaks as influenced by type of packaging. J. Food Protect. 45, 263–267.

Luno, M., Beltran, J.A., Roncales, P., 1998. Shelf-life extension and colour stabilisation of beef packaged in a low O_2 atmosphere containing CO:loin steaks and ground meat. Meat Sci. 48, 75–84.

Mackey, B.M., Kerridge, A.L., 1988. The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. Int. J. Food Microbiol. 6, 57–65.

Manu-Tawiah, W., Myers, D.J., Olson, D.G., Molins, R.A., 1993. Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified gas atmospheres. J. Food Sci. 58, 475–479.

Nesbakken, T., 2000. *Yersinia* spp. In: Lund, B.M., Baird-Parker, T.C., Gould, G.W. (Eds.), The Microbiological Safety and Quality of Food. Aspen Publishers, Inc, Gaithersberg, MD, USA, pp. 1363–1393.

Nissen, H., Holck, A.L., 1998. Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella kentucky* in Norwegian fermented, dry sausage. Food Microbiol. 15, 273–279.

Nychas, G.-J.E., Tassou, C.C., 1996. Growth/survival of *Salmonella enteritidis* on fresh poultry and fish stored under vacuum or modified atmosphere. Lett. Appl. Microbiol. 23, 115–119.

Silliker, J.H., Wolfe, S.K., 1980. Microbiological safety considerations in controlled-atmosphere storage of meats. Food Technol. 34, 59–63.

Stephens, P.J., Druggan, P., Nebe-von Caron, G., 1999. The significance of oxidative stress in food preservation. Food

Microbiology and food safety into the next millenium. In: Tuijtelaars, A.C.J., Samson, R.A., Rombouts, F.M., Notermans, S. (Eds.), Proceedings of the Seventeenth International Conference of the International Committee on Food Microbiology and Hygiene (ICFMH), Veldhoven, The Netherlands, 13–17 September, pp. 764–766.

Sutherland, J.P., Bayliss, A.J., Braxton, D.S., Beumont, A.L., 1997. Predictive modelling of *Escherichia coli* O157:H7: Inclusion of carbon dioxide as a fourth factor in a pre-existing model. Int. J. Microbiol. 37, 113–120.

Sørheim, O., Aune, T., Nesbakken, T., 1997. Technological, hygienic and toxicological aspects of carbon monoxide used in modified-atmosphere packaging of meat. Trends Food Sci. Technol. 8, 307–312.

Sørheim, O., Nissen, H., Nesbakken, T., 1999. The storage life of beef and pork packaged in an atmosphere with low carbon and high carbon dioxide. Meat Sci. 52, 157–164.