# THE EFFECT OF INITIAL GAS TO MEAT RATIO ON THE SPOILAGE FLORA OF BEEF PACKAGED UNDER $CO_2$

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## INTRODUCTION

Carbon dioxide is known to inhibit the growth of many microorganisms, although the susceptibility of microbial species to $CO_2$ varies widely. The inhibitory effects of $CO_2$ generally increase with partial pressure of the gas, but the relationship is not necessarily linear (Eklund, 1984). This inhibitory effect of $CO_2$ is exploited to retard meat spoilage in two types of packaging system: modified atmosphere retail display packs and packs designed for prolonged storage of meat. It is the latter type of packaging that is considered in this paper.

With packs designed for the prolonged storage of meat, an atmosphere composed solely of $CO_2$ is used with a gas-impermeable foil laminate film (Gill, 1986). Carbon dioxide is highly soluble in both water and oils, so the volume of gas initially added is reduced as equilibrium is attained between the atmosphere and the dissolved gas. If the amount of $CO_2$ added is insufficient to saturate the meat, the pack will collapse around the product and the partial pressure of $CO_2$ to which the spoilage flora is exposed will be less than atmospheric. The consequences for spoilage development of such reduction in $CO_2$ partial pressure have not been clearly defined. We therefore examined the effect on spoilage development of variations in the initial ratio of $CO_2$ volume to meat mass, to determine if $CO_2$ applied in less than saturating quantities could be effective in extending the storage life of chilled beef in anoxic packaging systems.

## THE SOLUBILITY OF $CO_2$ IN BEEF

The absorptive capacity of meat for $CO_2$ must be known to evaluate the effects of variation in the initial gas volume to meat ratio on the rate of spoilage development. The solubility of $CO_2$ in beef tissues was therefore determined by saturating weighed samples of beef tissue with $CO_2$ at defined temperatures, then treating the tissues with perchloric acid solution in a sealed system and absorbing the gas evolved in standard Ba(OH)2 solution. The volumes of gas evolved were determined by titrating the residual Ba(OH)2 with standard HCl solution.

The solubility of $CO_2$ in beef muscle tissue at O#C increased linearly with increasing tissue pH, by approximately 360 ml/kg for each pH unit rise (Fig. 1). The solubility of $CO_2$ in single samples of lamb and pork muscle tissue were in good agreement with this data.

The solubility of $CO_2$ in beef muscle tissue decreased with increasing temperature. The plot of $CO_2$ solubility against temperature is a shallow concave curve that is approximately linear for the temperature range to which chilled meat would be exposed during commercial handling and storage (-1.5 to 10°C). Within that temperature range, $CO_2$ solubility would decrease by about 19 ml/kg for each I#C rise in temperature (Fig. 2). Solubility vs temperature plots for muscle tissues of higher pH values and from different species essentially parallel the data for low pH beef muscle tissue (Gill, 1988).

The solubility of $CO_2$ in beef fat tissue increased slowly with temperature to reach a maximum value at about 22#C, and thereafter declined (Fig. 2). Solubility vs temperature curves for fat tissues from other species showed large initial increases and peak values at lower temperatures than were observed for beef fat tissue (Gill, 1988).

## SAMPLE PREPARATION AND STORAGE

Beef strip loins of normal pH (5.5-5.7) or high pH (6.0-6.4) were collected from a local abattoir, trimmed of all surface fat, then tumbled together in a tumbling machine to equalize the distribution of bacterial contaminants. After reassortment into normal- and high-pH groups, the strip loins were divided into 400 g cuts for packaging.

