EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON THE SURVIVAL OF PATHOGENS ON ARTIFICIALLY CONTAMINATED PORK

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SUMMARY

Boneless sliced pork loins were artificially contaminated with *Campylobacter jejuni/coli*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Yersinia enterocolitica*. Survival of these pathogens was studied during storage for 11 days under a high oxygen modified atmosphere and in air at 3°C. The approximate composition of the initial gas mixture was 65% O_2: 25% CO_2: 10% N_2. The numbers of each pathogen were determined with 3-tube decimal dilution series (MPN). The total aerobic colony count and the total number of Enterobacteriaceae were determined as well. Initial contamination levels were 2.3×10^6 for Campylobacter, 2.7×10^4 for Salmonella, 4.2×10^4 for Listeria and 7.8×10^2 for Yersinia, all in cfu/cm^2.

The total aerobic colony count remained stable during storage in the modified atmosphere but increased in air from 10^5 to 10^8 cfu/cm^2. Similar results were obtained for Enterobacteriaceae in the modified atmosphere. A moderate increase in the number of Enterobacteriaceae was seen when packaged in air. *C. jejuni/coli* decreased significantly in numbers during storage under both atmospheres. However, a lower Campylobacter survival rate was observed when pork was stored under gas. *L. monocytogenes* and *S. typhimurium* were not affected by both treatments and the numbers of these pathogens remained stable during the whole period. Similar results apparently were found for *Y. enterocolitica*.

Introduction

To enhance the bright red colour, fresh meats are more commonly packaged under a high oxygen/carbon dioxide modified atmosphere in Western Europe. Moreover such a mixture of O_2 and CO_2 improves shelf-life, although there is no guarantee pathogens will be controlled completely. Campylobacters, Salmonellae, Listeriae and Yersiniae can still be found in small percentages of retail meat samples. Given the longer shelf-life, the psychrotrophic pathogens like *Listeria monocytogenes* and *Yersinia enterocolitica* can, even at low storage temperatures, cause a problem. Furthermore there are no data that the survival rates of Salmonellae and Campylobacters will be different in a high oxygen/carbon dioxide modified atmosphere from the survival rates in air. The present contribution concerns the behaviour of those aforementioned pathogens on pork during storage in a gas mixture which is commercially used for fresh pork in the Netherlands. Therefore pork was artificially contaminated with *Campylobacter jejuni/coli*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Yersinia enterocolitica* and stored in 65%:25% O_2:CO_2 or in air at 3°C for 11 days.

Materials and Methods

The following strains of bacteria were used in this study: *C. jejuni* 81116 (human isolate; Dept. of Veterinary Bacteriology (DVB), Utrecht, The Netherlands) and LA32 (human isolate; RIVM, Bilthoven, The Netherlands), *C. coli* C59 and C63 (pig and chicken isolates; DVB) *S. typhimurium* V49 (meat isolate; Dept. of the Science of Food of Animal Origin (VVDO), Utrecht, The Netherlands), *L. monocytogenes* L2/93/4 and L2/93/12 (pork isolates; VVDO), *Y. enterocolitica* serotype O:3, biotype 4 and O:9 biotype 2 (meat isolates; L. de Zutter, University of Ghent, Belgium). Strains were cultured and a cocktail for each genus was prepared as previously described by Van Laack et al. (1993). Briefly, cells were centrifuged at 3500rpm for 20 min and resuspended in 0.85% phosphate buffered saline (PBS). Cell numbers were determined spectrophotometrically at 500nm and checked by direct plating on BHI agar (37°C; 24h) or in case of *C. jejuni/coli* checked by direct

plating on blood agar (37°C; 48h; micro-aerophilic). The inoculum was prepared in 100ml PBS. Cell suspensions were combined in such a way that target levels of 10^4 cfu/cm² for *S. typhimurium*, *Y. enterocolitica* and *L. monocytogenes* and 10^6 cfu/cm² for *C. jejuni/coli* would be achieved.

Four cold pork loins with a pH of 5.8-6.2, were obtained from a distribution plant on the morning of the inoculation. At the department, the loins were deboned and cut up into 40 pieces, each with a surface area of about 100cm². All pieces were mixed together to obtain a uniform contamination with background flora. The pieces were placed in trays made of PS/EVOH/PE (Cedap, EO2-pack, Apeldoorn, the Netherlands) and inoculated with 1ml of inoculum in drops, subsequently spreaded with a spatula over the 100cm². Fifteen trays were wrapped in oxygen permeable foil (air packaging). The other fifteen trays were filled with a gas mixture consisting of 70% O_2 and 30% CO_2 (MAP) and sealed with foil (VPA/PE-AF 15/75,1.5cc O_2/m²/24h/atm at 20°C 75%RH,2.5cc CO_2/m²/24h/atm at 20°C 75%RH, Südpack Verpackungen GmbH&Co, Ochenhausen, Germany). All thirty trays were stored at 3 ± 1°C. The temperature was registered by a thermograph during the whole experiment. The remaining trays were used as day 0 samples.

