

Inhibition of Anaerobic/Facultative Pathogen Growth in Meat by High Level Carbon-Monoxide Modified Atmosphere

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Background

Food spoilage by bacteria is due to growth of either organoleptic bacteria which change the appeal to consumer, or of pathogenic ones. The latter class of bacteria constitute the primary hazard of meat with extended shelf-life. The goal of modified atmosphere packing (MAP) of meat is extended shelf-life of refrigerated meat by appearance and safety criteria. Mixtures of oxygen to keep the fresh "blooming" color and carbon-dioxide to inhibit bacterial growth are used. However, these two gases have each their pitfall, oxygen as the initiator of oxidative rancidity and carbon dioxide as the cause of color loss. Our results indicated that meat kept under high concentration of CO has the benefit of oxidation prevention and color conservation plus reduced total bacterial growth. While growth of aerobic bacteria is expected due to lack of oxygen, the fate of anaerobic facultative bacteria under CO MAPs is less clear. Low concentration of 0.4% used in Norway (Sorheim et al. Meat Science 52, 157-164, 1999) does not affect bacterial growth (Sorheim et al, Meat Science 52, 157-164, 1999). In the current study we compared growth of anaerobic/ facultative meat pathogens under air and high CO MAP which retards oxidative rancidity.

Objectives

To evaluate the effect of CO MAP on growth of anaerobic/Facultative meat pathogens.

Methods

Materials: All chemicals used in this study were purchased in Sigma Ltd. Culture media were Difco- USA or BBL-USA products.

Meat preparation: Fresh meat (beef or poultry) was treated on the day of slaughter. Following slaughter meat was transferred on ice to the laboratory. The meat surface was quickly flamed and cut to small pieces weighing 25-35 grams. The meat pieces were individually placed on Petri dishes under sterile hood. Each sample was inoculated with 10^4 - 10^5 /gram of the desired ATCC pathogen bacteria strain. The inoculated meat sample was introduced into a gas nontransferable plastic bag (plastobar N 120 of Plastopil Ltd.). The bag was sealed and air exchanged by defined MAP.

Inoculation procedure: The bacterial strain tested was grown on a selective and a non-selective solid medium for 24 hours at 37° C. The typical growth of the bacterium on the selective agar confirmed its identity. Some colonies from the non-selective agar were diluted in peptone water (0.1%) to obtain turbidity of approximately 10 bacteria per ml. This inoculum was diluted further to obtain 10^5 bacteria per ml. From this dilution 0.2 - 0.3 ml. were spread on each meat piece so that the final concentration of bacteria per gram meat was 10^3 - 10^4 . In the case of *Clostridium perfringens* 0.2-0.3 ml. Inoculum was injected aseptically to the middle of each meat piece. The initial number of bacteria per gram meat was then determined using the selective agar according to FDA - BAM regulations.

3) Bacterial strains: The following ATCC bacterial strains were used: *Listeria monocytogenes*-7644; *Salmonella typhimurium*-14028; *Clostridium perfringens*-13124; *Staphylococcus aureus* - 29213; *Escherichia coli* O157-H7 - 35150

Results and discussions

Meat containing bags were filled with either air or nitrogen as control or the desired CO containing MAP and incubated at 10°C, representing non-strict chilled conditions. General bacterial count at time zero was 10^2 - 10^3 /gr. and inoculated pathogen in the range of 10^4 - 10^5 /gr. At time periods, samples were tested for total bacterial count and for the level of the inoculated strain according to FDA regulations described in the Bacteriological Analytical Methods (1995). At each time point bacterial growth was measured in three samples of meat sealed under air (control) and three samples sealed under CO MAP. Growth was followed for at least two weeks and mostly for three weeks. As a routine beef meat was used but for some strains like *Salmonella* experiments were carried out with poultry as well. Under air all pathogens grew several orders of magnitude within a few days at 10°C reaching a level of $\sim 10^9$ /gr. *Clostridium* as expected did not grow under air at this temperature. Under MAP of essentially all CO atmosphere (\square 95%), no growth could be observed for all pathogens even after three weeks. In figure 1 we demonstrate typical results for growth of *E. coli* O157 on beef meat under air and under MAP consisting of

CO only. MAP containing CO suggested to include up to 10% CO. To find out the effect of such atmospheres on the pathogen growth similar experiment was carried out for MAP containing 90% N₂ and only 10% CO. The results shown in Figure 2 Indicate that under this atmosphere growth of E.coli occurred although somewhat reduced as compared to control. These results indicate that CO is an effective inhibitor for anaerobic growth as well, but high levels of CO are required.

Figure 1: Growth of E.coli Under Air and Essentially-All CO

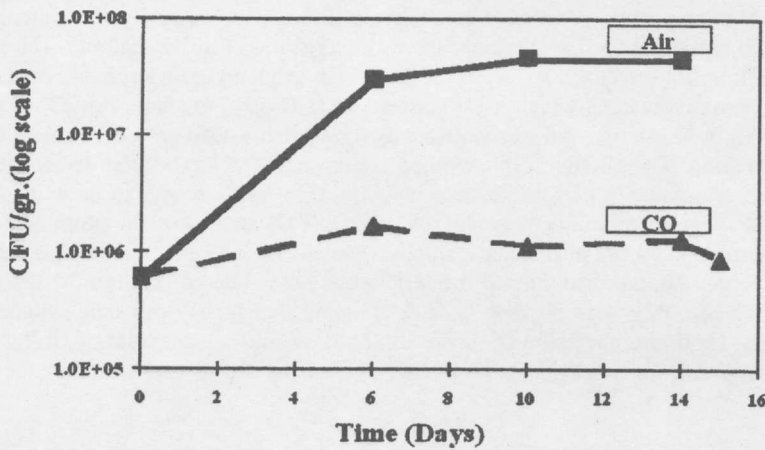
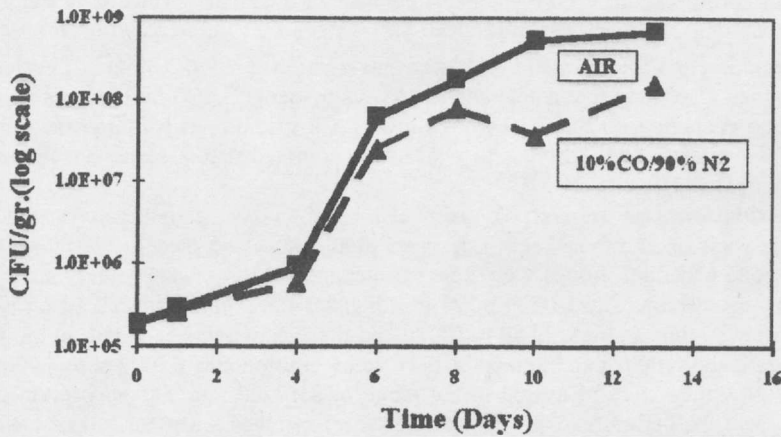


Figure 2: Growth of E.coli Under Air and Low CO



Conclusions

Our results show that under MAP containing essentially CO, growth of anaerobic/facultative bacteria mostly found in meats (beaf poultry fish etc.) is inhibited under refrigerated conditions. So far Norway is the only country in which CO is used in MAP and its level is only 0.4%. Our results suggest that high CO MAP atmosphere should be considered as an effective MAP which will provide protection against oxidative rancidity and bacterial growth. MAP of high CO provides the desired combination of simultaneous appeal by consumer senses and safety of product.