Each cut was vacuum packaged in a polyethylene film of high gas permeability before being packaged individually

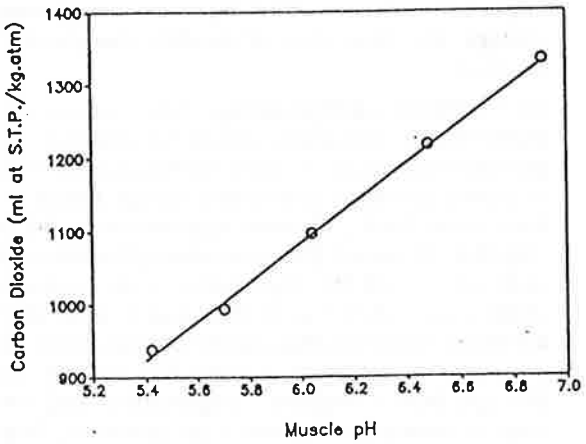


Fig. 1. The effect of the pH of the tissue on the solubility of $CO_2$ in beef muscle.

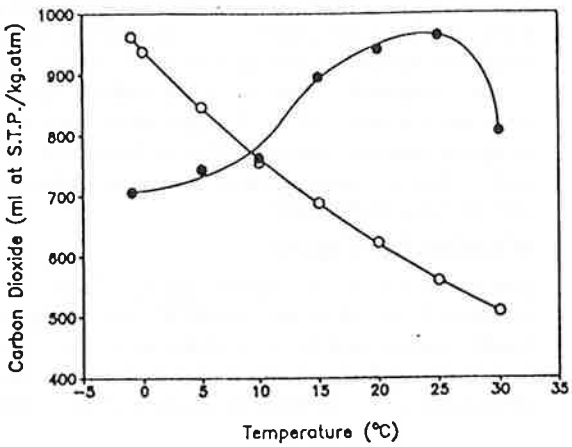


Fig 2. The effect of temperature on $CO_2$ solubility in beef muscle tissue, pH 5.42 ( O ) and beef fat tissue ( ● ).

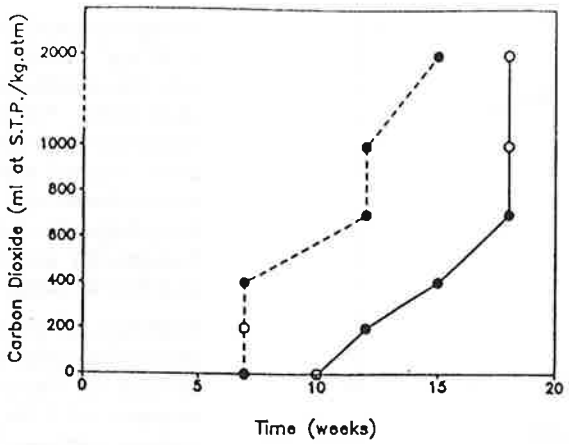


Fig 3. The effect of the added volume of $CO_2$ on the time of first detection of spoilage flavours in anoxically packaged beef. Stored at 1°C acid flavours ( O ), putrid flavours ( ● ), normal pH beef (—), high pH beef (- - -).

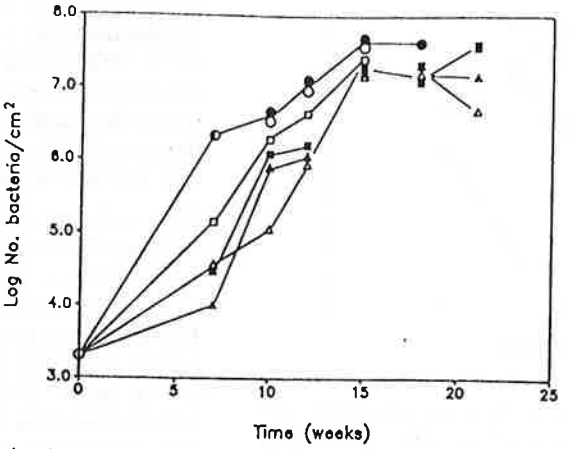


Fig 4. Growth of the spoilage flora on anoxically packaged normal pH beef stored at 1°C with $CO_2$ added at 0 ( O ), 200 ( ● ), 400 ( □ ), 700 ( ■ ), 1000 ( △ ) and 2000 ( ▲ ) ml/kg of meat.