On day 0, 2, 4, 7, 9 and 11, three trays for each type of packaging were opened for microbiological analysis. Before opening, gas compositions were measured with a Servomex® UK Ltd. Analyser (series 1400). The sampling method used, and determination of each pathogen with a 3-tube decimal dilution method (MPN) were in principle described by Van Laack et al. (1993). BPW was used as diluent in this study. Charcoal Cefaperazone Desoxycholate Broth (CCDB) was used instead of Preston broth for isolation of Campylobacter. The enrichment was streaked onto Charcoal Cefaperazone Desoxycholate Agar (CCDA; Oxoid #CM739 supplemented with 32 mg/l cefaperazone (Hutchinson and Bolton, 1984) and with 100 mg/l cycloheximide). CCDB contained 10 g lab lemco powder (Oxoid #L29), 10 g peptone (Difco #0118-01-8), 5 g sodium chloride, 4 g bacteriological charcoal, 3 g casein hydrolysate (Oxoid #L41), 1 g sodium desoxycholate (Oxoid #L57), 0.25 g sodiumpyruvate and 0.25 g ferro(II)sulphate per litre. This basal medium was supplemented with 32 mg/l cefaperazone and 100 mg/l cyclohexamide. For the isolation of Yersinia, 0.5 ml of the enrichment was added to 4.5 ml 0.25% KOH solution (Auliso et al., 1980: modified by Wauters at al., 1988) and a loopful of this material was streaked onto Celsulodin Irgasan Novobiocin agar (Schiemann, 1982: Oxoid #CM653 and #SR109). In addition to these microbiological assays, the total aerobic count (Plate Count Agar, Oxoid #CM463; 30°C; 72h) and number of Enterobacteriaceae (Violet Red Bile Glucose agar with similar overlay, Oxoid #CM485; 37°C; 24h) were determined.

Data were analysed by multiple analysis of variance.

Results and Discussion

The results of the microbiological analyses are shown in Table 1. The gas mixture in the gas packages consisted of 60-70% O_2 and 20-25% CO_2 on the starting day. This implies that no total vacuum was created with the applied machinery in the packages before they were flushed with the gas mixture consisting of 70% O_2 and 30% CO_2. The gas composition did not change during the 11 days of storage at 3°C. Pork stored under these conditions kept to a large extent a fresh red colour and the level of spoilage flora was not affected during storage. Meat discoloration appeared after 4 to 7 days of storage in air, whereupon the CO_2 concentration in the packs increased to 3-5% and the O_2 decreased to 2-7%. The changes in this gas composition are probably due to the metabolic activity of the bacteria, oxygen consumption by the mitochondria and lipid oxidation which apparently consumed more oxygen than the amount of this gas diffusing into the pack. After 7 days the aerobic spoilage flora was increased significantly ($p < 0.01$) to unacceptable levels but the number of Enterobacteriaceae showed only a slight increase.

The initial contamination levels were, with the exception of that for *Y. enterocolitica*, conform the target levels. The isolation of *Y. enterocolitica* appeared to be more successful with CIN agar used in this study than with the SSDC agar used by Van Laack et al. (1993). The number of *Y. enterolitica* showed a significant difference between both packaging treatments ($p < 0.05$). Air-packaging resulted in higher counts of *Y. enterocolitica* on pork but no effect between sampling days was registered. Manu-tawiah et al. (1993) showed that on pork chops packaged in 20:0:60, 40:10:50, 20:0:80 CO_2:O_2:N_2 and stored at 4°C, *Y. enterocolitica* count increased about log 3 cfu/cm² in 11 days. The absence of growth at 65% O_2 in our study may indicate the organisms were sensitive to high concentrations of oxygen. The sensitivity of the micro-aerophilic Campylobacters to oxygen is well known and substantiated again in this study. The number of *C. jejuni/coli* decreased significantly ($p < 0.01$) on pork under air as well as under the modified atmosphere, in addition to which also the number found on pork in air was significantly ($p < 0.01$) higher than the number found on pork in the modified atmosphere. Similar results were obtained by Tomancova et al. (1987) although in their

observations the survival rate of *C. jejuni* on pork stored in 85% O_2 and 15% CO_2 was higher compared to the survival rate in air at 4°C.

The numbers of *L. monocytogenes* on the air-packaged pork were not significantly different from numbers on pork in the gas atmosphere and no changes in numbers occurred during the 11 days of storage. However growth of *L. monocytogenes* was demonstrated by Wipfheimer et al. (1990) and Manu-tawiah et al. (1993) at 4°C on raw chicken and pork chops. This may be due to the type of meat (chicken vs. pork) or the type of inoculated strain. It appears that the *L. monocytogenes* strains used in our study were not able to grow on pork at 3°C although the strains were grown at 10°C and could become adapted to low temperatures. *L. monocytogenes* is a facultative anaerobe but prefers to grow under a reduced oxygen tension. Therefore little growth of *L. monocytogenes* was observed by Manu-tawiah et al. (1993) on pork stored in modified atmospheres either without O_2 or with a low O_2 concentration at 4°C and growth could not be detected on the gas-packaged pork in our study. For *S. typhimurium*, the same results were obtained as for *L. monocytogenes*. *S. typhimurium* is capable of growing above 5°C and consequently remained at constant levels on pork in air in this study. Furthermore we observed no die-off of the organism during the 11 days storage period at 3°C in the modified atmosphere.

Conclusion

This study confirms that the packaging of pork in this specific type of modified atmosphere extends shelf-life during refrigerated storage at 3°C. In addition, there appears to be no increased hazard with respect to the pathogens tested. This preliminary conclusion has to be verified by performing challenge experiments with contaminated pork stored at higher (abuse) temperatures.

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