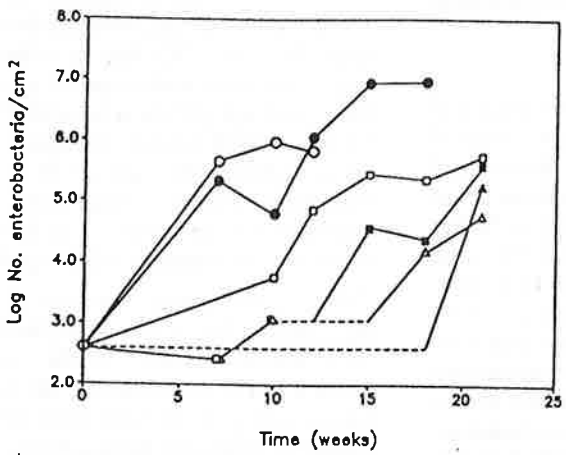


Fig. 5. Growth of the enterobacteria fraction of the spoilage flora on anoxically packaged, normal pH beef stored at 1°C with $CO_2$ added at 0 ( O ), 200 ( ● ), 400 ( □ ), 700 ( ■ ), 1000 ( △ ) and 2000 ( ▲ ) ml/kg of meat. Dashed lines connect to sampling times when enterobacteria were undetected.

in a gas barrier film of aluminium foil laminate. Cuts were packaged with $CO_2$ added at volumes of 0, 80, 160, 280, 400 or 800 ml per cut.

Three cuts of each pH type were examined to determine the numbers and composition of the initial flora. All other cuts were stored at 1 ± 0.5°C. Three cuts of each pH group in each type of packaging were removed for organoleptic assessment and microbiological analysis after storage periods of 7, 10, 12, 15, 18 and 21 weeks.

## SOLUBILITY OF $CO_2$ IN BEEF SAMPLES

The amount of $CO_2$ required to saturate the beef samples can be estimated from the solubility data for $CO_2$ in meat tissues. The samples were free of fat tissue, stored at 1°C, and had median pH values of 5.6 for normal-pH beef and 6.2 for high-pH beef. The amount of $CO_2$ required for tissue saturation would therefore be about 1000 ml/kg for the normal-pH samples and 1200 ml/kg for the high-pH samples.

## SPOILAGE OF THE BEEF SAMPLES

Spoilage was first detectable as flavour changes, generally without any accompanying odour changes. Most of these initial spoilage flavours were mildly putrid, but in some series of samples acid flavours were initially detected. With all series of samples, strong putrid flavours accompanied by putrid odours were observed at the sampling time after the initial detection of flavour changes.

With normal-pH beef, the time at which spoilage was detected increased with increasing amounts of added $CO_2$ up to 700 ml/kg. Larger amounts of $CO_2$ did not further extend the time at which spoilage was first detected, but with greater amounts, acid rather than putrid flavours were initially observed (Fig. 3).

With high-pH beef, the time at which spoilage was first detected did not differ for added amounts of $CO_2$ between 0 and 400 ml/kg. However, cuts packaged without added $CO_2$ were grossly spoiled when first examined at seven weeks, whereas at that sampling time, spoilage flavours were mild for cuts with $CO_2$ added at 200 and 400 ml/kg. Spoilage was first detected at later times with larger amounts of added $CO_2$ (Fig. 3).

The determination of times of spoilage onset was necessarily crude, as it relied on subjective judgements at relatively large time intervals. However, the information indicates that with both normal- and high-pH samples, the storage time before spoilage was extended by increasing the amount of $CO_2$ added until the meat was fully saturated at atmospheric pressure. Frank spoilage was essentially due to putrid odours and flavours resulting from the activities of enterobacteria, although such spoilage was sometimes shortly preceded by mild acid flavour changes ascribable to lactobacilli (Gill and Penney, 1985). Spoilage invariably developed in

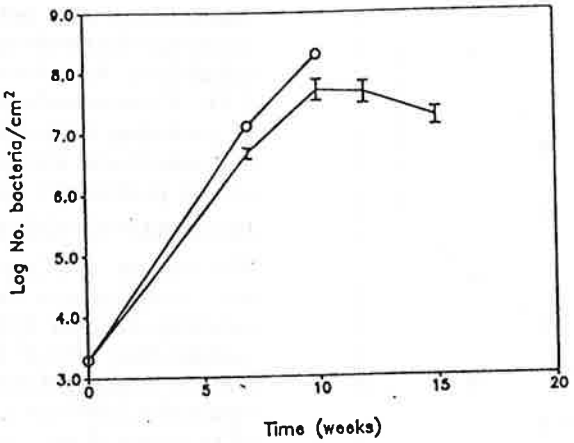


Fig. 6. Growth of the spoilage flora on anoxically packaged, high pH beef stored at 1°C with $CO_2$ added at 0 ( ○ ) or 200 to 2000 ( I ), ml/kg of meat.

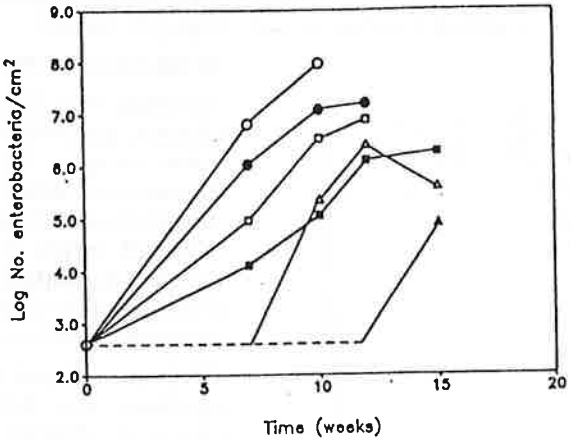


Fig. 7. Growth of the enterobacteria fraction of the spoilage flora on anoxically packaged, high pH beef stored at 1°C, with $CO_2$ added at 0 ( ○ ), 200 ( ● ), 400 ( □ ), 700 ( ■ ), 1000 ( △ ) and 2000 ( ▲ ) ml/kg of meat. Dashed lines connect to sampling times when enterobacteria were undetected.

high-pH beef before it developed in similarly packaged normal-pH beef.

## SPOILAGE FLORA DEVELOPMENT

Cuts were bisected immediately after packs were opened and one half of each cut used for microbiological examination. Each half cut was vigorously rinsed for 2 min with 100 ml of 0.1% peptone. Samples from serial dilutions of the rinse fluid were spread on plates of Plate Count Agar (Difco) that were then incubated for three days at 25°C. Flora numbers and composition were determined from plates bearing 20 to 100 colonies. Numbers of each distinctive colony type were estimated. Three representative colonies of each type were picked from each plate and identified to the generic level. Plates bearing approximately 1000 colonies were also examined for estimation of numbers of Gram negative bacteria when these organisms were few or absent on platings of higher dilutions. Relatively small numbers of Gram negative bacteria could be distinguished on such plates because their large colonies were easily distinguished amongst numerous small colonies of lactobacilli. Flora numbers per $cm^2$ were calculated on the basis that all half cuts had a similar surface area of 500 $cm^2$.

Initial numbers of bacteria were about 2 x $10^3/cm^2$. About 20% of the flora was enterobacteria, and lactobacilli were not detected. Apparently, the tumbling procedure had resulted in the cuts being contaminated with a flora enriched for enterobacteria. At all subsequent samplings, the floras were composed of lactobacilli and enterobacteria, or of lactobacilli alone.

With normal-pH beef, the proportion of enterobacteria in the spoilage flora tended to decrease with increasing amounts of $CO_2$, from about 15% for vacuum packaged cuts to undetectable numbers when $CO_2$ was added at 2000 ml/kg of meat. The rate of development of the total flora, in which lactobacilli greatly predominated, was similar for $CO_2$ added at 0 and 200 ml/kg, but declined with increasing amounts of $CO_2$ up to 1000 ml/kg. The slowing of flora development appeared to be due largely to an extension of the lag phase rather than a reduction of the maximum growth rate that was achieved (Fig. 4).

The enterobacteria fraction of the spoilage flora of normal pH beef was inhibited by increasing amounts of $CO_2$ to a greater extent than the total flora. The lag phase of the enterobacteria was greatly extended by increasing amounts of $CO_2$ and the growth rate also appeared to be reduced at the higher volumes of added $CO_2$ (Fig. 5).

The proportion of enterobacteria in the spoilage flora of high pH beef also tended to decrease with increasing amounts of $CO_2$. Enterobacteria formed about 50% of the total flora in packs with 0 $CO_2$ but, as with normal pH meat, these organisms were undetected at early sampling times in the floras of high pH meat to which the highest volumes of $CO_2$ had been added. Addition of $CO_2$ at 200 ml/kg of meat reduced the rate of flora development and the maximum numbers that were achieved. Increasing amounts of $CO_2$ appeared to have no further significant effect on development of the spoilage flora (Fig. 6).

The enterobacteria fraction of the spoilage flora of high-pH beef was inhibited by increasing amounts of $CO_2$ in a similar manner to the same flora fraction on normal-pH beef. However, the effects of $CO_2$ on the lag phase and growth rate were less, and the maximum numbers achieved were higher, for high-pH than for normal-pH meat (Fig. 7).

It has been suggested that enterobacteria cannot grow on muscle tissue of normal pH under strictly anoxic conditions (Grau 1983). However, it is clear that neither the low pH encountered in meat, nor high $CO_2$ concentrations, completely inhibited the growth of these organisms, although both together act to greatly increase the lag phase and slow the growth rate of enterobacteria.

With high-pH meat, the lesser inhibitory effects of $CO_2$ alone were observed.

Contrary to expectation, some inhibition of growth of lactobacilli due to the pH of normal-pH beef was indicated, with progressive enhancement of this inhibition by increasing $CO_2$ concentrations. There did not appear to be any significant inhibition by $CO_2$ of lactobacilli growing on high-pH beef.

The late appearance of enterobacteria in floras where previously only lactobacilli had been detected was also unexpected, as lactobacilli in high numbers are known to inhibit enterobacteria in meat spoilage floras (Reddy et al. 1975). There was some indication of inhibition of enterobacteria as flora numbers exceed $10^6/cm^2$ but, as has been observed on agar medium (Dubois et al. 1979), the inhibitory effects of lactobacilli apparently decay some time after these organisms have achieved their maximum numbers in the flora.

## PRACTICAL CONSIDERATIONS

Carbon dioxide can substantially extend the storage life of both normal-pH and high-pH meat. However, the inhibitory effects of relatively low $CO_2$ concentrations will be less pronounced with high pH than with normal pH meat, because of the additive inhibitory effects of low pH that occur with the latter type of meat.

Putrid spoilage due to enterobacteria will ultimately develop in all anoxically packaged meat, even when enterobacteria numbers remain below $10^5/cm^2$. However, if initial numbers of enterobacteria are sufficiently low, the meat can be spoiled by lactobacillus activities, or by non-microbially mediated flavour or textured deterioration, before spoilage due to enterobacteria develops (Egan and Shay, 1982).

The full effects of $CO_2$ addition will be achieved only if the gas is added to packs in quantities in excess of those required to saturate the meat. The maximum tissue pH, fat content and the anticipated minimum storage temperature will have to be considered when deciding the optimum quantity of $CO_2$ to be added to packs of particular meat products.

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